Edited by
Karl Esser

THE MYCOTA

A Comprehensive Treatise on Fungi
as Experimental Systems for Basic and Applied Research

Growth, Differentiation and Sexuality

I

Third Edition

Jürgen Wendland
Volume Editor

Springer

The Mycota

Edited by
K. Esser

The Mycota

I *Growth, Differentiation and Sexuality*
1st edition ed. by J.G.H. Wessels and F. Meinhardt
2nd edition ed. by U. Kües and R. Fischer
3rd edition ed. by J. Wendland

II *Genetics and Biotechnology*
1st and 2nd edition ed. by U. Kück

III *Biochemistry and Molecular Biology*
1st and 2nd edition ed. by R. Brambl and G. Marzluf
3rd edition ed. by D. Hoffmeister

IV *Environmental and Microbial Relationships*
1st edition ed. by D. Wicklow and B. Söderström
2nd edition ed. by C.P. Kubicek and I.S. Druzhinina
3rd edition ed. by I.S. Druzhinina and C.P. Kubicek

V *Plant Relationships*
1st edition ed. by G. Carroll and P. Tudzynski
2nd edition ed. by H.B. Deising

VI *Human and Animal Relationships*
1st edition ed. by D.H. Howard and J.D. Miller
2nd edition ed. by A.A. Brakhage and P.F. Zipfel

VII *Systematics and Evolution*
1st edition ed. by D.J. McLaughlin, E.G. McLaughlin, and P.A. Lemke†
2nd edition ed. by D.J. McLaughlin and J.W. Spatafora

VIII *Biology of the Fungal Cell*
1st and 2nd edition ed. by R.J. Howard and N.A.R. Gow

IX *Fungal Associations*
1st and 2nd edition ed. by B. Hock

X *Industrial Applications*
1st edition ed. by H.D. Osiewacz
2nd edition ed. by M. Hofrichter

XI *Agricultural Applications*
1st and 2nd edition ed. by F. Kempken

XII *Human Fungal Pathogens*
1st edition ed. by J.E. Domer and G.S. Kobayashi
2nd edition ed. by O. Kurzai

XIII *Fungal Genomics*
1st edition ed. by A.J.P. Brown
2nd edition ed. by M. Nowrousian

XIV *Evolution of Fungi and Fungal-Like Organisms*
Ed. by S. Pöggeler and J. Wöstemeyer

XV *Physiology and Genetics: Selected Basic and Applied Aspects*
Ed. by T. Anke and D. Weber

The Mycota

A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research

Edited by K. Esser

I

Growth, Differentiation and Sexuality
3rd Edition

Volume Editor:
Jürgen Wendland

Springer

Series Editor

Professor Dr. Dr. h.c. mult. Karl Esser
Allgemeine Botanik
Ruhr-Universität
44780 Bochum, Germany

Tel.: +49 (234)32-22211
Fax.: +49 (234)32-14211
e-mail: Karl.Esser@rub.de

Volume Editor

Professor Dr. Jürgen Wendland
Carlsberg Laboratory
Yeast Genetics
Gamle Carlsberg Vej 10
1799 Copenhagen V
Denmark

Tel.: +45 (3327) 5230
e-mail: Juergen.Wendland@carlsberglab.dk

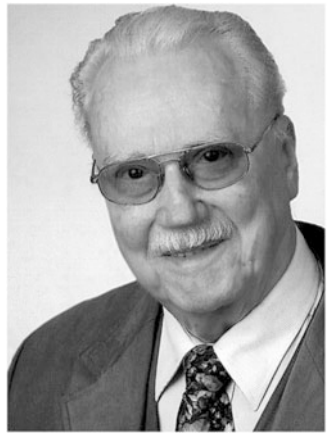
ISBN 978-3-319-25842-3 ISBN 978-3-319-25844-7 (eBook)
DOI 10.1007/978-3-319-25844-7

Library of Congress Control Number: 2015957987

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)



Karl Esser
(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

Jürgen Wendland
(born 1966) studied Biology at the Justus-Liebig-University in Giessen, Germany. He obtained his Ph.D. in Genetics at the Philipps University in Marburg in 1996 under the supervision of Prof. Albrecht Klein and Dr. Erika Kothe, working on the elucidation of the molecular basis of basidiomycete mating types using *Schizophyllum commune* as model system. The same year he joined Prof. Peter Philippsen at the Biozentrum of the University of Basel, turning his attention as a Post-Doc to genes controlling polarized hyphal growth in the flavinogenic fungus *Ashbya gossypii*. At the start of 2000, he joined Erika Kothe at the Friedrich-Schiller University in Jena and also became junior group leader at the Leibniz Institute of Natural Product Research and Infection Biology, Hans-Knöll Institute. Here, he started work on growth control of fungal pathogens, particularly, *Candida albicans*. In 2004, he completed his habilitation and earned the *Venia legendi* in Genetics and Microbiology. Since 2006, he works as Professor in Yeast & Fermentation at the Carlsberg Laboratory in Copenhagen, Denmark. His work there initially included *C. albicans* and *A. gossyppi*, but now is focused on comparative genomics and evolution of *Saccharomycetes*, lager yeast breeding, and industrial biotechnology.

Series Preface

Mycology, the study of fungi, originated as a sub discipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for self incompatibility, termed "heterothallism", and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with *Neurospora crassa*, led to the one gene: one enzyme hypothesis and to a second Nobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to *Saccharomyces cerevisiae*, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics and molecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology, medical mycology, and systematics. Today, there is much interest in the genetic manipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title *The Mycota*. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this Series of Volumes the names adopted for major groups of fungi (representative genera in parentheses) areas follows:

Pseudomycota

Division:	Oomycota (*Achlya, Phytophthora, Pythium*)
Division:	Hyphochytriomycota

Eumycota

Division:	Chytridiomycota (*Allomyces*)
Division:	Zygomycota (*Mucor, Phycomyces, Blakeslea*)
Division:	Dikaryomycota
Subdivision:	Ascomycotina
Class:	Saccharomycetes (*Saccharomyces, Schizosaccharomyces*)
Class:	Ascomycetes (*Neurospora, Podospora, Aspergillus*)
Subdivision:	Basidiomycotina
Class:	Heterobasidiomycetes (*Ustilago, Tremella*)
Class:	Homobasidiomycetes (*Schizophyllum, Coprinus*)

We have made the decision to exclude from *The Mycota* the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.

The Series throughout will address three basic questions: what are the fungi, what do they do, and what is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this Series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature of fungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather than ingest, nutrients and they have rigid cell walls. The vast majority of fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on their method of production have been used to delineate most fungal taxa. Although there is a multitude of spore forms, fungal spores are basically only of two types: (i) asexual spores are formed following mitosis (mitospores) and culminate vegetative growth, and (ii) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondary metabolites, is also noteworthy.The metabolic capacities of fungi have attracted much interest in natural products chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the microfungi that grow in the anaerobic environment of the rumen, the many insect associated fungi and the medically important pathogens afflicting humans. Yes, fungi are ubiquitous and important. There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contribute to this Series. We are especially indebted and grateful for the initiative and leadership shown by the Volume Editors in selecting topics and assembling the experts. We have all been a bit ambitious in producing these Volumes on a timely basis and therein lies the possibility of mistakes and oversights in this first edition. We encourage the readership to draw our attention to any error, omission or inconsistency in this Series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine Volumes.

Bochum, Germany — Karl Esser
Auburn, AL, USA — Paul A. Lemke
April 1994 — *Series Editors*

Volume Preface

The central aspects of fungal genetics and biology are fungal growth (either as yeast-like growth or hyphal growth), differentiation, generation of an elaborate fruiting-body structure, and sexuality, which is regulated by central mating loci and mating-type genes. Therefore, this volume was chosen as the first volume of *The Mycota* series more than 20 years ago. Since the second edition in 2006 another ten years have passed, necessitating an update and extension. This is now the third edition of Volume I of the series *The Mycota*. We have put great effort into renewing the chapters and have included new authors that have actively advanced novel research topics in the field.

We start the section on vegetative processes and growth from the inside of the fungal cell by looking at organelle inheritance and nuclear dynamics in multinucleate fungal cells. Both of these fields have progressed considerably in recent years. The molecular understanding of polarized hyphal growth at the hyphal tip has deepened over the last few years. Growth requires remodeling of the fungal cell wall and, at intervals, also the demarcation of hyphal cells by septation. A phenomenon that has been fascinating mycologists for decades has been hyphal fusion of vegetative cells. This can trigger incompatibility reactions or result in the generation of a network of interconnected hyphae. The general and molecular mechanisms and signal transduction cascades leading to hyphal fusion have been studied intensely and both are covered in this volume. This section is completed by a discussion on aspects of aging and longevity in fungi.

The section on signals in growth and development deals with autoregulatory signals, pheromone action, and abiotic signals of photomorphogenesis and gravitropism. This field is receiving growing attention and will gain ground over the next few years.

Reproductive processes in fungi are certainly of key interest for biotechnological applications and for the food industry. We cover both asexual and sexual development in fungi, with a close look at molecular genetics with respect to mating-type locus organization and regulatory genes.

With these sections, this volume of *The Mycota* covers specific topics that are presented in depth from their historic early days to today's detailed and mechanistic molecular characterizations. This volume thus contains information for both the student and advanced fungal biologist. It will, therefore, serve as a reference and as a starting point for further investigations.

With great delight we see that this field of molecular fungal biology is advancing rapidly and that new models are being established to allow representation of a greater biodiversity. We understand that it is always difficult to follow such rapidly changing fields, so we are indebted to the chapter authors for their

excellent contributions. Finally, we acknowledge the great collaboration with Springer and the project coordinator Dr. Andrea Schlitzberger, which made editing this book a pleasure.

Kopenhagen, Denmark Jürgen Wendland
October 2015

Contents

List of Contributors

Susanna M. Badalyan
Laboratory of Fungal Biology and Biotechnology, Yerevan State University, Yerevan, Armenia

Kirk Bartholomew
Department of Biology, Sacred Heart University, Fairfield, CT, USA

Albert D. de Boer
Regional Center for Biomedical Research, Albacete Science and Technology Park, University of Castilla–La Mancha, Albacete, Spain

Bernd W. Brandt
Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University, Amsterdam, The Netherlands

A. Burmester
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

Ana M. Calvo
Department of Biological Sciences, Northern Illinois University, Dekalb, IL, USA

Luis M. Corrochano
Departamento de Genética, Universidad de Sevilla, Sevilla, Spain

Bastian Dörnte
Department of Molecular Wood Biotechnology and Technical Mycology, Büsgen-Institute, University of Göttingen, Göttingen, Germany

R. Debuchy
Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette cedex, France

P. S. Dyer
School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

S. Ellenberger
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

K. Esser
Lehrstuhl für Allgemeine Botanik, Ruhr-Universität Bochum, Bochum, Germany

André Fleißner
Institut für Genetik, Technische Universität Braunschweig, Braunschweig, Germany

Thomas J. Fowler
Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL, USA

Daniela Freihorst
Institute for Microbiology, Microbial Communication, Friedrich Schiller University Jena, Jena, Germany

Paul Galland
Fachbereich Biologie, Philipps-Universität Marburg, Marburg, Germany

Luis J. García-Rodríguez
Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee, Scotland, UK

Alexander Gießler
Department of Molecular Wood Biotechnology and Technical Mycology, Büsgen-Institute, University of Göttingen, Göttingen, Germany

Amy S. Gladfelter
Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

Carolin Grimm
Department of Biosciences, Institute of Molecular Biosciences and Cluster of Excellence Frankfurt Macromolecular Complexes, J W Goethe University, Frankfurt, Germany

Piet W. J. de Groot
Regional Center for Biomedical Research, Albacete Science and Technology Park, University of Castilla–La Mancha, Albacete, Spain

Ryo Higuchi-Sanabria
Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

J. Stephen Horton
Department of Biological Sciences, Union College, Peter Irving Wold Center, Schenectady, NY, USA

Beatriz Ibarra
Department of Biological Sciences, Northern Illinois University, Dekalb, IL, USA

P. Inderbitzin
Department of Plant Pathology, University of California, Davis, CA, USA

Ursula Kües
Department of Molecular Wood Biotechnology and Technical Mycology, Büsgen-Institute, University of Göttingen, Göttingen, Germany

Erika Kothe
Institute for Microbiology, Microbial Communication, Friedrich Schiller University Jena, Jena, Germany

Jessica M. Lohmar
Department of Biological Sciences, Northern Illinois University, Dekalb, IL, USA

Luis G. Lugones
Microbiology, Utrecht University, Utrecht, The Netherlands

Fabienne Malagnac
Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette cedex, France

Míriam Osés-Ruiz
Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

Heinz D. Osiewacz
Department of Biosciences, Institute of Molecular Biosciences and Cluster of Excellence Frankfurt Macromolecular Complexes, J W Goethe University, Frankfurt, Germany

Jordi F. Pelkmans
Microbiology, Utrecht University, Utrecht, The Netherlands

Leonardo Peraza-Reyes
Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México, D.F., Mexico

Liza A. Pon
Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Marjatta Raudaskoski
Department of Biochemistry, Plant Molecular Biology, University of Turku, Biocity A, Tykistök. 6A, Turku, Finland

M. Riquelme
Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Baja California, Mexico

R. W. Roberson
School of Life Sciences, Arizona State University, Tempe, AZ, USA

Samantha E. Roberts
Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

Ana Belén Rodriguez-Urra
Biofungitek, SL, Parque Científico y Tecnológico de Bizkaia, Zamudio, Spain

E. Sánchez-León
Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Baja California, Mexico

Wasin Sakulkoo
Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

Timothy Satterlee
Department of Biological Sciences, Northern Illinois University, Dekalb, IL, USA

C. Schimek
Fernhochschule Riedlingen, Riedlingen, Germany

Monika Schmoll
Department Health and Environment, Bioresources, Austrian Institute of Technology GmbH, Tulln, Austria

E. Schulz
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

Antonio Serrano
Institut für Genetik, Technische Universität Braunschweig, Braunschweig, Germany

L. Siegmund
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

Pallavi Srivastava
Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Nicholas J. Talbot
Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

U. Ugalde
Biochemistry Laboratory II, University of the Basque Country, Donostia-San Sebastian, Spain

Eulogio Valentín
Departamento de Microbiología y Ecología, Universidad de Valencia, Burjassot, Valencia, Spain

J. Voigt
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

J. Wöstemeyer
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

Han A. B. Wösten
Microbiology, Utrecht University, Utrecht, The Netherlands

Ting-Fang Wang
Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

J. Wetzel
Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Jena, Germany

Matthias Wiemer
Department of Biosciences, Institute of Molecular Biosciences and Cluster of Excellence Frankfurt Macromolecular Complexes, J W Goethe University, Frankfurt, Germany

Dana M. Alessi Wolken
Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA

Vegetative Processes and Growth

1 Organelle Inheritance in Yeast and Other Fungi

Pallavi Srivastava[1], Dana M. Alessi Wolken[2], Luis J. García-Rodríguez[3], Ryo Higuchi-Sanabria[1], Liza A. Pon[1]

CONTENTS

PS and DMAW contributed equally to the work.
[1]Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA; e-mail: lap5@cumc.columbia.edu
[2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591, USA
[3]Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, UK

I. Introduction

The compartmentalization of cytoplasm into membrane-bound organelles creates order within a cell, prevents undesirable interactions between cellular activities, and enables specialized functions. While each organelle has a defined cellular role, its localization, copy number, size, and morphology can vary. For instance, variations in the carbon source for growth of budding yeast affects peroxisomal biosynthesis and can result in a tenfold change in the abundance of mitochondria (Einerhand et al. 1992).

Nuclear inheritance, which uses the mitotic spindle to segregate nuclei and chromosomes, occurs by a conserved and tightly regulated mechanism. The inheritance of other organelles occurs by more diverse and less-understood mechanisms. Organelles like mitochondria and chloroplasts cannot be produced de novo. This is true, in part, because they contain DNA, which must undergo template-dependent replication. As a result, these organelles must be transferred from mother to daughter cells to ensure normal daughter cell viability and/or function, and more than one mechanism for these essential inheritance events has been identified. Additionally, checkpoints are in place to help to ensure the inheritance of some organelles prior to the completion of cell division. Similarly, there are mechanisms that ensure the retention of newly inherited organelles in developing daughter cells during cell division.

Advances in fluorescence microscopy along with classic genetic and molecular biology tools make fungi a powerful model system to

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

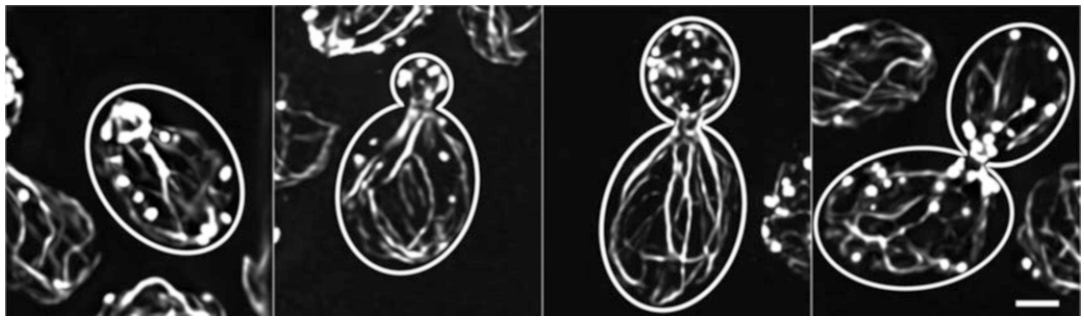


Fig. 1.1 Cell cycle-linked changes in the localization of actin patches and actin cables in *S. cerevisiae*. Alexa488-stained actin in wild-type BY4741 cells undergoing division: unbudded, small bud, medium bud, and large bud from *left to right panel*. Cells were fixed in 3.7 % paraformaldehyde for 50 min and stained with 2.5 μM Alexa488-phalloidin for 35 min. Maximum-intensity projection of a super-resolution structured illumination microscopy (SIM) Z-series. Scale bar represents 1 μM

understand the process of organelle inheritance. Time-lapse imaging provides spatiotemporal analysis of organelles during cell division. This chapter describes the mechanisms and regulation of organelle inheritance in the budding yeast, *Saccharomyces cerevisiae*, and other fungi.

II. Cytoskeletal Organization and Function in Organelle Inheritance

The cytoskeleton is essential for the maintenance of cell shape and polarity, chromosome segregation, cytokinesis, and organelle transport. It is also essential for directed movement of organelles during their inheritance and of components of the organelle inheritance machinery. The cytoskeleton controls organelle movement by two mechanisms. Cytoskeletal fibers function as tracks for the transport of organelles. Cytoskeletal dynamics also generate forces for directed organelle movement. Finally, the cytoskeleton is involved in the anchorage of organelles in mother and daughter cells, which is critical for retention of organelles at those sites.

A. Organization of the Actin Cytoskeleton in Fungi

The actin cytoskeleton is involved in cellular functions including cell polarity, cell shape, endocytosis, cytokinesis, and vesicular trafficking. In filamentous and unicellular fungi, actin is also required for cell wall deposition and septation and for polarized growth of hyphal tips and buds. F-actin is a highly dynamic polymer formed from G-actin monomers. F-actin is a double-stranded filament with a fast-growing barbed end (plus end) and a slow-growing pointed end (minus end). F-actin assembles into three major structures: actin patches, actin cables, and the actomyosin ring (Fig. 1.1). These structures perform several critical functions in all fungi including *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Aspergillus nidulans*, *Neurospora crassa*, and *Ustilago maydis* (Xiang and Plamann 2003; Mishra et al. 2014; Berepiki et al. 2011).

Actin patches are endosomes that contain a dense network of branched actin filaments and are usually concentrated at sites of polarized growth and cell wall remodeling (Huckaba et al. 2004; Young et al. 2004; Kaksonen et al. 2006). In *S. cerevisiae*, actin patches undergo cell cycle-linked changes in localization (Fig. 1.1) (Kilmartin and Adams 1984). They form a ring at the incipient bud site. During apical bud growth, which occurs in yeast with small buds, actin patches are enriched in the bud tip. As the bud grows by isotropic growth, actin patches localize throughout the cortex of the bud. Finally, they localize to the bud neck prior to cytokinesis. Although the precise

function of actin patches and endosomes at each of these sites is not well understood, it is likely that they function in membrane protein recycling.

More than 60 endosomal proteins, including clathrin, endocytic adapters, and scaffolds, also localize to actin patches (Burston et al. 2009; Weinberg and Drubin 2012). The force for overcoming cytoplasmic turgor pressure during membrane invagination and actin patch formation is produced by Arp2/3 complex-driven actin polymerization (Aghamohammadzadeh and Ayscough 2009; Winter et al. 1997). Arp2/3 complex nucleates actin from the side of a preexisting actin filament to form a dendritic network and is activated by nucleation-promoting factors (NPFs) (Volkmann et al. 2001). The four NPFs of *S. cerevisiae*, WASp, Myo1p, Pan1p, and Abp1p, increase but are not required for Arp2/3 complex nucleation activity (Winter et al. 1999; Evangelista et al. 2000; Duncan et al. 2001; Goode et al. 2001; Wen and Rubenstein 2005). Since actin-binding proteins are conserved between *S. cerevisiae*, *N. crassa*, and *A. nidulans*, it is likely that the composition of actin patches of filamentous fungi is similar to that found in *S. cerevisiae*. Interestingly, although Arp2/3 complex is essential in other fungi, *C. albicans* bearing deletions in Arp2 and Arp3 are viable and can carry out endocytosis, albeit at reduced rates.

Actin cables are bundles of F-actin arranged along the long axis of the cell (Fig. 1.1). They contain conserved proteins including the actin-bundling proteins fimbrin (Sac6p) and Abp140p and two tropomyosin isoforms (Tpm1p and Tpm2p) and are similar to actin bundles in microvilli, filopodia, and stereocilia (Adams et al. 1991; Drees et al. 1995; Asakura et al. 1998; Sagot et al. 2002a; Pruyne et al. 2002). Through most of the cell cycle in *S. cerevisiae*, actin filaments within actin cables are oriented with their barbed (plus) ends toward the bud or bud neck. However, immediately before cytokinesis, actin cables rearrange such that their barbed ends of actin cables in mother cells and buds are directed toward the bud neck (Fig. 1.1). This reorganization is triggered by the relocation of the polarisome from bud tip to bud neck (Kamei et al. 1998) and is critical for transport of secretory vesicles and other cargoes to the bud neck (Chuang and Schekman 1996; Bretscher 2003).

Actin cables are essential for organelle inheritance in *S. cerevisiae*, in part through their function as tracks for the transport of mitochondria, secretory vesicles, actin patches, Golgi apparatus, peroxisomes, and vacuoles from mother cells to buds (Pruyne et al. 1998; Bretscher 2003; Fehrenbacher et al. 2003; Huckaba et al. 2004). Myosin V motors drive the polarized movement of many of these cargoes along actin cables (Bretscher 2003; Matsui 2003). Although much of the characterization of actin cables was carried out in *S. cerevisiae,* the polarized localization and morphology of the Golgi apparatus require actin in *C. albicans* and *A. nidulans.* Therefore, it is possible that Golgi elements may move using actin cables in these filamentous fungi.

Actin cables are assembled at the bud tip and bud neck of *S. cerevisiae* and require the formins Bni1p and Bnr1p for nucleation of F-actin during actin cable assembly and elongation (Evangelista et al. 2002; Sagot et al. 2002b; Vavylonis et al. 2006; Buttery et al. 2007) (Fig. 1.2). Bni1p and Bnr1p localize to different sites within yeast cells, have different biochemical properties, and are regulated by different mechanisms. For example, Bud14p triggers removal of Bnr1p but not Bni1p from the barbed end of F-actin (Chesarone et al. 2009). Moreover, recent studies indicate that Bni1p function in actin cable assembly is cell cycle-regulated and support a role for cyclin-dependent kinase 1 in that process (Miao et al. 2013). There are also multiple formins that drive actin assembly in distinct regions of the cell in *S. pombe* and *A. gossypii*. However, in *A. nidulans* and *N. crassa,* there is only one formin (Berepiki et al. 2011). Actin cable disassembly is driven by Aip1p and cofilin (Ono and Ono 2002; Okada et al. 2006; Kueh et al. 2008).

Actin cables undergo continuous retrograde movement from their assembly site in the bud tip or bud neck into the mother cell (Yang and Pon 2002) (Fig. 1.2). This movement, retrograde actin cable flow (RACF), was identified and characterized in *S. cerevisiae*

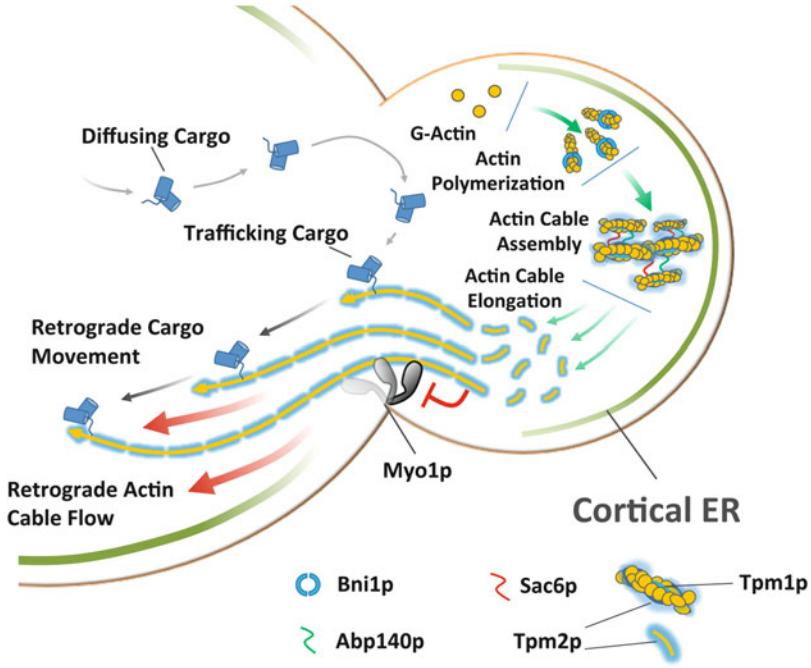


Fig. 1.2 Schematic representation of actin cable dynamics in budding yeast. Actin cables undergo retrograde actin cable flow from mother to the bud cell. This process is driven by actin assembly at the bud tip and neck from G-actin and stimulated by formins (Bni1p and Bnr1p). Pulling force for RACF is provided by Myo1p localized at the bud neck in a Tmp2p-dependent manner. From Higuchi-Sanabria et al. (2014)

(Huckaba et al. 2006). It is driven by two forces, like retrograde actin flow in other eukaryotes. The elongation of actin cables by insertion of new material at the tip of the actin cable in the bud tip or bud neck provides the pushing force for RACF. This pushing force requires formin-stimulated actin polymerization. The type II myosin at the bud neck, Myo1p, provides the pulling force for RACF and is regulated by a specific tropomyosin isoform, Tpm2p. As described below, RACF drives movement of mitochondria and endosomes (actin patches) from buds toward mother cells in *S. cerevisiae* and contributes to lifespan control as a filter to prevent lower-functioning mitochondria from moving from mother cells to buds (Higuchi et al. 2013; Liu et al. 2011).

In addition to actin patches and cables, the actin cytoskeleton also forms an actomyosin ring involved in cytokinesis. The actomyosin ring is composed of linear actin filaments nucleated by formins (Bni1p in the budding yeast *S. cerevisiae* and Cdc12p in the fission yeast *S. pombe*), which are activated by RhoA family GTPases (Tolliday et al. 2002; Yoshida et al. 2006). The Arp2/3 complex is localized to the ring in *S. pombe*, but this complex does not appear to play a role in ring assembly in *S. cerevisiae* (Winter et al. 1999; Pelham and Chang 2002). Actin, actin cross-linking proteins, and myosin II all play major roles in the assembly and constriction of the ring (Barr and Gruneberg 2007; Bi and Park 2012). Studies on *S. pombe* suggest a search-capture-pull-release model for ring constriction, in which actin filaments nucleated at one node are captured by myosin II in the adjacent node to condense the nodes together (Vavylonis et al. 2008).

B. Organization of the Microtubule Cytoskeleton in Fungi

Microtubules are highly dynamic polymers of conserved α-and β-tubulins. Microtubules display dynamic instability through a process of

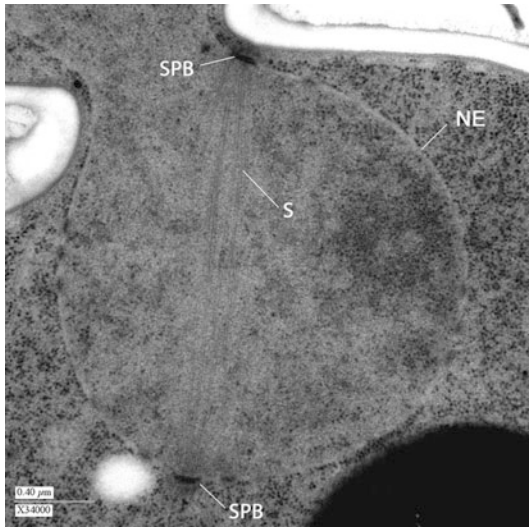


Fig. 1.3 The spindle pole body and spindle apparatus in budding yeast. Transmission electron micrograph of the nucleus in a dividing yeast cell. *NE* nuclear envelope, *S* spindle, and *SPB* spindle pole body. Image generously provided by M. Winey (University of Colorado)

continuous growth and shrinkage (Desai and Mitchison 1997). The spindle pole body (SPB) is the microtubule-organizing center (MTOC) for the spindle apparatus in fungi. Although they are functionally equivalent to centrosomes, SPBs do not contain centrioles. The SPB of *S. cerevisiae* is embedded in the nuclear envelope, where it nucleates microtubules that extend into the cytoplasm and into the nucleus (Fig. 1.3). It consists of 5 sub-complexes: a central core, membrane anchors that tether the central core to the nuclear envelope, 2 γ-tubulin complexes (one on each side of the central core), and linker proteins that connect γ-tubulin to the central core. Microtubule nucleation occurs at each of the γ-tubulin complexes (Jaspersen and Ghosh 2012). In *S. pombe*, the SPB is tethered to the nucleus through most of the cell cycle. It is inserted into the nuclear envelope prior to entry into mitosis and extruded from the nuclear envelope upon mitotic exit (Ding et al. 1997).

In *S. cerevisiae,* all microtubules nucleate from SPBs that are embedded in the nuclear envelope. In contrast, microtubules are also nucleated from cytoplasmic MTOCs in *S. pombe*, *A. nidulans*, and *U. maydis* (Tran et al. 2001; Straube et al. 2003; Veith et al. 2005). In *S. pombe*, three to six bundles of microtubules are arranged in an antiparallel manner along the longitudinal axis of the cell during interphase (Tran et al. 2001). The plus end of microtubules is more dynamic and faces the cell cortex at the poles. They are responsible for delivering polarity proteins to the pole and positioning the nucleus in the cell center (Tran et al. 2001). In filamentous fungi, microtubules are either arranged in a uniform array or in an antiparallel fashion (Xiang 2003).

The microtubule cytoskeleton undergoes reorganization as the cell enters the cell division cycle (Fig. 1.4). At the start of mitosis, interphase microtubules disappear and duplicated SPBs nucleate spindle microtubules in the nucleus. During metaphase, the spindle spans the length of the nucleus and microtubules capture kinetochores. The bipolar spindle is composed of kinetochore and pole microtubules. Rapid elongation of the spindle during anaphase leads to chromosome segregation (Mallavarapu et al. 1999; Ovechkina et al. 2003). Astral microtubules projecting from both SPBs play important roles in spindle orientation and chromosome separation (Markus et al. 2012).

III. Organelle-Specific Inheritance in Fungi

In *S. cerevisiae*, the system in which organelle inheritance has been studied most extensively, there are two fundamental mechanisms underlying organelle inheritance: movement of organelles from mother cells to daughter cells and anchorage of newly inherited organelles within developing daughter cells. The cytoskeleton is critical for organelle movement and for localization of anchorage proteins to the daughter cell. In the case of mitochondrial inheritance, movement and region-specific anchorage of the organelle are also critical for inheritance of higher functioning mitochondria by daughter cells, which in turn affects daughter cell fitness and lifespan.

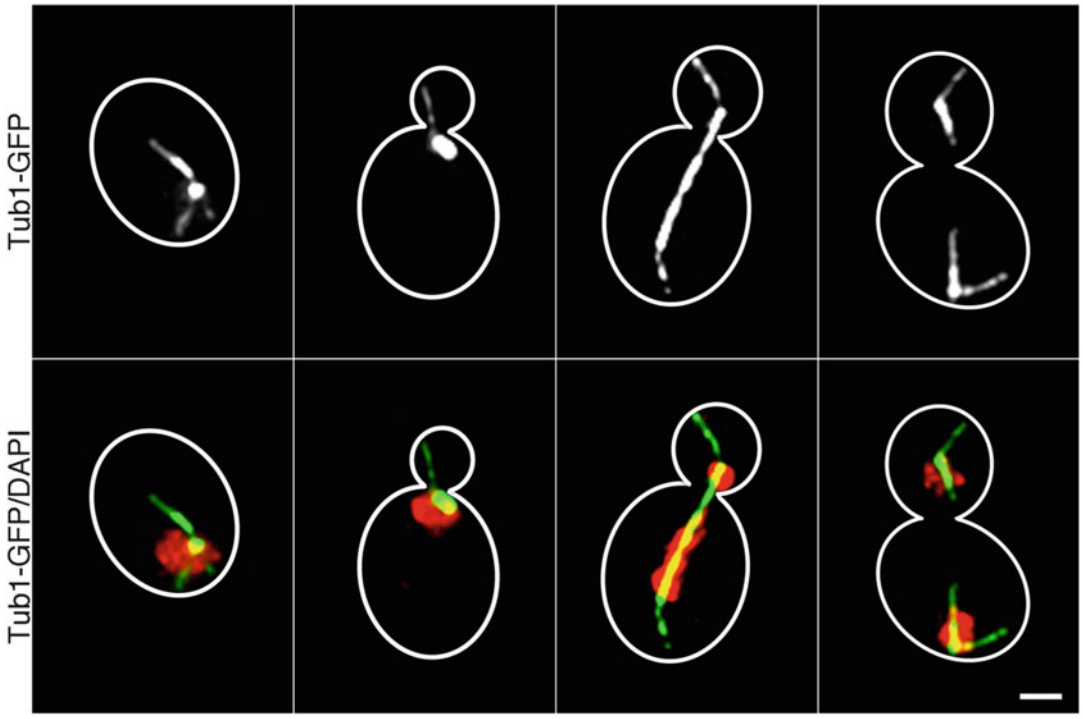


Fig. 1.4 Cell cycle-linked changes in the localization of the microtubule cytoskeleton in *S. cerevisiae*. Tub1-GFP-speckled tubulin in wild-type BY4741 cells undergoing division: unbudded, small bud, medium bud, and large bud from left to right panel. A GFP-tagged *TUB1* gene was integrated into the *LEU2* site of the genome. Cells were grown to mid-log phase and fixed in 3.7 % paraformaldehyde for 50 min and stained with 1 μg/mL DAPI in mounting solution. Maximum-intensity projection of a deconvolved wide-field Z-series, showing tubulin-GFP (upper panels; green in lower panels) and DAPI (red in lower panels). DAPI is pseudo-colored in red for reference. Scale bar represents 1 μM

A. Nuclear Inheritance in *S. cerevisiae*

Nuclei are dynamic organelles that undergo directed movement during cell division, development, and establishment of cell polarity. Fungi have been used extensively for studies on nuclear positioning because they are genetically manipulatable and because the defined shape of fungi provides landmarks for the analysis of nuclear migration. The widely studied function of the cytoskeleton in spindle formation and nuclear migration in fungi is the topic of numerous reviews (Xiang and Fischer 2004; Pavin and Tolić-Nørrelykke 2013). Here, we provide an overview for the role of the cytoskeleton in nuclear movement during cell division in *S. cerevisiae*.

During G2 and early M phase in *S. cerevisiae*, the nucleus undergoes a series of oscillations across the bud neck, which ultimately leads to alignment of the spindle along the mother-bud axis and migration of the nucleus into the bud. There are two functionally redundant mechanisms for nuclear migration during anaphase (Fig. 1.5). In one process, Kar9p and Bim1p tether the type V myosin Myo2p to the plus ends of astral microtubules. Myo2p then drives movement of the astral microtubules and their associated nucleus toward the bud tip using actin cables as tracks, which leads to migration of the nucleus into the bud (Miller and Rose 1998; Miller et al. 2000).

Dynein and microtubules also drive nuclear migration in *S. cerevisiae* (Fig. 1.5). During anaphase, dynein relocalizes from the plus ends of astral microtubules to the bud tip through interactions with the cortical anchor Num1p. At the bud tip, dynein binds to and

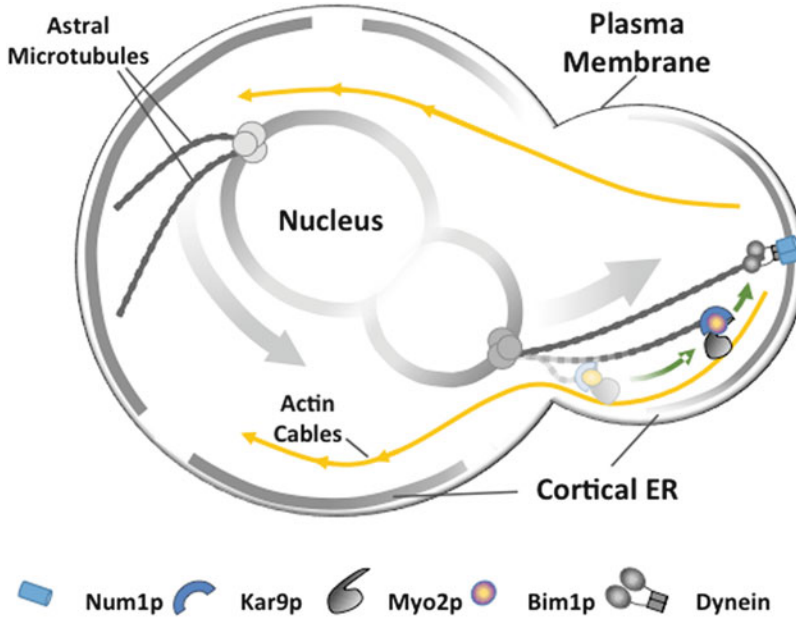


Fig. 1.5 Nuclear migration during cell division in *S. cerevisiae*. A schematic representation of the mechanism of action of key players involved in nuclear migration during cell division in budding yeast. Refer to the text for more details

generates pulling forces on astral microtubules that emanate from SPBs on the nucleus, which leads to migration of the nucleus into the bud (Lee et al. 2003; Sheeman et al. 2003). Dynein, dynactin, NUDF/LIS1, and NUDE/RO11 all form comet-like structures on the plus ends of microtubules, and microtubule plus ends are less dynamic in dynein mutants (Han et al. 2001; Zhang et al. 2002, 2003; Efimov 2003). Therefore, it is possible that dynein may also control nuclear position through its effects on microtubule dynamics. In this model, dynamic microtubules may exert pushing forces on nuclei, affecting their position by a mechanism that is similar to microtubule control of nuclear position in interphase cells in *S. pombe* (Tran et al. 2000; Xiang 2003).

B. Endoplasmic Reticulum

The ER is the major site of lipid biosynthesis and is essential for protein secretion. In addition, it stores Ca^{2+}, an ion that functions in many signal transduction cascades (Meldolesi and Pozzan 1998). Proteins that follow the secretory pathway are glycosylated in the ER lumen before they are exported from the organelle (Hegde and Lingappa 1999). The ER is also the final destination for vesicles that transport ER resident proteins from the Golgi apparatus to the ER (Pelham 1996). Interestingly, the ER maintains contact sites with nearly every membrane-bound structure within the cell, including the plasma membrane, Golgi apparatus, peroxisomes, endosomes, and mitochondria. As will be discussed later, the ER-mitochondria contact has implications for the regulation of organelle inheritance.

Structurally, the ER is a large, continuous, membrane-bound organelle enclosing a single lumen. It consists of different subdomains that are unique in morphology. More specifically, the ER consists of a reticulum underlying the plasma membrane (cortical ER, cER), ER associated with the nuclear envelope (nuclear ER,

nER), and thin projections connecting cER and nER (Preuss et al. 1991; Friedman and Voeltz 2011; English and Voeltz 2013). Ribosomes are associated with both cER and nER. To date, there is no evidence for the existence of smooth ER in *S. cerevisiae* (Baba and Osumi 1987).

Time-lapse microscopy experiments revealed that tubular ER structures undergo sliding, branching, and fusion (Prinz et al. 2000). Since treatment of cells with the actin-destabilizing drug Latrunculin-A (Lat-A) decreases ER motility, it is clear that the actin cytoskeleton plays a role in cER dynamics and morphology in budding yeast (Prinz et al. 2000; Fehrenbacher et al. 2002). However, the precise function of the actin cytoskeleton in ER dynamics is not well understood.

U. maydis serves as an example of ER organization in mycelial fungi. In *U. maydis,* the ER forms a polygonal network of tubules that associates with the cell cortex and makes contact with the nuclear envelope. As in *S. cerevisiae* (Prinz et al. 2000), the ER in *U. maydis* is highly dynamic. Analysis of the effect of the destabilization of microtubules or mutations in microtubule-dependent motors revealed that ER motility in *U. maydis* requires microtubules and cytoplasmic dynein (Wedlich-Söldner et al. 2002a, b), and microtubules support ER movement in *U. maydis* and in many vertebrate cells (Dabora and Sheetz 1988). However, ER tubules are present in the growing bud in cells with conditional mutations in tubulin at permissive and restrictive conditions (Wedlich-Söldner et al. 2002a). Thus, microtubules are not required for ER inheritance in *U. maydis*.

In *S. cerevisiae*, nER undergoes spindle-driven inheritance in conjunction with the nucleus. In contrast, inheritance of cER occurs by a fundamentally different, actin-dependent mechanism. cER is the first organelle inherited during the cell cycle (Preuss et al. 1991; Koning et al. 1996; Du et al. 2001). Shortly after bud emergence, a tubule of ER extends from the nER along the mother-bud axis and into the bud. The tubule makes stable contact with the bud tip and then spreads around the cortex of the bud forming cER at that site (Fehrenbacher et al. 2002; Estrada et al. 2003).

Extension of the ER tubule into the bud tip is dependent upon the actin cytoskeleton and a type V myosin, Myo4p. Early studies revealed that deletion of *MYO4* results in defects in actin cable-dependent movement of the mRNA from mother to daughter cells (Bertrand et al. 1998; Böhl et al. 2000). The She2p/She3p protein complex serves as an adaptor to bind mRNA to a region in the Myo4p C-terminal tail (Böhl et al. 2000). Other studies revealed that a point mutation in the ATP-binding region of the motor domain of Myo4p or a mutation in She3p inhibits ER inheritance (Estrada et al. 2003). Specifically, these mutations result in defects in formation of the ER segregation tubule, alignment of ER tubules along the mother-bud axis, and extension of ER tubules into the bud. Moreover, both She3p and Myo4p are recovered in fractions enriched in ER-derived membranes after subcellular fractionation. These findings raise the possibility that myosin may drive transport of ER tubules from mother to bud, where they form the cER in the daughter cell. Myo4p and She3p may also mediate the transport of ER anchoring proteins or mRNAs that encode ER anchoring proteins from the mother to the bud tip.

The mechanism underlying anchorage of ER in the bud tip is not well understood. However, recent studies indicate that five proteins tether cER to the plasma membrane in *S. cerevisiae* (Manford et al. 2012). The mRNA and proteins of two of those tethers, Ist2p and Tcb2p, localize to the bud tip (Shepard et al. 2003). Therefore, it is possible that one or both of these proteins mediate stable attachment of ER tubules to the bud tip during cER inheritance. Anchorage of ER in the bud tip is also dependent upon Sec3p, a nonessential component of the exocyst, a tethering complex that is required for polarized transport of secretory vesicles into buds (TerBush et al. 1996; Guo et al. 1999). ER tubules form from the nER in *sec3Δ* yeast. However, they fail to form stable contacts with the bud tip and can recede from buds to mother cells. Conversely, overexpression of *SEC3* promotes ER accumulation in the bud tip (Wiederkehr et al. 2003; Reinke et al. 2004). Since Sec3p serves as a spatial landmark

for polarized secretion in yeast, it may also serve as a landmark for ER anchorage in the bud tip.

Spreading of ER that is anchored to the bud tip around the bud cortex requires the serine/threonine protein phosphatase Ptc1p through effects on Slt2p, the MAP kinase of the PKC pathway that is needed for cell wall integrity (CWI) (Du et al. 2006). Slt2p localizes to the nucleus and to the bud tip, and its localization to the bud tip requires Pea2p and Spa2p, two components of the polarisome, a protein complex that localizes to the bud tip and promotes assembly and polarization of the actin cytoskeleton at that site (Li et al. 2013). Slt2p is also activated at the stage in the cell cycle when the ER tubule is anchored to the bud tip and it must be inactivated for ER to spread from the bud tip to the bud cortex. Therefore, it is possible that Ptc1p promotes release of these ER tubules from the actin cytoskeleton, which allows ER to spread around the bud cortex.

Recent studies revealed that ER stress, which leads to accumulation of unfolded proteins in the ER, inhibits cER inheritance and cytokinesis (Babour et al. 2010). Specifically, ER stress results in activation of Slt2p and defects in inheritance of cER by buds. Since deletion of *SLT2* promotes cER inheritance in yeast undergoing ER stress, it is clear that the defect in ER inheritance observed in ER stressed cells is due to activation of Slt2p. Finally, ER stress also inhibits cytokinesis through effects on the septins. These studies revealed a surveillance checkpoint that inhibits cell cycle progression and ER inheritance when the ER is compromised. Additionally during ER inheritance, a lateral diffusion barrier formed at the bud neck by sphingolipids in a septin-dependent manner promotes the retention of misfolded proteins in the mother cell (Clay et al. 2014). Other components of this barrier include Epo1p, Scs2p, and Shs1p (Clay et al. 2014).

Recent work has shown a link between cER inheritance and mitochondrial inheritance in budding yeast that is consistent with the temporal inheritance of these organelles, namely, that cER is trafficked to the bud tip prior to mitochondrial inheritance. This interesting dependency will be discussed in greater detail below in the section on mitochondrial inheritance.

C. Mitochondria

Mitochondria are involved in many vital functions in cells including aerobic respiration; synthesis of molecules such as fatty acids, amino acids, iron-sulfur clusters, and heme; and apoptosis. Mitochondria cannot be synthesized de novo, making their inheritance essential during cell division. Mitochondria use the cytoskeleton for their inheritance from mother to daughter cell during division. In *S. cerevisiae* and *A. nidulans*, mitochondrial movement is dependent upon the actin cytoskeleton. In contrast, mitochondrial movement in *Fusarium acuminatum*, *Uromyces phaseoli*, *Nectria haematococca*, *N. crassa*, *S. pombe*, and *Allomyces macrogynus* is microtubule-dependent (Howard and Aist 1980; Aist and Bayles 1991; Steinberg and Schliwa 1993; Yaffe et al. 1996; Suelmann and Fischer 2000; McDaniel and Roberson 2000; Fuchs et al. 2002; Fehrenbacher et al. 2004).

1. Microtubule-Dependent Mitochondrial Movement

Mitochondrial movement in *N. crassa* requires the two kinesin family members, Nkin2p and Nkin3p (Fuchs and Westermann 2005). In contrast, no motor has been identified for mitochondria in *S. pombe* and *A. nidulans* and mitochondrial inheritance in these fungi occurs by a motor-independent process.

Fluorescent imaging and electron tomography revealed a direct interaction between mitochondria and microtubules in *S. pombe* (Yaffe et al. 1996; Hoog et al. 2007). During mitosis, when interphase microtubules reorganize and assemble into a spindle, mitochondria accumulate at the spindle pole. As the spindle elongates during anaphase, mitochondria move toward the opposite pole along microtubules. However, mitochondria do not exhibit track-dependent movement along microtubules. Rather, they bind to dynamic microtubule bundles and

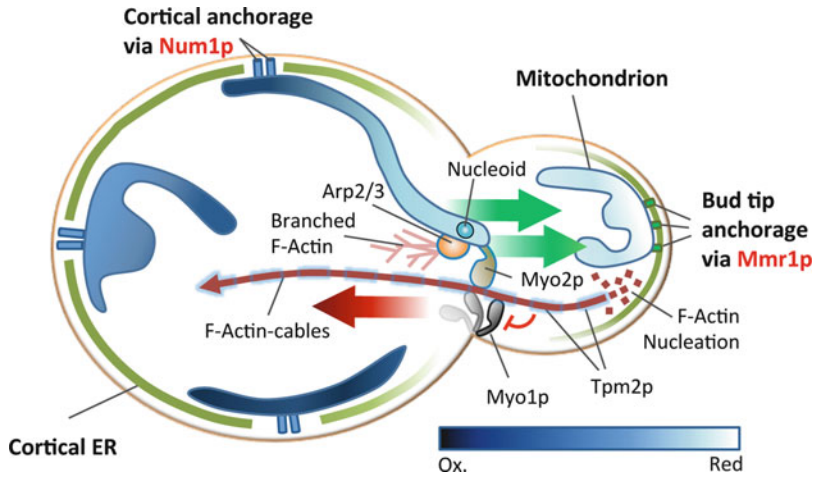


Fig. 1.6 Segregation of mitochondria in *S. cerevisiae*. Mitochondria use actin as a track for anterograde movement toward the bud during inheritance that is mediated by Arp2/3-driven actin dynamics and the motor Myo2p. Proper segregation requires retention of mitochondria at both mother and daughter cells. RACF provides a filter to ensure the inheritance of fitter mitochondria. From Higuchi-Sanabria et al. (2014)

move to and from the cell tips as their associated microtubule bundle elongates and shortens (Yaffe et al. 2003).

Earlier reports (Chiron et al. 2008) suggest the conserved CLASP protein Peg1p is a linker between mitochondria and plus ends of microtubules. More recent studies support a role for the protein Mmb1p in linking mitochondria to microtubules in *S. pombe* (Fu et al. 2011). Mmb1p binds to the lateral surface of the microtubule. During interphase, mitochondria are aggregated in 80 % of *mmb1Δ* cells examined. In approximately 10 % of cells, mitochondria aggregate at only one pole of the cell, which results in asymmetric segregation of mitochondria leaving one daughter cell devoid of mitochondria. Mmb1p also affects microtubule dynamics. Specifically, it stabilizes the microtubule lattice against shrinkage.

2. Actin-Dependent Mitochondrial Movement and Quality Control During Inheritance in *S. cerevisiae*

In *S. cerevisiae*, during S phase, mitochondria align along the longitudinal axis of the cell. At G1 phase, mitochondria orient toward the site of bud emergence (Simon et al. 1997). During G2 and M phase, mitochondria undergo anterograde (toward the bud tip) and retrograde (away from the bud tip) movements (Fehrenbacher et al. 2004). Mitochondria also become anchored at the bud tip and at the distal mother tip. At the end of cell division, mitochondria are released from these retention sites and re-align along the axis of the cell during S phase (Yang et al. 1999; Boldogh et al. 2004; Swayne et al. 2011).

Mitochondria use actin cables as tracks for anterograde movement, from mother cells to buds, during cell division in *S. cerevisiae* (Fig. 1.6). Specifically, mitochondria co-localize with actin cables as they undergo anterograde movement and require actin cables for movement and inheritance. Moreover, alteration in actin cable dynamics affects both the velocity and direction of anterograde mitochondrial movement (Fehrenbacher et al. 2004). Anterograde mitochondrial movement in mother cells is driven by pushing forces generated by Arp2/3 complex-driven actin polymerization at the interface between actin cables and the organelle (Boldogh et al. 2001; Senning and Marcus 2010). The Pumilio family protein Jsn1p is the Arp2/3 complex receptor on the mitochondrial surface (Fehrenbacher et al. 2005). Anterograde movement of mitochondria across the bud neck requires the type V myosin motor Myo2p (Altmann et al. 2008; Fortsch et al. 2011). Two

proteins, Mmr1p and Ypt11p, have been described as possible cargo adapters for Myo2p on mitochondria because they can bind to Myo2p and are required for normal mitochondrial distribution (Itoh et al. 2002, 2004). However, as described below, there is also evidence that these proteins control mitochondrial position through effects on anchorage of the organelle in the bud tip. Finally, a protein complex consisting of Mdm10p, Mdm12p, and Mmm1p is required for association of mitochondria with actin cables in vivo and F-actin in vitro (Boldogh et al. 2003). These proteins are also required for normal mitochondrial morphology in *S. cerevisiae*, *A. nidulans*, and *N. crassa* and for maintenance of mitochondrial DNA (mtDNA), assembly of beta-barrel proteins in the mitochondrial outer membrane, and interaction of mitochondria with ER in *S. cerevisiae* (Sogo and Yaffe 1994; Prokisch et al. 2000; Hobbs et al. 2001; Koch et al. 2003; Meisinger et al. 2004; Kornmann et al. 2009).

Retrograde mitochondrial movement in *S. cerevisiae* is driven by actin cable dynamics (Fig. 1.6). As described above, actin cables undergo retrograde flow, assembly- and myosin-driven movement from their assembly site in the bud tip or bud neck toward the distal tip of the mother cell. Several lines of evidence support the model that retrograde actin cable flow (RACF) drives retrograde mitochondrial movement. Mitochondria co-localize with dynamic actin cables as they undergo retrograde flow and require actin cables for retrograde movement. Moreover, the velocity of retrograde mitochondrial movement is similar to that of RACF. Finally, mutations that increase or decrease the rate of RACF also increase or decrease the rate of retrograde mitochondrial movement (Huckaba et al. 2006; Higuchi et al. 2013).

Since actin cables, the tracks for anterograde mitochondrial movement, undergo retrograde flow, mitochondria are effectively "swimming upstream," against the opposing force of RACF as they move from mother cells to buds (Fig. 1.6). This raises the interesting possibility that RACF serves as a filter to prevent low-functioning mitochondria from entering the bud or to drive movement of low-functioning mitochondria from the bud into the mother cell. Indeed, recent studies indicate that mitochondria that are more reduced and have less reactive oxygen species (ROS) are preferentially inherited by buds in *S. cerevisiae* (McFaline-Figueroa et al. 2011). Other studies indicate that increasing the rate of RACF results in inheritance of mitochondria that are higher functioning compared to mitochondria in wild-type cells. It also results in extended lifespan and improved healthspan through effects on mitochondrial quality control (Higuchi et al. 2013). Thus, the machinery for mitochondrial motility in *S. cerevisiae* also contributes to inheritance of fitter mitochondria by daughter cells, which affects daughter cell fitness and lifespan.

3. Anchorage of Mitochondria in Mother Cells and Buds in *S. cerevisiae*

For the proper inheritance of mitochondria, mitochondria must anchor to the bud tip in order to prevent their movement to the mother through retrograde flow of actin cables. Light and electron microscopy studies revealed that mitochondria are anchored to the cER at the bud tip (Swayne et al. 2011). These studies also revealed a role for Mmr1p, a member of the DSL1 family of tethering proteins, in anchorage of mitochondria to cER in the bud tip. Deletion of *MMR1* results in defects in anchorage of mitochondria in the bud tip and accumulation of mitochondria in mother cells, while overexpression of *MMR1* causes excessive accumulation of mitochondria at the bud tip (Swayne et al. 2011; Frederick et al. 2008). Consistent with this, *MMR1* protein and mRNA localize exclusively in the bud tip. Localization of *MMR1* mRNA to the bud tip relies on Myo4p-dependent transport (Shepard et al. 2003). In contrast, localization of *MMR1* protein to the bud tip requires Myo2p and Ptc1p, a protein that has been implicated in assembly of Myo2p with its cargo-specific adapters (Jin et al. 2009; Swayne et al. 2011). Finally, Mmr1p localizes to apposed surfaces of mitochondria and cER at the bud tip and is recovered with mitochondria and ER upon subcellular fractionation (Swayne et al. 2011). Together, these findings suggest

that Mmr1p serves as an anchor protein for the retention of mitochondria at the bud tip through its association with cER.

Interestingly, defects in anchorage of mitochondria in the bud tip affect mitochondrial quality control during inheritance and lifespan control in *S. cerevisiae*. As described above, fitter mitochondria, which are more reduced and have less ROS, are preferentially inherited by daughter cells. Deletion of *MMR1* results in two populations of cells. One population is long-lived and inherits mitochondria that are higher functioning compared to those in wild-type cells. The other population is short-lived and contains mitochondria that are severely compromised (McFaline-Figueroa et al. 2011). Thus, inheritance of fitter mitochondria by daughter cells relies on transport of fitter organelles from mother cells to buds and retention of those fitter organelles within the bud.

Ypt11p is a Rab-like GTPase that binds to the cargo-binding domain of Myo2p. It is involved in the inheritance of Golgi apparatus, ER, and mitochondria (Itoh et al. 2002; Boldogh et al. 2004; Buvelot Frei et al. 2006). Currently, the role of Ypt11p in mitochondrial inheritance is controversial. Some studies suggest that Ypt11p plays a direct role in inheritance of mitochondria by linking mitochondria to type V myosin motors (Lewandowska et al. 2013). Other findings support the model that Ypt11p affects cER inheritance, which in turn affects anchorage of mitochondria to cER in the bud tip. Specifically, Ypt11p, when overexpressed, localizes to cER in the bud. These studies also showed that deletion of *YPT11* results in severe defects in cER inheritance, while overexpression of *YPT11* results in increased cER inheritance (Buvelot Frei et al. 2006). Other studies revealed that deletion of *YPT11* results in defects in anchorage of mitochondria in the bud tip without affecting the velocity of mitochondrial movement (Boldogh et al. 2004; Swayne et al. 2011). Finally, there are two populations of *ypt11Δ* cells: one long-lived and the other short-lived (Rafelski et al. 2012). Thus, the aging phenotype observed in *ypt11Δ* cells is similar to that observed in *mmr1Δ* cells.

Proper segregation of mitochondria also requires the retention of mitochondria in the mother cell. Num1p is a large protein that localizes to the cell cortex and is involved in dynein-dependent nuclear migration (Farkasovsky and Kuntzel 1995; Heil-Chapdelaine et al. 2000). A role for Num1p in mitochondrial distribution was obtained in a high-copy suppressor screen for a dominant negative mutant of the dynamin-like mitochondrial fusion protein, Dnm1p (Cerveny et al. 2007). Other studies support a role for Num1p in anchorage of mitochondria to the mother cell cortex. Num1p localizes to punctate structures at the cortex of mother cells and sites where mitochondria are closely opposed to the cell cortex (Klecker et al. 2013; Lackner et al. 2013). Deletion of *NUM1* results in reduced localization of mitochondria to the mother cell cortex, increased mitochondrial motility in mother cells, and restoration of the distribution of mitochondria in mother cells and buds in *mmr1Δ* yeast (Klecker et al. 2013). Finally, tethering mitochondria to the plasma membrane rescues the *num1Δ* defects. These studies revealed retention machinery for mitochondria in yeast mother cells, a requirement for this retention machinery in mitochondrial inheritance and a role for Num1p in this process.

4. Checkpoints That Inhibit Cell Cycle Progression in Response to Defects in Mitochondrial Inheritance

There are many checkpoints that inhibit cell cycle progression when there are defects in inheritance of the nucleus or nuclear DNA. Recent studies revealed two checkpoints that block cell cycle progression in response to defects in mitochondrial inheritance in *S. cerevisiae* (Garcia-Rodriguez et al. 2009; Peraza-Reyes et al. 2010). The mitochondrial inheritance checkpoint inhibits contractile ring closure in response to severe defects in mitochondrial inheritance. This checkpoint is regulated by a conserved checkpoint signaling pathway, the mitotic exit network (MEN), which regulates cell cycle progression in response to spindle alignment and elongation and to the transfer of the nucleus from mother to daughter cell during the anaphase-to-telophase transition (Segal 2011). Two lines of evidence suggest that the MEN pathway regulates cytokinesis when there is a defect in mitochon-

drial inheritance. First, cells with defects in mitochondrial inheritance have a delay in the release of the MEN component, Cdc14p, from the nucleolus during anaphase. Second, hyperactivation of MEN by deleting *BUB2* can rescue defects in cytokinesis (Garcia-Rodriguez et al. 2009).

The mitochondrial DNA (mtDNA) inheritance checkpoint inhibits progression from G1 to S phase in response to loss of mtDNA (Crider et al. 2012). Interestingly, this checkpoint responds to loss of DNA within buds and not to loss of genes encoded by mtDNA or loss of mitochondrial respiratory activity. This checkpoint is regulated by Rad53p, a component of the DNA damage checkpoint, which arrests the cell cycle at G1, S, and G2 phase and activates DNA repair pathways in response to nuclear DNA damage or replication interference events including DNA breaks, adducts, cross-links, and inhibition of DNA polymerases (Elledge 1996). Specifically, loss of mtDNA results in activation of Rad53p and Rad53p-dependent phosphorylation of Pif1p, a helicase that is activated by the DNA damage checkpoint. Conversely, Rad53p is required for inhibition of cell cycle progression in response to loss of mtDNA.

Thus, there are two checkpoints that monitor mitochondrial inheritance in *S. cerevisiae*. These checkpoints respond to different aspects of mitochondrial inheritance, inhibit cell cycle progression at different stages in the cell division cycle, and are regulated by different checkpoint signaling pathways. Since the signaling pathways that regulate these checkpoints are conserved, it is possible that these checkpoints exist in other eukaryotes. Indeed, Chk2, the mammalian homologue of Rad53p, has known effects on mtDNA content (Lebedeva and Shadel 2007).

D. Vacuoles, the Lysosomes of Yeast

Vacuoles are evenly distributed between mother and daughter cells in *S. cerevisiae*. While the vacuole is an essential organelle, its inheritance is not essential and cells that fail to inherit the organelle are able to generate one through an unknown mechanism (Weisman 2006). Vacuoles remain relatively constant in size during cell division, and the primary event during vacuole inheritance is the formation of a tubular, vacuole-derived "segregation structure" (Weisman et al. 1987; Weisman and Wickner 1988). The segregation structure forms near the bud and extends from the mother cell to the bud before the nucleus enters the mother-bud neck. Initially, the vacuole in the bud remains connected to the vacuole in the mother via the segregation structure (Weisman and Wickner 1988). Thereafter, the segregation structure resolves, and fusion of vacuolar vesicles produces the large vacuoles typical of daughter cells (Conradt et al. 1992; Jones et al. 1993). Resolution of the vacuolar segregation structure requires Cal4p, a kinase that localizes to the bud and is activated at segregation structures in the bud (Bartholomew and Hardy 2009).

Many of the genes required for vacuolar inheritance affect the actin cytoskeleton (for review see Weisman 2003). Specifically, mutations in genes encoding actin (*ACT1*), profilin (*PFY1*), and a type V myosin (*MYO2*) affect inheritance, but not morphology, of the organelle (Hill et al. 1996). Some *MYO2* mutations that interfere with vacuolar inheritance are in the Myo2p motor and therefore affect many Myo2p-dependent processes. However, other mutations in the Myo2p tail (e.g., *myo2-2*) appear to be vacuole-specific (Catlett and Weisman 1998; Catlett et al. 2000). Ptc1p/Vac10p is required for vacuolar inheritance and plays several roles in regulating this process. These include the regulation of Myo2p distribution, regulation of Vac17p steady-state levels, and promoting the association of Myo2p with Vac17p (Jin et al. 2009).

Other studies support a role for the peripheral and integral vacuolar membrane proteins Vac17p and Vac8p in recruiting Myo2p to vacuoles. *VAC17* was isolated as a multi-copy suppressor of the vacuole inheritance defect of the *myo2-2* allele. Vac17p is required for normal vacuolar inheritance and is not required for the inheritance of peroxisomes, late Golgi apparatus, or secretory vesicles (Ishikawa et al. 2003). Vac17p immunoprecipitates with Myo2p,

can bind to the Myo2p tail in two-hybrid tests, and is required to recruit Myo2p to vacuolar membranes. Vac8p, an integral vacuolar membrane protein, interacts with Vac17p in co-immunoprecipitation experiments and two-hybrid tests and is required for vacuole inheritance and recruitment of Vac17p to the organelle (Tang et al. 2003). Together, these studies support the model that binding of Vac17p to the vacuole-specific region of the globular tail of Myo2p and to the integral vacuolar membrane protein Vac8p results in recruitment of Myo2p to the vacuolar membrane, which in turn allows for Myo2p-driven movement of the vacuolar segregation structure from mother to developing bud using actin cables as tracks (Tang et al. 2003).

Association of Vac17p with Myo2p is a tightly regulated process. Phosphorylation of Vac17p by the cyclin-dependent kinase Cdk1 early in the cell cycle promotes binding of Vac17 to Myo2 (Peng and Weisman 2008). Conversely, cell cycle-regulated degradation of Vac17p blocks association of Myo2p with vacuoles in late stages in the cell cycle. This degradation relies on a PEST sequence in Vac17p (Tang et al. 2003). Recent studies also revealed a role for Dma1p in cell cycle-dependent degradation of Vac17p. Dma1p is an E3 ubiquitin ligase that contains a RING finger domain and regulates the level of proteins that function in the spindle positioning checkpoint protein, septin deposition, and cytokinesis (Fraschini et al. 2004). Dma1p is recruited to Vac17p and is required for degradation of Vac17p by the proteasome. Vac17p degradation is dependent upon the ubiquitin ligase activity of Dma1p and is ubiquitinated in vivo. Interestingly, recruitment of Dma1p to Vac17p requires (1) phosphorylation of Vac17p by Cdk1, (2) association of Myo2p with Vac17p, and (3) resolution of the segregation structure. Together, these findings suggest that Dma1p is recruited to Vac17p after Myo2p has driven transport of the vacuole from mother cells to bud. Once Dma1p is recruited to Vac17p, it catalyzes ubiquitination of Vac17p, which triggers degradation of Vac17p by the proteasome, release of Myo2p from the vacuole, and termination of vacuolar transport.

In *A. nidulans* DigA, a protein involved in vacuolar trafficking, was cloned as a potential homologue to *S. cerevisiae* Pep3p (Vps18p) (Geissenhoner et al. 2001). *digA* mutants show pleiotropic defects like abnormal mitochondria and clustered nuclei in addition to defects in polarization of the actin cytoskeleton. Thus, normal vacuolar function may be necessary for organelle positional control and polarized growth in this filamentous fungus.

Vacuole inheritance and hyphal branching require Vac8p in *C. albicans* (Barelle et al. 2006). Time-lapse microscopy and three-dimensional imaging studies indicate that vacuoles are inherited asymmetrically during germ tube formation. After the first division, the subapical cell in the incipient hypha inherits most of the vacuolar compartment and arrests in G_1. Subapical cells are released from their arrest when the vacuoles decrease in size. Thus, vacuole segregation may be coordinated with the cell cycle, by cell cycle-regulatory machinery that monitors vacuole size (Barelle et al. 2003). Indeed, several genes including *VAC1, VAC7, VAC8, FAB1, VAM2, VAM, ABG1, VSP11*, and *VSP34* have been implicated in a regulatory process that links vacuole inheritance to cell cycle progression in *C. albicans* (Bruckmann et al. 2001; Augsten et al. 2002; Veses et al. 2005, 2009; Palmer et al. 2005; Barelle et al. 2006; Franke et al. 2006).

E. The Golgi Apparatus

Inheritance of Golgi apparatus has been studied extensively in two types of budding yeast, *P. pastoris* and *S. cerevisiae*. In *P. pastoris*, the Golgi apparatus is composed of ordered stacks of cisternae. Golgi stacks are present in close association with transitional ER (tER). Fluorescence imaging of Sec7p-GFP confirmed that the Golgi apparatus is synthesized de novo from the tER in *P. pastoris* (Bevis et al. 2002).

The Golgi apparatus in *S. cerevisiae* is not arranged in stacks but instead comprises multiple cisternae that are dispersed in the cytoplasm. There are two sets of Golgi cisternae present in the cell: early and late cisternae. They utilize two different mechanisms for

inheritance. Early Golgi apparatus cisternae are present in the bud at very early stages of the cell cycle. These cisternae are synthesized de novo from ER membranes. In mutants with defects in ER inheritance, small buds lack Golgi cisternae (Reinke et al. 2004). Inheritance of late Golgi cisternae is an ordered cell cycle-dependent process that uses the actin cytoskeleton. *CDC1* mutants have depolarized actin cables and show defects in the inheritance of late Golgi cisternae (Rossanese et al. 2001).

Studies of Myo2p mutants support a role for this motor in Golgi localization in mother cells and buds (Rossanese et al. 2001). Other studies support a role for Ypt11p, a protein that binds to Myo2p and is implicated in cER inheritance and anchorage of mitochondria in the bud tip, as well as in Golgi inheritance (Arai et al. 2008). Ypt11p exhibits high-affinity binding to Ret2p, a subunit of the COPI coatomer complex on the Golgi apparatus. Consistent with this, deletion of *YPT11* results in defects in localization of late Golgi apparatus to the bud. Moreover, overexpression of *YPT11* results in enhanced accumulation of Ret2p and late Golgi apparatus in the buds, and mutations in *RET2* that reduce its affinity for Ypt11p suppress this *YPT11* overexpression effect. Finally, Golgi membranes undergo linear, bud-directed movement in dividing yeast, and deletion of *YPT11* or destabilization of actin inhibits this movement. Overall, this supports the model that Ypt11p and Ret2p are receptors for Myo2p on late Golgi membranes and that Ret2p-dependent recruitment of Myo2p to the late Golgi apparatus is required for actin-dependent polarized movement and inheritance of this organelle. Since Ret2p is also a component of COPI vesicles that are transported from the cis Golgi apparatus to the ER, this study may also reveal why deletion of *YPT11* also affects cER inheritance.

There is also evidence for actin control of Golgi localization in other fungi. In *A. nidulans* Golgi equivalents (GEs) are polarized during hyphae growth (Breakspear et al. 2007) and that organization is disrupted after treatment with latrunculin, an agent that disrupts the actin cytoskeleton (Pantazopoulou and Penalva 2009). In *C. albicans* the majority of the Golgi apparatus localizes to the distal region of the hyphae. This pattern of localization also depends on the actin cytoskeleton (Rida et al. 2006). Recent studies indicate that polarization of the Golgi apparatus toward the hyphal tips during development in *C. albicans* depends on the cAMP-PKA signaling pathway and on the phosphorylation of Gyp1, a protein that localizes to the Golgi apparatus (Huang et al. 2014).

F. Peroxisomes

Peroxisomes are single-membrane-bound organelles that are required for many essential metabolic pathways including β-oxidation of fatty acids, homeostasis of ROS, glyoxylate cycle, and penicillin biosynthesis (van den Bosch et al. 1992). Different processes determine peroxisome abundance in fungi: biogenesis, division, turnover, and active transport to daughter cells. Peroxisome abundance is also controlled by environmental signals. For example, in *Candida boidinii*, a yeast that can grow using methanol as a sole carbon source and is frequently found on the surface of plants, peroxisome abundance correlates with methanol oscillations during the daily light-dark cycle (Oku et al. 2014).

1. Peroxisome Biogenesis

The number of peroxisomes remains fairly constant in budding yeast, and peroxin (PEX) genes control peroxisome biogenesis and proliferation (Kiel et al. 2006). Pex3p is a peroxisomal membrane protein that is essential for peroxisome biogenesis. Deletion of *PEX3* results in complete loss of peroxisomes, and introduction of *PEX3* to *pex3Δ* yeast results in peroxisome biogenesis (Hettema et al. 2000). Early studies revealed that newly synthesized Pex3p localizes first to discrete foci within the ER and later to mature peroxisomes (Hoepfner et al. 2005). Other groups found that ER is critical for peroxisome biogenesis and that other peroxisomal membrane but not matrix proteins are transported from ER to peroxisomes in organisms ranging from fungi to mammalian cells (Geuze et al. 2003; Kim et al. 2006; Karnik and Trelease 2007; Kragt et al. 2005; Tam et al. 2005; Thoms et al. 2012). Studies in the yeast *Yarrowia lipo-*

lytica revealed immature vesicles containing an incomplete peroxisome proteasome budding from the ER and a role for ER protein trafficking in peroxisome biogenesis (Titorenko et al. 2000; Titorenko and Rachubinski 2000; van der Zand et al. 2010).

These findings support the model that peroxisome membrane proteins that are imported into ER form at least two different cargo groups, which bud from the ER. Fusion of vesicles containing different cargo groups then leads to the formation of a membrane-bound compartment with a complete peroxisome membrane protein proteome. Finally, matrix proteins are imported into the organelle using the peroxisome import machinery. In *S. cerevisiae*, fission of peroxisomes involves the dynamin-like protein Vps1p. Deletion of *VPS1* results in fewer than normal but oversized peroxisomes in the cell. In *S. pombe*, peroxisome number is controlled by two dynamin-like proteins, Dnm1p and Vps1p, that act in a redundant manner (Hoepfner et al. 2001).

2. Peroxisome Inheritance

Peroxisome inheritance in fungi is coordinated with the transport of other organelles. For example, in the yeast *Hansenula polymorpha*, Emp24, a member of the p24 family, localizes not only to the ER and the Golgi apparatus but also to peroxisomes, where it regulates peroxisome fission and inheritance (Kurbatova et al. 2009). In *S. cerevisiae*, peroxisomes associate with mitochondria and their movement is coupled with mitochondrial dynamics (Jourdain et al. 2008). However, peroxisomes are transported into buds early in the cell cycle, before mitochondria are detected in the bud (Knoblach and Rachubinski 2013). Time-lapse imaging of GFP bearing the peroxisomal targeting signal type 1 (PTS1) reveals cell cycle-dependent peroxisome movement. Specifically, peroxisomes accumulate at the incipient bud site. As the bud emerges, peroxisomes move immediately into the bud, initially localizing to the bud tip but later presenting throughout the bud. Before cytokinesis, they are also present at the bud neck.

Transport of peroxisomes from mother to buds is an ordered, actin-dependent process. Time-lapse imaging revealed that peroxisomes undergo linear, polarized movement along actin cables in *S. cerevisiae* and that treatment with latrunculin-A, an agent that results in rapid loss of actin filaments, or shift of a temperature-sensitive Myo2p mutant to restrictive temperatures, abolishes peroxisome movements in *S. cerevisiae* (Hoepfner et al. 2001). Thus, Myo2p drives movement of peroxisomes from mother cells to buds, using actin cables as tracks in *S. cerevisiae*.

Other studies support a role for Inp2p as the Myo2p receptor on peroxisomes in *S. cerevisiae*. These studies revealed sites on the Myo2p tail that are required for its association with peroxisomes and that Inp2p, an integral peroxisomal membrane protein, binds to Myo2p and is required to recruit Myo2p to peroxisomes and for peroxisome movement (Fagarasanu et al. 2006). Interestingly, Inp2p undergoes cell cycle-linked changes in abundance and phosphorylation (Fagarasanu et al. 2009). Another protein, Pex19p, has also been reported to be important, in addition to Inp2p, for binding of peroxisomes to Myo2p (Otzen et al. 2012). Finally, a role for the E3 ubiquitin ligases Dma1 and Dma2 in the release of peroxisomes in the bud has been reported (Yau et al. 2014). Because Dma1 is also required for termination of vacuole transport into the bud and for Vac17 degradation, the authors suggest that degradation of adaptor proteins by the proteasome is a general mechanism to detach myosin V cargoes at daughter cells.

Inp1p is a peripheral membrane protein of peroxisomes that anchors the peroxisome to the mother cell cortex. Deletion of *INP1* causes defects in the retention of peroxisomes in the mother cell and overexpression of Inp1p has the opposite effect (Fagarasanu et al. 2005). A role for the peroxin Pex3p in peroxisome inheritance via Inp1p has also been reported. Pex3p and Inn1p directly interact, and Pex3p seems to serve as an anchor protein recruiting Inp1p to the peroxisomal membrane (Munck et al. 2009). Finally, recent studies indicate that Inp1p anchors peroxisomes to cER in the mother cell by serving as a hinge to link Pex3p

in ER membranes to Pex3p in peroxisome membranes (Knoblach et al. 2013).

In filamentous fungi like *A. nidulans*, peroxisomes move along microtubules in a kinesin-3 and dynein-dependent way. The dynein-associated protein Lis1 is critical for the initiation of dynein-dependent peroxisome motility (Egan et al. 2012). Interestingly, ApsB, a component of the septal microtubule-organizing centers (sMTOCs), interacts with a subpopulation of peroxisomes in *A. nidulans* (Zekert et al. 2010). This observation suggests that peroxisome transport might contribute to the formation of sMTOCs in filamentous fungi.

IV. Conclusion

While the study of organelle inheritance has long been a topic of active investigation, there are still many outstanding questions. Do the mechanisms found in *S. cerevisiae* for initiation and termination of organelle movement from mother cells to buds, as well as anchorage of organelles in mother cells and buds, occur in other fungi? Recent studies indicate that the machinery for mitochondrial inheritance in *S. cerevisiae* promotes inheritance of fitter mitochondria by daughter cells and promotes daughter cell fitness and lifespan. Are there other inheritance-linked organelle quality control mechanisms and how does this affect mother and daughter cell fitness? Several checkpoints are in place to ensure the faithful segregation of chromosomes. Recent studies have illustrated similar robust surveillance mechanisms for the inheritance of various organelles such as mitochondria, cER, Golgi apparatus, and vacuoles. Are there other organelle inheritance checkpoints in fungi and other eukaryotes? How do defects in organelle inheritance, organelle quality control, and/or localization of organelles to specific regions within cells contribute to neurodegenerative and metabolic diseases in mammals? Continued analysis of organelle inheritance in fungi will not only guide us in understanding this complex phenomenon in other organisms but will also help in finding therapies for these diseases.

Acknowledgments We would like to thank the members of the Pon Lab for their support and critical evaluation, Mark Winey (University of Colorado) for providing images, and Wolfgang Pernice for his scientific artwork. This work was supported by awards from the Ellison Medical Foundation (AG-SS-2465) and the NIH (GM45735 and GM096445) to LAP.

References

Adams AE, Botstein D, Drubin DG (1991) Requirement of yeast fimbrin for actin organization and morphogenesis in vivo. Nature 354:404–408

Aghamohammadzadeh S, Ayscough KR (2009) Differential requirements for actin during yeast and mammalian endocytosis. Nat Cell Biol 11:1039–1042

Aist JR, Bayles CJ (1991) Organelle motility within mitotic asters of the fungus Nectria haematococca. Eur J Cell Biol 56:358–363

Altmann K, Frank M, Neumann D et al (2008) The class V myosin motor protein, Myo2, plays a major role in mitochondrial motility in Saccharomyces cerevisiae. J Cell Biol 181:119–130

Arai S, Noda Y, Kainuma S et al (2008) Ypt11 functions in bud-directed transport of the Golgi by linking Myo2 to the coatomer subunit Ret2. Curr Biol 18:987–991

Asakura T, Sasaki T, Nagano F et al (1998) Isolation and characterization of a novel actin filament-binding protein from Saccharomyces cerevisiae. Oncogene 16:121–130

Augsten M, Hübner C, Nguyen M et al (2002) Defective hyphal induction of a Candida albicans phosphatidylinositol 3-phosphate 5-kinase null mutant on solid media does not lead to decreased virulence. Infect Immun 70:4462–4470

Baba M, Osumi M (1987) Transmission and scanning electron microscopic examination of intracellular organelles in freeze-substituted Kloeckera and Saccharomyces cerevisiae yeast cells. J Electron Microsc Technol 5:249–261

Babour A, Bicknell AA, Tourtellotte J, Niwa M (2010) A surveillance pathway monitors the fitness of the endoplasmic reticulum to control its inheritance. Cell 142:256–269

Barelle CJ, Bohula EA, Kron SJ, Wessels D, Soll DR, Schafer A, Brown AJ, Gow NA (2003) Asynchronous cell cycle and asymmetric vacuolar inheritance in true hyphae of Candida albicans. Eukaryot Cell 2:398–410

Barelle CJ, Richard ML, Gaillardin C et al (2006) Candida albicans VAC8 is required for vacuolar inheritance and normal hyphal branching. Eukaryot Cell 5:359–367

Barr FA, Gruneberg U (2007) Cytokinesis: placing and making the final cut. Cell 131:847–860

Bartholomew CR, Hardy CFJ (2009) p21-activated kinases Cla4 and Ste20 regulate vacuole inheritance in Saccharomyces cerevisiae. Eukaryot Cell 8:560–572

Berepiki A, Lichius A, Read ND (2011) Actin organization and dynamics in filamentous fungi. Nat Rev Microbiol 9(12):876–887

Bertrand E, Chartrand P, Schaefer M et al (1998) Localization of ASH1 mRNA particles in living yeast. Mol Cell 2:437–445

Bevis BJ, Hammond AT, Reinke CA, Glick BS (2002) De novo formation of transitional ER sites and Golgi structures in Pichia pastoris. Nat Cell Biol 4:750–756

Bi E, Park HO (2012) Cell polarization and cytokinesis in budding yeast. Genetics 191:347–387

Böhl F, Kruse C, Frank A et al (2000) She2p, a novel RNA-binding protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p. EMBO J 19:5514–5524

Boldogh IR, Yang HC, Nowakowski WD et al (2001) Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast. Proc Natl Acad Sci USA 98:3162–3167

Boldogh IR, Nowakowski DW, Yang HC et al (2003) A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. Mol Biol Cell 14:4618–4627

Boldogh IR, Ramcharan SL, Yang HC, Pon LA (2004) A type V myosin (Myo2p) and a Rab-like G-protein (Ypt11p) are required for retention of newly inherited mitochondria in yeast cells. Mol Biol Cell 15 (9):3994–4002

Breakspear A, Langford KJ, Momany M, Assinder SJ (2007) CopA:GFP localizes to putative Golgi equivalents in Aspergillus nidulans. FEMS Microbiol Lett 277:90–97

Bretscher A (2003) Polarized growth and organelle segregation in yeast: the tracks, motors, and receptors. J Cell Biol 160:811–816

Bruckmann A, Künkel W, Augsten K et al (2001) The deletion of CaVPS34 in the human pathogenic yeast Candida albicans causes defects in vesicle-mediated protein sorting and nuclear segregation. Yeast 18:343–353

Burston HE, Maldonado-Baez L, Davey M et al (2009) Regulators of yeast endocytosis identified by systematic quantitative analysis. J Cell Biol 185:1097–1110

Buttery SM, Yoshida S, Pellman D (2007) Yeast formins Bni1 and Bnr1 utilize different modes of cortical interaction during the assembly of actin cables. Mol Biol Cell 18:1826–1838

Buvelot Frei S, Rahl PB, Nussbaum M et al (2006) Bioinformatic and comparative localization of Rab proteins reveals functional insights into the uncharacterized GTPases Ypt10p and Ypt11p. Mol Cell Biol 26:7299–7317

Catlett NL, Weisman LS (1998) The terminal tail region of a yeast myosin-V mediates its attachment to vacuole membranes and sites of polarized growth. Proc Natl Acad Sci USA 95:14799–14804

Catlett NL, Duex JE, Tang F, Weisman LS (2000) Two distinct regions in a yeast myosin-V tail domain are required for the movement of different cargoes. J Cell Biol 150:513–526

Cerveny KL, Studer SL, Jensen RE, Sesaki H (2007) Yeast mitochondrial division and distribution require the cortical num1 protein. Dev Cell 12:363–375

Chesarone M, Gould CJ, Moseley JB, Goode BL (2009) Displacement of formins from growing barbed ends by bud14 is critical for actin cable architecture and function. Dev Cell 16:292–302

Chiron S, Bobkova A, Zhou H, Yaffe MP (2008) CLASP regulates mitochondrial distribution in Schizosaccharomyces pombe. J Cell Biol 182:41–49

Chuang JS, Schekman RW (1996) Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p. J Cell Biol 135:597–610

Clay L, Caudron F, Denoth-Lippuner A et al (2014) A sphingolipid-dependent diffusion barrier confines ER stress to the yeast mother cell. Elife 3, e01883

Conradt B, Shaw J, Vida T et al (1992) In vitro reactions of vacuole inheritance in Saccharomyces cerevisiae. J Cell Biol 119:1469–1479

Crider DG, García-Rodríguez LJ, Srivastava P et al (2012) Rad53 is essential for a mitochondrial DNA inheritance checkpoint regulating G1 to S progression. J Cell Biol 198:793–798

Dabora SL, Sheetz MP (1988) The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts. Cell 54:27–35

Desai A, Mitchison TJ (1997) Microtubule polymerization dynamics. Annu Rev Cell Dev Biol 13:83–117

Ding R, West RR, Morphew DM, Oakley BR, McIntosh JR (1997) The spindle pole body of Schizosaccharomyces pombe enters and leaves the nuclear envelope as the cell cycle proceeds. Mol Biol Cell 8:1461–1479

Drees B, Brown C, Barrell BG, Bretscher A (1995) Tropomyosin is essential in yeast, yet the TPM1 and TPM2 products perform distinct functions. J Cell Biol 128:383–392

Du Y, Pypaert M, Novick P, Ferro-Novick S (2001) Aux1p/Swa2p is required for cortical endoplasmic reticulum inheritance in Saccharomyces cerevisiae. Mol Biol Cell 12:2614–2628

Du Y, Walker L, Novick P, Ferro-Novick S (2006) Ptc1p regulates cortical ER inheritance via Slt2p. EMBO J 25:4413–4422

Duncan MC, Cope MJ, Goode BL et al (2001) Yeast Eps15-like endocytic protein, Pan1p, activates the Arp2/3 complex. Nat Cell Biol 3:687–690

Efimov VP (2003) Roles of NUDE and NUDF proteins of Aspergillus nidulans: insights from intracellular

localization and overexpression effects. Mol Biol Cell 14:871–888
Egan MJ, McClintock MA, Reck-Peterson SL (2012) Microtubule-based transport in filamentous fungi. Curr Opin Microbiol 15:637–645
Einerhand AW, Van Der Leij I, Kos WT et al (1992) Transcriptional regulation of genes encoding proteins involved in biogenesis of peroxisomes in Saccharomyces cerevisiae. Cell Biochem Funct 10:185–191
Elledge SJ (1996) Cell cycle checkpoints: preventing an identity crisis. Science 274:1664–1672
English AR, Voeltz GK (2013) Endoplasmic reticulum structure and interconnections with other organelles. Cold Spring Harb Perspect Biol 5:a013227
Estrada P, Kim J, Coleman J et al (2003) Myo4p and She3p are required for cortical ER inheritance in Saccharomyces cerevisiae. J Cell Biol 163:1255–1266
Evangelista M, Klebl BM, Tong AH et al (2000) A role for myosin-I in actin assembly through interactions with Vrp1p, Bee1p, and the Arp2/3 complex. J Cell Biol 148:353–362
Evangelista M, Pruyne D, Amberg DC et al (2002) Formins direct Arp2/3-independent actin filament assembly to polarize cell growth in yeast. Nat Cell Biol 4:260–269
Fagarasanu M, Fagarasanu A, Tam YY et al (2005) Inp1p is a peroxisomal membrane protein required for peroxisome inheritance in Saccharomyces cerevisiae. J Cell Biol 169:765–775
Fagarasanu A, Fagarasanu M, Eitzen GA et al (2006) The peroxisomal membrane protein Inp2p is the peroxisome-specific receptor for the myosin V motor Myo2p of Saccharomyces cerevisiae. Dev Cell 10:587–600
Fagarasanu A, Mast FD, Knoblach B, Jin Y, Brunner MJ, Logan MR, Glover JN, Eitzen GA, Aitchison JD, Weisman LS, Rachubinski RA (2009) Myosin-driven peroxisome partitioning in S. cerevisiae. J Cell Biol 186:541–554
Farkasovsky M, Kuntzel H (1995) Yeast Num1p associates with the mother cell cortex during S/G2 phase and affects microtubular functions. J Cell Biol 131:1003–1014
Fehrenbacher KL, Davis D, Wu M et al (2002) Endoplasmic reticulum dynamics, inheritance, and cytoskeletal interactions in budding yeast. Mol Biol Cell 13:854–865
Fehrenbacher KL, Boldogh IR, Pon LA (2003) Taking the A-train: actin-based force generators and organelle targeting. Trends Cell Biol 13:472–477
Fehrenbacher KL, Yang H-C, Gay AC et al (2004) Live cell imaging of mitochondrial movement along actin cables in budding yeast. Curr Biol 14:1996–2004
Fehrenbacher KL, Boldogh IR, Pon LA (2005) A role for Jsn1p in recruiting the Arp2/3 complex to mitochondria in budding yeast. Mol Biol Cell 16:5094–5102
Fortsch J, Hummel E, Krist M, Westermann B (2011) The myosin-related motor protein Myo2 is an essential mediator of bud-directed mitochondrial movement in yeast. J Cell Biol 194:473–488
Franke K, Nguyen M, Härtl A et al (2006) The vesicle transport protein Vac1p is required for virulence of Candida albicans. Microbiology 152:3111–3121
Fraschini R, Bilotta D, Lucchini G, Piatti S (2004) Functional characterization of Dma1 and Dma2, the budding yeast homologues of Schizosaccharomyces pombe Dma1 and human Chfr. Mol Biol Cell 15:3796–3810
Frederick RL, Okamoto K, Shaw JM (2008) Multiple pathways influence mitochondrial inheritance in budding yeast. Genetics 178:825–837
Friedman JR, Voeltz GK (2011) The ER in 3D: a multifunctional dynamic membrane network. Trends Cell Biol 21:709–717
Fu C, Jain D, Costa J et al (2011) mmb1p binds mitochondria to dynamic microtubules. Curr Biol 21:1431–1439
Fuchs F, Westermann B (2005) Role of Unc104/KIF1-related motor proteins in mitochondrial transport in Neurospora crassa. Mol Biol Cell 16:153–161
Fuchs F, Prokisch H, Neupert W, Westermann B (2002) Interaction of mitochondria with microtubules in the filamentous fungus Neurospora crassa. J Cell Sci 115:1931–1937
Garcia-Rodriguez LJ, Crider DG, Gay AC et al (2009) Mitochondrial inheritance is required for MEN-regulated cytokinesis in budding yeast. Curr Biol 19:1730–1735
Geissenhoner A, Sievers N, Brock M, Fischer R (2001) Aspergillus nidulans DigA, a potential homolog of Saccharomyces cerevisiae Pep3 (Vps18), is required for nuclear migration, mitochondrial morphology and polarized growth. Mol Genet Genomics 266:672–685
Geuze HJ, Murk JL, Stroobants AK, Griffith JM, Kleijmeer MJ, Koster AJ, Verkleij AJ, Distel B, Tabak HF (2003) Involvement of the endoplasmic reticulum in peroxisome formation. Mol Biol Cell 14:2900–2907
Goode BL, Rodal AA, Barnes G, Drubin DG (2001) Activation of the Arp2/3 complex by the actin filament binding protein Abp1p. J Cell Biol 153:627–634
Guo W, Roth D, Walch-Solimena C, Novick P (1999) The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. EMBO J 18:1071–1080
Han G, Liu B, Zhang J et al (2001) The Aspergillus cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics. Curr Biol 11:719–724
Hegde RS, Lingappa VR (1999) Regulation of protein biogenesis at the endoplasmic reticulum membrane. Trends Cell Biol 9:132–137
Heil-Chapdelaine RA, Oberle JR, Cooper JA (2000) The cortical protein Num1p is essential for dynein-dependent interactions of microtubules with the cortex. J Cell Biol 151:1337–1344

Hettema EH, Girzalsky W, van Den Berg M, Erdmann R, Distel B (2000) Saccharomyces cerevisiae pex3p and pex19p are required for proper localization and stability of peroxisomal membrane proteins. EMBO J 19:223–233

Higuchi R, Vevea JD, Swayne TC et al (2013) Actin dynamics affect mitochondrial quality control and aging in budding yeast. Curr Biol 23:2417–2422

Higuchi-Sanabria R, Pernice WM, Vevea JD, Alessi Wolken DM, Boldogh IR, Pon LA (2014) Role of asymmetric cell division in lifespan control in Saccharomyces cerevisiae. FEMS Yeast Res 14:1133–1146

Hill KL, Catlett NL, Weisman LS (1996) Actin and myosin function in directed vacuole movement during cell division in Saccharomyces cerevisiae. J Cell Biol 135:1535–1549

Hobbs AE, Srinivasan M, McCaffery JM, Jensen RE (2001) Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. J Cell Biol 152:401–410

Hoepfner D, van den Berg M, Philippsen P et al (2001) A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in Saccharomyces cerevisiae. J Cell Biol 155:979–990

Hoepfner D, Schildknegt D, Braakman I et al (2005) Contribution of the endoplasmic reticulum to peroxisome formation. Cell 122:85–95

Hoog JL, Schwartz C, Noon AT et al (2007) Organization of interphase microtubules in fission yeast analyzed by electron tomography. Dev Cell 12:349–361

Howard RJ, Aist JR (1980) Cytoplasmic microtubules and fungal morphogenesis: ultrastructural effects of methyl benzimidazole-2-ylcarbamate determined by freeze-substitution of hyphal tip cells. J Cell Biol 87:55–64

Huang ZX, Wang H, Wang YM, Wang Y (2014) Novel mechanism coupling cyclic AMP-protein kinase a signaling and Golgi trafficking via gyp1 phosphorylation in polarized growth. Eukaryot Cell 13:1548–1556

Huckaba TM, Gay AC, Pantalena LF et al (2004) Live cell imaging of the assembly, disassembly, and actin cable-dependent movement of endosomes and actin patches in the budding yeast, Saccharomyces cerevisiae. J Cell Biol 167:519–530

Huckaba TM, Lipkin T, Pon LA (2006) Roles of type II myosin and a tropomyosin isoform in retrograde actin flow in budding yeast. J Cell Biol 175:957–969

Ishikawa K, Catlett NL, Novak JL et al (2003) Identification of an organelle-specific myosin V receptor. J Cell Biol 160:887–897

Itoh T, Watabe A, Toh EA, Matsui Y (2002) Complex formation with Ypt11p, a rab-type small GTPase, is essential to facilitate the function of Myo2p, a class V myosin, in mitochondrial distribution in Saccharomyces cerevisiae. Mol Cell Biol 22:7744–7757

Itoh T, Toh EA, Matsui Y (2004) Mmr1p is a mitochondrial factor for Myo2p-dependent inheritance of mitochondria in the budding yeast. EMBO J 23:2520–2530

Jaspersen SL, Ghosh S (2012) Nuclear envelope insertion of spindle pole bodies and nuclear pore complexes. Nucleus 3:226–236

Jin Y, Taylor Eves P, Tang F, Weisman LS (2009) PTC1 is required for vacuole inheritance and promotes the association of the myosin-V vacuole-specific receptor complex. Mol Biol 20(5):1312–1323

Jones HD, Schliwa M, Drubin DG (1993) Video microscopy of organelle inheritance and motility in budding yeast. Cell Motil Cytoskeleton 25:129–142

Jourdain I, Sontam D, Johnson C et al (2008) Dynamin-dependent biogenesis, cell cycle regulation and mitochondrial association of peroxisomes in fission yeast. Traffic 9:353–365

Kaksonen M, Toret CP, Drubin DG (2006) Harnessing actin dynamics for clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 7:404–414

Kamei T, Tanaka K, Hihara T et al (1998) Interaction of Bnr1p with a novel Src homology 3 domain-containing Hof1p. Implication in cytokinesis in Saccharomyces cerevisiae. J Biol Chem 273:28341–28345

Karnik SK, Trelease RN (2007) Arabidopsis peroxin 16 trafficks through the ER and an intermediate compartment to pre-existing peroxisomes via overlapping molecular targeting signals. J Exp Bot 58:1677–1693

Kiel JA, Veenhuis M, van der Klei IJ (2006) PEX genes in fungal genomes: common, rare or redundant. Traffic 7:1291–1303

Kilmartin JV, Adams AE (1984) Structural rearrangements of tubulin and actin during the cell cycle of the yeast Saccharomyces. J Cell Biol 98:922–933

Kim PK, Mullen RT, Schumann U, Lippincott-Schwartz J (2006) The origin and maintenance of mammalian peroxisomes involves a de novo PEX16-dependent pathway from the ER. J Cell Biol 173:521–532

Klecker T, Scholz D, Fortsch J, Westermann B (2013) The yeast cell cortical protein Num1 integrates mitochondrial dynamics into cellular architecture. J Cell Sci 126:2924–2930

Knoblach B, Rachubinski RA (2013) Doing the math: how yeast cells maintain their peroxisome populations. Commun Integr Biol 6, e26901

Knoblach B, Sun X, Coquelle N, Fagarasanu A, Poirier RL, Rachubinski RA (2013) An ER-peroxisome tether exerts peroxisome population control in yeast. EMBO J 32:2439–2453

Koch KV, Suelmann R, Fischer R (2003) Deletion of mdmB impairs mitochondrial distribution and morphology in Aspergillus nidulans. Cell Motil Cytoskeleton 55:114–124

Koning AJ, Roberts CJ, Wright RL (1996) Different subcellular localization of Saccharomyces cerevisiae HMG-CoA reductase isozymes at elevated levels

corresponds to distinct endoplasmic reticulum membrane proliferations. Mol Biol Cell 7:769–789
Kornmann B, Currie E, Collins SR et al (2009) An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325(5939):477–481
Kragt A, Voorn-Brouwer T, van den Berg M, Distel B (2005) Endoplasmic reticulum-directed Pex3p routes to peroxisomes and restores peroxisome formation in a Saccharomyces cerevisiae pex3-Delta strain. J Biol Chem 280:34350–34357
Kueh HY, Charras GT, Mitchison TJ, Brieher WM (2008) Actin disassembly by cofilin, coronin, and Aip1 occurs in bursts and is inhibited by barbed-end cappers. J Cell Biol 182:341–353
Kurbatova E, Otzen M, van der Klei IJ (2009) p24 proteins play a role in peroxisome proliferation in yeast. FEBS Lett 583:3175–3180
Lackner LL, Ping H, Graef M et al (2013) Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria. Proc Natl Acad Sci USA 110: E458–E467
Lebedeva MA, Shadel GS (2007) Cell cycle- and ribonucleotide reductase-driven changes in mtDNA copy number influence mtDNA inheritance without compromising mitochondrial gene expression. Cell Cycle 6:2048–2057
Lee W-L, Oberle JR, Cooper JA (2003) The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. J Cell Biol 160:355–364
Lewandowska A, Macfarlane J, Shaw JM (2013) Mitochondrial association, protein phosphorylation, and degradation regulate the availability of the active Rab GTPase Ypt11 for mitochondrial inheritance. Mol Biol Cell 24:1185–1195
Li X, Ferro-Novick S, Novick P (2013) Different polarisome components play distinct roles in Slt2p-regulated cortical ER inheritance in Saccharomyces cerevisiae. Mol Biol Cell 24:3145–3154
Liu B, Larsson L, Franssens V, Hao X, Hill SM, Andersson V, Hoglund D, Song J, Yang X, Oling D, Grantham J, Winderickx J, Nystrom T (2011) Segregation of protein aggregates involves actin and the polarity machinery. Cell 147:959–961
Mallavarapu A, Sawin K, Mitchison T (1999) A switch in microtubule dynamics at the onset of anaphase B in the mitotic spindle of Schizosaccharomyces pombe. Curr Biol 9:1423–1426
Manford AG, Stefan CJ, Yuan HL et al (2012) ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology. Dev Cell 23:1129–1140
Markus SM, Kalutkiewicz KA, Lee WL (2012) Astral microtubule asymmetry provides directional cues for spindle positioning in budding yeast. Exp Cell Res 318:1400–1406
Matsui Y (2003) Polarized distribution of intracellular components by class V myosins in Saccharomyces cerevisiae. Int Rev Cytol 229:1–42
McDaniel DP, Roberson RW (2000) Microtubules Are required for motility and positioning of vesicles and mitochondria in hyphal tip cells of Allomyces macrogynus. Fungal Genet Biol 31:233–244
McFaline-Figueroa JR, Vevea J, Swayne TC et al (2011) Mitochondrial quality control during inheritance is associated with lifespan and mother-daughter age asymmetry in budding. Aging Cell 10(5):885–895
Meisinger C, Rissler M, Chacinska A et al (2004) The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. Dev Cell 7:61–71
Meldolesi J, Pozzan T (1998) The endoplasmic reticulum Ca2+ store: a view from the lumen. Trends Biochem Sci 23:10–14
Miao Y, Wong CCL, Mennella V et al (2013) Cell-cycle regulation of formin-mediated actin cable assembly. Proc Natl Acad Sci USA 110:E4446–E4455
Miller RK, Cheng SC, Rose MD (2000) Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. Mol Biol Cell 11:2949–2959
Miller RK, Rose MD (1998) Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. J Cell Biol 140:377–390
Mishra M, Huang J, Balasubramanian MK (2014) The yeast actin cytoskeleton. FEMS Microbiol Rev 38:213–227
Munck JM, Motley AM, Nuttall JM, Hettema EH (2009) A dual function for Pex3p in peroxisome formation and inheritance. J Cell Biol 187:463–471
Okada K, Ravi H, Smith EM, Goode BL (2006) Aip1 and cofilin promote rapid turnover of yeast actin patches and cables: a coordinated mechanism for severing and capping filaments. Mol Biol Cell 17:2855–2868
Oku M, Takano Y, Sakai Y (2014) The emerging role of autophagy in peroxisome dynamics and lipid metabolism of phyllosphere microorganisms. Front Plant Sci 5:81
Ono S, Ono K (2002) Tropomyosin inhibits ADF/cofilin-dependent actin filament dynamics. J Cell Biol 156:1065–1076
Otzen M, Rucktaschel R, Thoms S et al (2012) Pex19p contributes to peroxisome inheritance in the association of peroxisomes to Myo2p. Traffic 13:947–959
Ovechkina Y, Maddox P, Oakley CE et al (2003) Spindle formation in Aspergillus is coupled to tubulin movement into the nucleus. Mol Biol Cell 14:2192–2200
Palmer GE, Kelly MN, Sturtevant JE (2005) The Candida albicans vacuole is required for differentiation and efficient macrophage killing. Eukaryot Cell 4:1677–1686
Pantazopoulou A, Penalva MA (2009) Organization and dynamics of the Aspergillus nidulans Golgi during apical extension and mitosis. Mol Biol Cell 20:4335–4347
Pavin N, Tolić-Nørrelykke IM (2013) Dynein, microtubule and cargo: a ménage à trois. Biochem Soc Trans 41:1731–1735

Pelham HR (1996) The dynamic organisation of the secretory pathway. Cell Struct Funct 21:413–419

Pelham RJ, Chang F (2002) Actin dynamics in the contractile ring during cytokinesis in fission yeast. Nature 419:82–86

Peng Y, Weisman LS (2008) The cyclin-dependent kinase Cdk1 directly regulates vacuole inheritance. Dev Cell 15:478–485

Peraza-Reyes L, Crider DG, Pon LA (2010) Mitochondrial manoeuvres: latest insights and hypotheses on mitochondrial partitioning during mitosis in Saccharomyces cerevisiae. Bioessays 32:1040–1049

Preuss D, Mulholland J, Kaiser CA et al (1991) Structure of the yeast endoplasmic reticulum: localization of ER proteins using immunofluorescence and immunoelectron microscopy. Yeast 7:891–911

Prinz WA, Grzyb L, Veenhuis M et al (2000) Mutants affecting the structure of the cortical endoplasmic reticulum in Saccharomyces cerevisiae. J Cell Biol 150:461–474

Prokisch H, Neupert W, Westermann B (2000) Role of MMM1 in maintaining mitochondrial morphology in Neurospora crassa. Mol Biol Cell 11:2961–2971

Pruyne DW, Schott DH, Bretscher A (1998) Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J Cell Biol 143:1931–1945

Pruyne D, Evangelista M, Yang C et al (2002) Role of formins in actin assembly: nucleation and barbed-end association. Science 297:612–615

Rafelski SM, Viana MP, Zhang Y et al (2012) Mitochondrial network size scaling in budding yeast. Science 338:822–824

Reinke CA, Kozik P, Glick BS (2004) Golgi inheritance in small buds of Saccharomyces cerevisiae is linked to endoplasmic reticulum inheritance. Proc Natl Acad Sci USA 101:18018–18023

Rida PC, Nishikawa A, Won GY, Dean N (2006) Yeast-to-hyphal transition triggers formin-dependent Golgi localization to the growing tip in Candida albicans. Mol Biol Cell 17:4364–4378

Rossanese OW, Reinke CA, Bevis BJ et al (2001) A role for actin, Cdc1p, and Myo2p in the inheritance of late Golgi elements in Saccharomyces cerevisiae. J Cell Biol 153:47–62

Sagot I, Klee SK, Pellman D (2002a) Yeast formins regulate cell polarity by controlling the assembly of actin cables. Nat Cell Biol 4:42–50

Sagot I, Rodal AA, Moseley J et al (2002b) An actin nucleation mechanism mediated by Bni1 and profilin. Nat Cell Biol 4:626–631

Segal M (2011) Mitotic exit control: a space and time odyssey. Curr Biol 21:R857–R859

Senning EN, Marcus AH (2010) Actin polymerization driven mitochondrial transport in mating S. cerevisiae. Proc Natl Acad Sci USA 107:721–725

Sheeman B, Carvalho P, Sagot I et al (2003) Determinants of S. cerevisiae dynein localization and activation: implications for the mechanism of spindle positioning. Curr Biol 13:364–372

Shepard KA, Gerber AP, Jambhekar A et al (2003) Widespread cytoplasmic mRNA transport in yeast: identification of 22 bud-localized transcripts using DNA microarray analysis. Proc Natl Acad Sci USA 100:11429–11434

Simon VR, Karmon SL, Pon LA (1997) Mitochondrial inheritance: cell cycle and actin cable dependence of polarized mitochondrial movements in Saccharomyces cerevisiae. Cell Motil Cytoskeleton 37:199–210

Sogo LF, Yaffe MP (1994) Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. J Cell Biol 126:1361–1373

Steinberg G, Schliwa M (1993) Organelle movements in the wild type and wall-less fz;sg;os-1 mutants of Neurospora crassa are mediated by cytoplasmic microtubules. J Cell Sci 106(Pt 2):555–564

Straube A, Brill M, Oakley BR et al (2003) Microtubule organization requires cell cycle-dependent nucleation at dispersed cytoplasmic sites: polar and perinuclear microtubule organizing centers in the plant pathogen Ustilago maydis. Mol Biol Cell 14:642–657

Suelmann R, Fischer R (2000) Mitochondrial movement and morphology depend on an intact actin cytoskeleton in Aspergillus nidulans. Cell Motil Cytoskeleton 45:42–50

Swayne TC, Zhou C, Boldogh IR et al (2011) Role for cER and Mmr1p in anchorage of mitochondria at sites of polarized surface growth in budding yeast. Curr Biol 21:1994–1999

Tam YY, Fagarasanu A, Fagarasanu M, Rachubinski RA (2005) Pex3p initiates the formation of a preperoxisomal compartment from a subdomain of the endoplasmic reticulum in Saccharomyces cerevisiae. J Biol Chem 280:34933–34939

Tang F, Kauffman EJ, Novak JL et al (2003) Regulated degradation of a class V myosin receptor directs movement of the yeast vacuole. Nature 422:87–92

TerBush DR, Maurice T, Roth D, Novick P (1996) The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J 15:6483–6494

Thoms S, Harms I, Kalies KU, Gartner J (2012) Peroxisome formation requires the endoplasmic reticulum channel protein Sec61. Traffic 13:599–609

Titorenko VI, Chan H, Rachubinski RA (2000) Fusion of small peroxisomal vesicles in vitro reconstructs an early step in the in vivo multistep peroxisome assembly pathway of Yarrowia lipolytica. J Cell Biol 148:29–44

Titorenko VI, Rachubinski RA (2000) Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p. J Cell Biol 150:881–886

Tolliday N, VerPlank L, Li R (2002) Rho1 directs formin-mediated actin ring assembly during budding yeast cytokinesis. Curr Biol 12:1864–1870

Tran PT, Doye V, Chang F, Inoué S (2000) Microtubule-dependent nuclear positioning and nuclear-dependent septum positioning in the fission yeast

Schizosaccharomyces [correction of Saccharomyces] pombe. Biol Bull 199:205–206
Tran PT, Marsh L, Doye V et al (2001) A mechanism for nuclear positioning in fission yeast based on microtubule pushing. J Cell Biol 153:397–411
van den Bosch H, Schutgens RB, Wanders RJ, Tager JM (1992) Biochemistry of peroxisomes. Annu Rev Biochem 61:157–197
van der Zand A, Braakman I, Tabak HF (2010) Peroxisomal membrane proteins insert into the endoplasmic reticulum. Mol Biol Cell 21:2057–2065
Vavylonis D, Kovar DR, O'Shaughnessy B, Pollard TD (2006) Model of formin-associated actin filament elongation. Mol Cell 21:455–466
Vavylonis D, Wu JQ, Hao S et al (2008) Assembly mechanism of the contractile ring for cytokinesis by fission yeast. Science 319:97–100
Veith D, Scherr N, Efimov VP, Fischer R (2005) Role of the spindle-pole-body protein ApsB and the cortex protein ApsA in microtubule organization and nuclear migration in Aspergillus nidulans. J Cell Sci 118:3705–3716
Veses V, Casanova M, Murgui A et al (2005) ABG1, a novel and essential Candida albicans gene encoding a vacuolar protein involved in cytokinesis and hyphal branching. Eukaryot Cell 4:1088–1101
Veses V, Richards A, Gow NAR (2009) Vacuole inheritance regulates cell size and branching frequency of Candida albicans hyphae. Mol Microbiol 71:505–519
Volkmann N, Amann KJ, Stoilova-McPhie S et al (2001) Structure of Arp2/3 complex in its activated state and in actin filament branch junctions. Science 293:2456–2459
Wedlich-Söldner R, Schulz I, Straube A, Steinberg G (2002a) Dynein supports motility of endoplasmic reticulum in the fungus Ustilago maydis. Mol Biol Cell 13:965–977
Wedlich-Söldner R, Straube A, Friedrich MW, Steinberg G (2002b) A balance of KIF1A-like kinesin and dynein organizes early endosomes in the fungus Ustilago maydis. EMBO J 21:2946–2957
Weinberg J, Drubin DG (2012) Clathrin-mediated endocytosis in budding yeast. Trends Cell Biol 22:1–13
Weisman LS (2003) Yeast vacuole inheritance and dynamics. Annu Rev Genet 37:435–460
Weisman LS (2006) Organelles on the move: insights from yeast vacuole inheritance. Nat Rev Mol Cell Biol 7:243–252
Weisman LS, Wickner W (1988) Intervacuole exchange in the yeast zygote: a new pathway in organelle communication. Science 241:589–591
Weisman LS, Bacallao R, Wickner W (1987) Multiple methods of visualizing the yeast vacuole permit evaluation of its morphology and inheritance during the cell cycle. J Cell Biol 105:1539–1547
Wen KK, Rubenstein PA (2005) Acceleration of yeast actin polymerization by yeast Arp2/3 complex does not require an Arp2/3-activating protein. J Biol Chem 280:24168–24174
Wiederkehr A, Du Y, Pypaert M et al (2003) Sec3p is needed for the spatial regulation of secretion and for the inheritance of the cortical endoplasmic reticulum. Mol Biol Cell 14:4770–4782
Winter D, Podtelejnikov AV, Mann M, Li R (1997) The complex containing actin-related proteins Arp2 and Arp3 is required for the motility and integrity of yeast actin patches. Curr Biol 7:519–529
Winter DC, Choe EY, Li R (1999) Genetic dissection of the budding yeast Arp2/3 complex: a comparison of the in vivo and structural roles of individual subunits. Proc Natl Acad Sci USA 96:7288–7293
Xiang X (2003) LIS1 at the microtubule plus end and its role in dynein-mediated nuclear migration. J Cell Biol 160:289–290
Xiang X, Fischer R (2004) Nuclear migration and positioning in filamentous fungi. Fungal Genet Biol 41:411–419
Xiang X, Plamann M (2003) Cytoskeleton and motor proteins in filamentous fungi. Curr Opin Microbiol 6:628–633
Yaffe MP, Harata D, Verde F et al (1996) Microtubules mediate mitochondrial distribution in fission yeast. Proc Natl Acad Sci USA 93:11664–11668
Yaffe MP, Stuurman N, Vale RD (2003) Mitochondrial positioning in fission yeast is driven by association with dynamic microtubules and mitotic spindle poles. Proc Natl Acad Sci USA 100:11424–11428
Yang HC, Palazzo A, Swayne TC, Pon LA (1999) A retention mechanism for distribution of mitochondria during cell division in budding yeast. Curr Biol 9:1111–1114
Yang HC, Pon LA (2002) Actin cable dynamics in budding yeast. Proc Natl Acad Sci U S A 99:751–756
Yau RG, Peng Y, Valiathan RR, Birkeland SR, Wilson TE, Weisman LS (2014) Release from myosin V via regulated recruitment of an E3 ubiquitin ligase controls organelle localization. Dev Cell 28:520–533
Yoshida S, Kono K, Lowery DM et al (2006) Polo-like kinase Cdc5 controls the local activation of Rho1 to promote cytokinesis. Science 313:108–111
Young ME, Cooper JA, Bridgman PC (2004) Yeast actin patches are networks of branched actin filaments. J Cell Biol 166:629–635
Zekert N, Veith D, Fischer R (2010) Interaction of the Aspergillus nidulans microtubule-organizing center (MTOC) component ApsB with gamma-tubulin and evidence for a role of a subclass of peroxisomes in the formation of septal MTOCs. Eukaryot Cell 9:795–805
Zhang J, Han G, Xiang X (2002) Cytoplasmic dynein intermediate chain and heavy chain are dependent upon each other for microtubule end localization in Aspergillus nidulans. Mol Microbiol 44:381–392
Zhang J, Li S, Fischer R, Xiang X (2003) Accumulation of cytoplasmic dynein and dynactin at microtubule plus ends in Aspergillus nidulans is kinesin dependent. Mol Biol Cell 14:1479–1488

2 Nuclear Dynamics and Cell Growth in Fungi

Samantha E. Roberts[1], Amy S. Gladfelter[1]

CONTENTS

I. Introduction

Nuclei are the repositories of genetic information in eukaryotic cells. These organelles are highly dynamic in terms of both physical movement and genetic change from the level of gene expression to large-scale genome alterations. Fungal systems provide an especially excellent model for understanding the fundamentals of nuclear behavior in all cells and display a range of strategies for manipulating both the position and content of genetic information to adapt to the external environment as well as the constantly changing cellular context during growth. From the first characterizations of nuclear motility within **basidiomycete** mycelia to the present day, fungal cells have provided researchers with countless complex problems, many still awaiting inquiry. Investigations into fundamental activities within fungi have yielded insights into processes such as the mechanical dynamics of **growth**, **evolution**, and mechanisms of **pathogenesis** that have furthered our understanding of eukaryotic cell biology as a whole. While there are numerous excellent reviews on various aspects of nuclear mobility, exchange of genetic material, the interplay between nuclear behavior and cell growth, and principles of evolution in the fungal kingdom, we seek in this chapter to communicate a more integrative view of these processes to illustrate the remarkable adaptability and dynamic behaviors of nuclei.

II. Physical Nuclear Mobility

Throughout the entire spectrum of morphologies exhibited by fungi from **uninucleate** baker's yeast only microns in diameter to **multinucleate mycelia** of mushroom species that can cover multiple square miles, nuclei are strategically and actively positioned to facilitate their interactions with other cellular components and contribute to successful growth and reproduction of the organism. Nuclei are transported throughout the cell for a variety of purposes by a combination of precise active and bulk passive mechanisms.

A. Mechanics of Nuclear Movement

The **microtubule cytoskeleton** is essential for nuclear positioning in fungi. One of the most

[1]Department of Biological Sciences, Dartmouth College, Hanover, NH, 03755, USA; e-mail: Amy.Gladfelter@dartmouth.edu

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

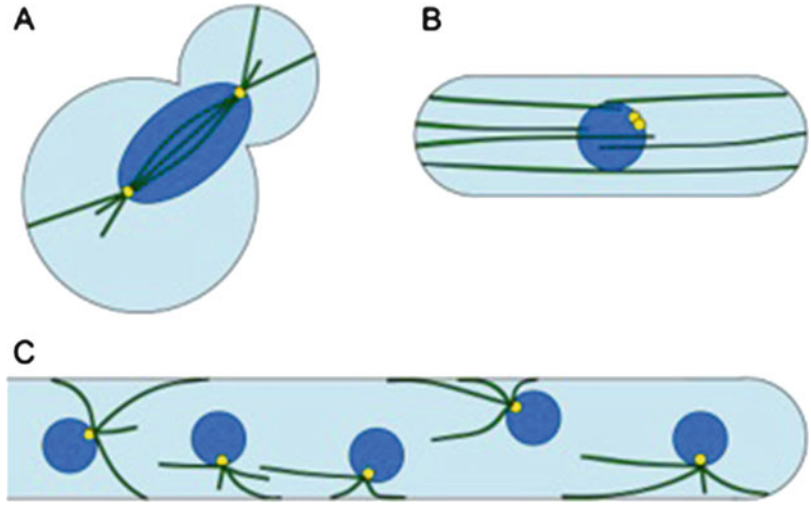


Fig. 2.1 Overview of the microtubule cytoskeleton in different species. (**a**) *S. cerevisiae* microtubules emanate from the spindle pole bodies and interact with the cell cortex to orient the nucleus during mitosis. (**b**) *S. pombe* microtubules associate with the nuclear envelope and push against the cell cortex to center the nucleus within the cell. (**c**) Microtubules emanating from SPBs in filamentous fungi interact with the cell cortex and dynamically position nuclei within a constantly flowing cytoplasm

extensively studied microtubule-dependent nuclear processes is **migration during mitosis** in *Saccharomyces cerevisiae*. Astral microtubules emanating from the **spindle pole body** (SPB, the microtubule-organizing center of fungal cells) embedded within the nuclear envelope are captured by cortical dynein and used to orient the nucleus so that the daughter nucleus will traverse the bud neck during spindle elongation (Fig. 2.1a). **Microtubule defects** or **dynein mutations** cause misalignment of the spindle and generation of a binucleate mother and an anucleate daughter cell. The details of this process can be found in many excellent review articles on the subject, but here it is essential to note that conserved functions of microtubules, associated motors, and accessory proteins primarily coordinate these nuclear movements (ten Hoopen et al. 2012; Ananthanarayanan et al. 2013).

In the antithesis to mitosis, **karyogamy**, two haploid nuclei must be brought together for fusion prior to **meiosis**. Genetic and molecular analyses of the *S. cerevisiae kar* mutants have revealed that this process also depends upon microtubules and microtubule-associated motors (Rose and Fink 1987; Meluh and Rose 1990; Endow et al. 1994; Miller and Rose 1998). The kinesin Kar3 associates with the SPB of each nucleus and captures microtubules emanating from the SPB of the partner nucleus. The force generated by these motors brings the two nuclei together prior to fusion (Melloy et al. 2007; Gibeaux et al. 2013). The process of nuclear envelope fusion begins with fusion of the separate SPBs so that after completion of karyogamy, the diploid nucleus contains one SPB with a half-bridge (the construction site of a new SPB). In this way, the number of microtubule nucleation sites is regulated to successfully segregate chromosomes during meiosis (Byers and Goetsch 1975; Gibeaux et al. 2013; Gibeaux and Knop 2014).

In contrast to budding yeast, which have the hourglass of the mother-bud neck as a cellular landmark, nuclei in fission yeast cells must achieve proper positioning through alternative mechanisms. In *Schizosaccharomyces pombe*, **dynamically unstable microtubules** associated with the nuclear envelope generate pushing forces against the plasma membrane and keep the nucleus centered within the cell (Fig. 2.1b) (Tran et al. 2001). In the case of a nucleus that has been displaced from the center

(e.g., by centrifugation), microtubules on one side must grow much longer than those on the alternate side to reach the cortex. This increases the chance of shorter microtubules reaching the cortex on the closer end of the cell, and these stiffer, shorter microtubules exert more force on the closer cell end. These interactions are sufficient to return the nucleus to the center of the cell (Daga et al. 2006). However, microtubule motors are important for nuclear positioning during other processes in *S. pombe*. During meiosis, **nuclear oscillations** within the cell promote chromosome pairing and recombination. These oscillations are generated by changes in **dynein localization** that exert forces on the nucleus from different directions through time (Vogel et al. 2009).

It is clear that the **cell shape** can influence nuclear positioning, and therefore **dimorphic** fungi encounter unique challenges with respect to nuclear mobility. For example, in the **yeast form** of *Candida albicans*, the nucleus must be positioned properly during mitosis to produce uninucleate progeny. It has been shown that, as in *S. cerevisiae*, dynein-dependent nuclear oscillations coordinate nuclear positioning in the yeast form during mitosis (Finley and Berman 2005; Finley et al. 2008). Indeed, the velocity of nuclear movement in both yeast and hyphae is reduced in dynein mutants (Finley et al. 2008). Often, dynein mutants in the yeast form are able to successfully segregate nuclei during mitosis because although nuclear division may proceed in the mother cell, a spindle checkpoint prevents cytokinesis before a nucleus eventually moves into the bud (Finley and Berman 2005). However, nuclei must also undergo long-range transport after transition to the **hyphal** growth form. In *C. albicans*, microtubule-dependent nuclear migration within hyphae is important for ensuring appropriate timing of **septum** formation relative to mitosis, and this process is not successful in dynein mutants (Finley and Berman 2005). Similarly, in the corn pathogen *Ustilago maydis*, microtubules are not essential during initial **germ tube** formation, but are required for hyphal growth after **infection** initiation (Fuchs et al. 2005). Dimorphic species are able to adapt the same machinery to transport nuclei within very different spatial contexts. Investigation into the different behavior of microtubules and microtubule-associated motors between these two growth strategies can provide insight into adaptations during the evolution of multinucleated cells and other cells with complex geometries.

Microtubule-dependent nuclear transport in **multinucleate** fungi serves to properly distribute nuclei throughout the mycelium. Compromised microtubules, dynein, or dynein activators in the filamentous fungi *Neurospora crassa*, *Aspergillus nidulans*, *Ashbya gossypii*, and *Nectria haematococca* result in a disruption of normal nuclear spatial distribution (Oakley and Morris 1980; Plamann et al. 1994; Tinsley et al. 1996; Inoue et al. 1998; Grava et al. 2011). In these organisms, SPB-associated microtubules interact with dynein at the cortex to generate force required for nuclear movement (Fig. 2.1c). In the yeast *S. cerevisiae*, the protein Num1 localizes to the cortex and interacts with cytoplasmic microtubules and dynein to facilitate nuclear positioning during mitosis (Farkasovsky and Küntzel 1995, 2001). Homologues of this protein in *A. gossypii* (Num1) and *A. nidulans* (ApsA) are also required for normal nuclear positioning, suggesting a conserved role for cortical dynein anchoring in nuclear positioning (Fischer and Timberlake 1995; Grava et al. 2011). In *A. gossypii*, it has been demonstrated that microtubules are required for active redistribution of nuclei and maintenance of consistent internuclear distance, though the precise nature of microtubule interactions remains unclear and involves the activity of kinesins as well as dynein (Anderson et al. 2013). There is also evidence for the involvement of kinesins in nuclear positioning from studies in *A. nidulans*, but further investigation is required to understand their involvement in these processes (Requena et al. 2001; Zhang et al. 2003).

Nuclear movement and division must be coupled to **cell growth**. In some species both nuclear movement and polarized secretion require microtubules, providing a strategy for linking these processes. For example, in addition to nuclear distribution, polarized growth becomes compromised when microtubules are disrupted in *N. crassa*, *A. nidulans*, or

N. haematococca (Plamann et al. 1994; Inoue et al. 1998; Horio and Oakley 2005). However, in *A. gossypii*, microtubule-based nuclear transport and actin-based polarized growth are sufficiently uncoupled to allow continuation of growth despite aberrantly positioned nuclei in mutant strains or pharmacologic disruption of microtubules (Gladfelter et al. 2006; Grava et al. 2011). This may be because *A. gossypii* is more closely related to the yeast *S. cerevisiae*, in which nuclear movements and polarized growth are also relatively distinct processes (Dietrich et al. 2004). It may be that the more divergent filamentous fungi from *S. cerevisiae* have adapted to more closely coordinate nuclear dynamics with polarized growth by using the same cytoskeleton for both. Interestingly, the **rates of polarized growth** differ in all of these species from 0.1 to 0.5 μm/min in *A. nidulans*, 1–2 μm/min in *A. gossypii*, 3 μm/min in *N. haematococca*, and 1.3–12.9 μm/min of individual *N. crassa* hyphae, though mature mycelium in race tubes has been measured at up to 80 μm/min (Ryan et al. 1943; Wu et al. 1998; Horio and Oakley 2005; Araujo-Palomares et al. 2007; Kohli et al. 2008). The mechanistic differences behind this variation are currently unclear, though they likely depend upon differential allocation of resources for growth, vesicle production and fusion rates, cytoskeletal dynamics, and differences between cytoskeletal motors in each species. In the species for which it has been measured (*A. nidulans*, *N. crassa*, and *A. gossypii*), the growth rate is highly dependent upon the age of the cell, probably due to increased capacity for transport of components required for growth, such as vesicles, to hyphal tips (Horio and Oakley 2005; Araujo-Palomares et al. 2007; Kohli et al. 2008).

An additional transportive force upon nuclei in filamentous fungal cells is that produced by **cytoplasmic streaming**. The cytoplasm of these cells continually moves toward the growing tips indiscriminately carrying cytosolic components and organelles (Ramos-Garcia et al. 2009; Lang et al. 2010; Abadeh and Lew 2013). This promotes transport of necessary components to growth sites and guides nuclei, cytosol, and other organelles into new hyphae upon symmetry breaking and polarity establishment. Investigations of **nutrient transport** on the millimeter and centimeter scale have indicated that osmotic and pump-driven water uptake during growth induces mass flow throughout the mycelial network, contributing to nutrient dispersal throughout the organism (Heaton et al. 2010). The pressure generated by this flow is thought to promote apical growth (Lew 2005). Many species are capable of vegetative and/or sexual **hyphal fusion**, generating even more complex networks and fluid flows. During vegetative fusion in *N. crassa*, nuclei from each partner move into the other, rapidly redistributing the genotypes throughout the fused network (Fig. 2.2a). It has been demonstrated that the combination of complex network geometry and bulk cytoplasmic flow strongly influences this nuclear mixing (Roper et al. 2013). It remains to be thoroughly investigated how large-scale fluid movements throughout mycelial networks relate to dynamic flow within individual hyphae. The activity of the same cytoskeletal motors that actively transport nuclei throughout the mycelium has been shown to generate **complex fluid flows** on the order of microns that promote nutrient uptake and mixing throughout the cells of some plant species. For example, in cells of the algal weed *Chara corallina*, cytoplasmic streaming has been described as "barber pole flow," because two bands of flow with opposite polarity spiral along the length of the cell (Goldstein et al. 2008). Additionally, it has been demonstrated using various experimental and modeling techniques that the activity of motors within eukaryotic cells contributes significantly to patterns and rates of diffusion within the cytoplasm (Brangwynne et al. 2008). It is likely that similar processes contribute to dynamic cytoplasmic flow over micron-scale regions of mycelial networks, and the role these play in nuclear distribution throughout large networks is an area open for investigation.

The placement of **septal pores** along hyphae provides another mechanism for the cell to regulate fluid flow between compartments. In species such as *N. crassa*, pores in healthy cells are left open allowing for cytoplasmic flow and

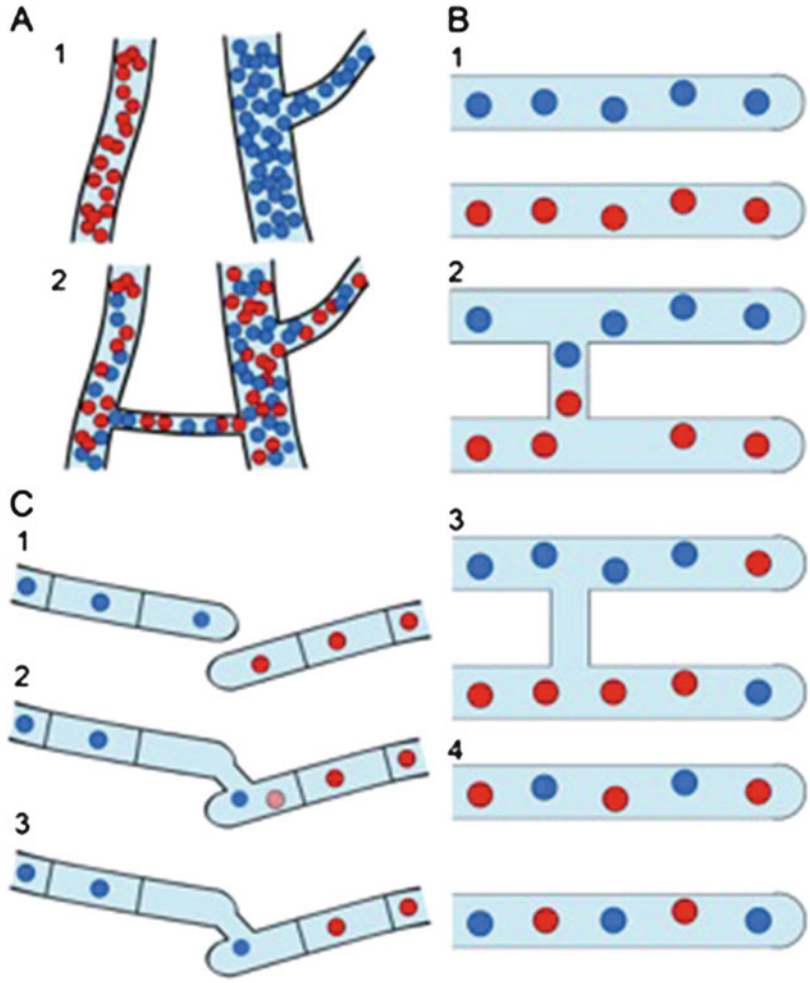


Fig. 2.2 Nuclear interactions during vegetative fusion of fungal cells. (**a**) Upon fusion of compatible *N. crassa* hyphae, the two nuclear genotypes (*denoted in red and blue*) rapidly intermix within the fused mycelium. (**b**) During formation of a dikaryon in basidiomycetes, a nucleus from each fusion partner moves into the other fusion partner (2). These nuclei move to the growing tips of the cell (3). Subsequent mitotic events result in alternation of genotypes in these hyphae (4). (**c**) After migration of a donor nucleus into a compartment of a host cell of *F. oxysporum* (2), the host cell nucleus is degraded (3)

nuclear movement between compartments (Shatkin and Tatum 1959). Upon damage to a portion of the cell, or signaling during the **heterokaryon incompatibility response**, a specialized proteinaceous plug termed the **Woronin body** blocks septal pores to prevent damage to and loss of cytoplasm from other regions (Jedd and Chua 2000; Tenney et al. 2000). However, not all cells utilize this type of flow for nuclear transport. Some cells lay down complete septa between compartments, such as *Fusarium oxysporum* (Ruiz-Roldan et al. 2010). In these cells, cytoplasmic components do not move between different parts of the mycelium, even in healthy cells, and nuclei in different hyphal compartments cannot intermix.

B. Functions of Movement in Reproduction

One of the most universal roles of nuclear mobility is **segregation of sister chromosomes** into separate cells during mitosis. Many fungal cells undergo completely **closed mitosis**, in

which the nuclear envelope does not degrade (Heath 1980). The SPB's location within the nuclear envelope allows nuclear transportation via force generation by cytoskeletal motors on associated microtubules. This motor activity is coordinated with **cell cycle checkpoints** and the **cytokinetic machinery** to ensure daughter cells do not separate until each contains a nucleus. Failure to properly position the nucleus throughout this process results in the production of multinucleate and anucleate cells (Morris et al. 1995). In uninucleate species, the resulting **ploidy** changes can lead to **genome instability** and **cell death**. In some cell types, nuclear placement itself helps specify the plane of division. The position of the nucleus coordinates the placement of the actomyosin ring during *S. pombe* cytokinesis, promoting creation of equally sized daughter cells (Paoletti and Chang 2000). Nuclear positioning in this species, therefore, is essential to normal partitioning of resources during cytokinesis.

Not all fungi undergo closed mitosis, however. Some partially degrade the nuclear envelope during mitosis, such as the disassembly of portions of the **nuclear pore complex** (NPC) in *A. nidulans* (Osmani et al. 2006). Others such as *S. pombe* open a **fenestra** in the nuclear envelope during mitosis for transient SPB insertion (Ding et al. 1997). In *U. maydis*, extranuclear microtubule-organizing centers (MTOCs) orchestrate mitosis along with SPBs. To allow cytoplasmic microtubules access to kinetochores, the nuclear envelope is torn open in this species during prophase by dynein-generated forces (Straube et al. 2005). The differences among fungal mitotic strategies and comparisons with other eukaryotes have furthered our understanding of the evolution of mitosis.

Sexual reproduction involves the recombination of genetic material from two individuals. This requires bringing nuclei from two parents into physical proximity for nuclear fusion to occur. Though mating increases the genetic diversity essential for the survival of the species, it involves certain risks on the part of the individual. Fusion of separate individuals in order to bring different nuclei together can entail mixing of cytoplasm and potential transmission of deleterious elements such as **viruses**, **prions**, or **parasitic mitochondrial DNA genotypes**. Uninucleate cells must accept the consequences of taking these risks and will often only initiate sexual reproduction in the event of environmental adversity. This has the twofold benefit in many species of increasing genetic variation, which will hopefully help the next generation survive the stress and generation of environmentally resistant spores.

Many multinucleate ascomycetes permit nuclear encounters only in specialized **mating structures**. It has been hypothesized that this, in combination with **mating type** and **heterokaryon incompatibility** mechanisms, serves to protect the rest of the organism from parasitic elements (Buss 1982, 1987; Debets and Griffiths 1998). Nuclei enter the mating structure to form a dikaryon and subsequently undergo karyogamy (nuclear fusion) and meiosis to produce sexual spores. In basidiomycetes, donor nuclei move into a host cell upon fusion (**anastomosis**) of two compatible mates and rapidly migrate to hyphal tips, 2–3 mm per hour in *Schizophyllum commune* (Niederpruem 1980) and an astonishing 4 cm per hour in *Coprinellus congregatus* (Ross 1976). Both host and donor genotypes then exist together and replicate in a stable dikaryotic mycelium. Through formation of specialized "clamp cells" in some species and regulation of nuclear positioning and mitotic spindle length, the nuclei with different genotypes in these regions alternate positions along the hypha (Fig. 2.2b). Each hyphal compartment contains one nucleus of each type, and upon reception of environmental cues, genetically different nuclei will pair up, fuse, and undergo meiosis to generate sexual spores (Brown and Casselton 2001). Donor nuclei do not necessarily propagate throughout the entire mycelium, which serves a similar function to specialized sexual structures in ascomycetes. In *Termitomyces* species, a dikaryotic mycelium is only generated at the interface between mating monokaryons (Nobre et al. 2014). Subapical regions retain their monokaryon identity, and in the event that fusion is detrimental to the survival of the genotype, these regions of the mycelium are free to make contacts with other mating partners.

Exchange of nuclei between **basidiomycete monokaryons** is often reciprocal, with hyphae of both individuals acting as both a nuclear donor and recipient. Fusion with a compatible mating partner induces nuclear migration into the partner cell. If incompatible hyphae fuse with each other, nuclear migration does not occur. It is likely that products of the mating-type loci interact with dynein and the microtubule cytoskeleton to regulate nuclear transport during these events. Interestingly, during fusion between a dikaryon and a monokaryon, the dikaryon will donate a nucleus to the monokaryon generating a new dikaryon. However, the individual that was already a dikaryon does not accept additional nuclei from the monokaryon (Swiezynski and Day 1960). Furthermore, under these conditions, the monokaryon preferentially accepts nuclei from the dikaryon that are more different from itself (Ellingboe and Raper 1962; Raper 1966). In another example of how nuclear identity influences events after cell fusion, hyphal fusion in the basidiomycete *F. oxysporum* can be followed by **degradation** of the nucleus from the host compartment (Fig. 2.2c) (Ruiz-Roldan et al. 2010). Interestingly, although nuclear genetic material is shared during dikaryotization, the host cell generally retains its **mitochondrial genome** and other **cytoplasmic elements** through unknown processes (Lee and Taylor 1993; Marcinko-Kuehn et al. 1994). The mechanisms by which cells identify the genotypes present within them, limit this number to a maximum of two, and ensure proper pairing upon the decision to undertake karyogamy and spore production are unknown.

Generation and release of **spores** or **conidia** is a highly regulated process, but control mechanisms vary between species. Uninucleate *S. cerevisiae* and *S. pombe* produce a set of four haploid spores within an ascus membrane. Many fungal species produce uninucleate spores, but *Aspergillus* species contain two and some mycorrhizal fungal spores contain 2000–20,000 nuclei with multiple different genotypes (Burggraaf and Beringer 1989; Becard and Pfeffer 1993; Hijri et al. 1999). During either sexual or asexual production of spores or conidia, the appropriate number of nuclei must be moved and packaged into specialized compartments and sometimes into specialized structures prior to release into the environment. This transport is dependent upon the microtubule cytoskeleton. Multinucleate *A. gossypii* designates specialized spore producing hyphal compartments that fill up with spores prior to bursting to release them into the environment (Wendland and Walther 2005; Kemper et al. 2011). *Aspergillus* and *Neurospora* species produce elegant structures upon which conidia are poised for maximum dispersal, though these are dwarfed by the elaborate fruiting bodies common among the basidiomycetes (mushrooms). Dynein mutants of *Aspergillus oryzae* form abnormally shaped anucleate and multinucleate conidia (Maruyama et al. 2003). *A. nidulans* mutants with abnormal nuclear distribution likewise create malformed metulae and phialides (parts of the **conidiophore**) and anucleate or multinucleate conidia (de Queiroz and de Azevedo 1998; Castiglioni Pascon et al. 2001) or anucleate sterigmata (also part of the conidiophore), resulting in the arrest of further conidial development (Fischer and Timberlake 1995). In all of these cases, the meiosis must be coordinated with a specific developmental program to trigger the morphological changes necessary to produce the environmentally resistant spore and (if applicable) its dispersion structure, further indicating the coupling of nuclear mobility and growth programs in multinucleate fungi.

Chytrid fungi, recently appreciated as major **amphibian pathogens**, create **flagellated spores** that are capable of active movement through their environment (Letcher et al. 2008; Letcher and Powell 2014). These motile sexual structures find each other for mating. *Rhizophydiales* produce multinucleate sporangia that undergo multiple cytokinetic events during maturation to release uninucleate zoospores (Berger et al. 2005; Letcher et al. 2008). It has been demonstrated that subgroups of chytrids exhibit differences in nuclear positioning and microtubule cytoskeleton organization, but further research is required to determine the mechanisms of nuclear positioning in this poorly understood fungal lineage (Letcher and Powell 2014). Examinations of these fungi in

the early 1900s yielded many qualitative observations about their vegetative, sexual, and parasitic interactions. From these, it is clear that there are many types of interesting nuclear interactions in these species that have not been investigated in more recent years, but merit further research. For example, during *Olpidiopsis* sexual reproduction, one **multinucleate thallus** donates all of its cytoplasm to another to create the resting zoospore. The two sets of nuclei then undergo karyogamy, meiosis, and the cytokinetic events necessary to produce uninucleate zoospores (Sparrow 1935). The process by which nuclei of opposite mating types find each other in this large multinucleate cytoplasm and how cytokinesis is linked to the events following meiosis remain completely mysterious, but will likely provide insight into similar processes observed in the basidiomycetes.

The first chytrid **genome sequencing project** was undertaken for the important amphibian pathogen *Batrachochytrium dendrobatidis*. Most of the *B. dendrobatidis* genome was sequenced and assembled by the Broad Institute Fungal Genome Initiative. More recently, the genomes of two other chytrids are being sequenced as part of the Broad Institute Origins of Multicellularity Project: *Spizellomyces punctatus* and *Allomyces macrogynus* (Ruiz-Trillo et al. 2007). This genomic information will allow more research into chytrid pathogenicity and control strategies, as well as general chytrid and fungal biology.

C. Functions of Nuclear Movement During Vegetative Growth

In addition to the necessity of nuclear movement during reproduction, cells require regulated and dynamic nuclear positioning during **vegetative growth**. As previously discussed, dynein activity is required in the filamentous fungi *N. crassa*, *A. nidulans*, *A. gossypii*, and *N. haematococca* to maintain regular internuclear spacing. In cells with compromised microtubules, dynein, or dynein activators, nuclei form large clusters, precluding effective regulation of nuclear activity, mitosis, and polarized growth. Although these cells all actively move nuclei within the cytoplasm, the average distance differs between different species, and internuclear spacing is dynamic within a single cell (Suelmann et al. 1997; Grava et al. 2011; Anderson et al. 2013). This suggests that different cells have adapted nuclear mobility and mitosis in different ways to optimize the fitness of the organism.

In *A. gossypii*, nuclei actively repulse their neighbors via SPB-associated microtubules to maintain regular internuclear spacing and a consistent **nuclear-cytoplasmic ratio** (Gladfelter et al. 2006; Anderson et al. 2013). This poses a conundrum when it comes to mitotic strategies within multinucleate cells. If nuclei duplicate either **synchronously** (e.g., *Physarum polycephalum*) or in **waves of synchrony** that progress at a different rate than apical growth (e.g., *A. nidulans*), the cell experiences rapid changes of nuclear-cytoplasmic ratio and gene dosage (Nygaard et al. 1960; Clutterbuck 1970). This is in stark contrast with studies of the tightly regulated nuclear-cytoplasmic ratio in *S. pombe* (Neumann and Nurse 2007). Obviously these organisms have developed ways to tolerate these fluctuations, but other species employ various strategies to avoid these problems. *N. crassa* and *A. gossypii* exhibit **asynchronous** mitosis within the common mycelium. Mitotic events happen only frequently enough to maintain the nuclear-cytoplasmic ratio, and nuclei continually bypass each other and mix within these hyphae (Grava and Philippsen 2010; Roper et al. 2013). In other species, such as *Alternaria solani*, only nuclei within the most apical compartment undergo mitosis (King and Alexander 1969). In *N. crassa*, *A. gossypii*, and *A. solani*, nuclei maintain constant internuclear distances while producing sufficient nuclei to populate new growth regions. The optimal internuclear distance in a given species can even change in response to its environment, similar to how cell size can be nutrient controlled in single-celled yeasts (Unger and Hartwell 1976; Johnston et al. 1977; Fantes and Nurse 1977). In *A. gossypii*, starvation prolongs the G2 phase of the cell cycle, leading to increased average internuclear spacing (Helfer and Gladfelter 2006).

Internuclear distance in *S. commune* varies depending on the growth substrate, and cell secretes different types of **hydrophobins** to aid in surface attachment (Schuurs et al. 1998). It is tempting to speculate that the altered internuclear distance contributes to the regulation of the production of these proteins, though further investigation is required. Interactions between environment, gene expression, and nuclear spacing are fascinating areas for future research. It is clear that nuclear division is linked with cellular growth and nutrient availability, but recently it has been shown that other external signals also regulate mitosis. In the **circadian** model organism *N. crassa*, asynchronously dividing nuclei can be synchronized using periodic light exposure (Hong et al. 2014). The intersection between the highly studied circadian rhythm regulation and cell cycle control is only beginning to be understood (Zamborszky et al. 2014).

Nuclear positioning can also influence the organization of the surrounding cytosol. Recent studies have shown that organization of cytoplasmic factors and regular internuclear spacing are both important in the generation of nuclear asynchrony in *A. gossypii* (Lee et al. 2013; Anderson et al. 2013). Transcripts encoding the G1 cyclin protein, Cln3, are concentrated in the vicinity of nuclei via an aggregation-prone RNA-binding protein. It is hypothesized that by forming RNA-protein complexes, transcript diffusivity is limited and neighboring nuclei are prevented from sharing gene products (Lee et al. 2013). It is intriguing to speculate that the assembly of **RNA-protein aggregates** of different sizes and degrees of diffusivity is tuned to match the spacing between nuclei. The mechanisms by which these **membrane-free domains** are created and associated with a specific nucleus are areas of active study but are known to involve the formation of higher-order assemblies between RNAs and polyQ tracts in RNA-binding proteins (Lee et al. 2013). Furthermore, it raises the possibility that fungi have mechanisms to restrict cooperation between genetically distinct nuclei in a common cytoplasm.

Multinucleate cells have the opportunity to contain nuclei with different genotypes. This **heterokaryon** state has been hypothesized to confer fitness advantages, as the rest of the population can dilute deleterious alleles. Indeed, it has been demonstrated in several species that heterokaryons grow more rapidly than their homokaryon counterparts (Jinks 1952; James et al. 2008; Samils et al. 2014; Nobre et al. 2014). A prime example of nuclear cooperation in a heterokaryon is that of mating-type loci within *S. commune*. In this organism, nuclei with nonfunctional genes at the B mating locus have a growth advantage in the homokaryon state, but these genes contribute to hook cell fusion and nuclear migration during mating, meaning these homokaryons are at a disadvantage for sexual reproduction (Raper 1985). In heterokaryons with nuclei containing functional and nonfunctional alleles of these mating genes, nuclei with the functional alleles are overrepresented, but the mycelium grows better than the homokaryon. This suggests that dilution of the functional B alleles can be advantageous to an individual. In *N. crassa*, nuclei from different parents rapidly disseminate throughout the mycelium after fusion (Fig. 2.2a) (Roper et al. 2013). The benefits of this are surely to share beneficial and dilute deleterious alleles throughout the whole cell, but the mechanisms for this active redistribution are unknown.

In general, **heterokaryosis** seems to provide a cell with the advantage of as many potentially beneficial alleles as possible, without having to "decide" on any specific genotype. In a system with multiple genotypes contained in a single nucleus, all of these must be inherited together, given the physical limitations of mitosis. When multiple nuclei coexist within the cell, there is the potential for selection at the level of each nucleus to optimize the balance of genotypes that will benefit the organism. Sister nuclei sharing the cytoplasm provide buffering capacity for mutational exploration, but only the fittest genotypes will be able to generate new individuals after sexual or asexual spore production.

III. Genome Content Dynamics

In any discussion about the genetics of multinucleate fungi, we must constantly bear in mind the varying levels of **selection** at play.

Each nucleus is a potential unit of selection, and yet different genotypes may come together in one cell to increase the fitness of the organism as a whole. In multinucleate as well as uninucleate cells, the genetic identity of a nucleus and each cell is constantly in flux due to mutation, random drift, and active response to environmental cues.

A. Inter-organism Interactions

Fungi must react rapidly and appropriately to interactions with each other and with the environment in order to survive. Different species have developed elaborate **mating** and **vegetative fusion** strategies in order to maximize the benefits of interacting with other individuals. In order to discern the suitability of a potential mate, fungi have developed various **mating-type** structures. Most ascomycetes utilize a system with only two mating types, while many basidiomycetes have four, and some even have thousands (Raper 1985; Glass and Kuldau 1992; Casselton and Challen 2006). More mating types in the population means that an encounter between two individuals is more likely to result in successful mating. Often karyogamy, meiosis, and sexual spore production are heavily dependent upon environmental cues such as nutrient starvation. In these scenarios, generation of genetic variability by mating combined with the production of environmentally resistant spores improves the chances of survival in the next generation. In some cases, mating-type mechanisms have been co-opted by **fungal parasites** (Burgeff 1924). *Parasitella parasitica* parasitizes zygomycetous fungi and uses the regulatory pathways involved in sexual fusion to initiate infection (Schultze et al. 2005). Interestingly, this means that the "mating type" of a *P. parasitica* individual must be the opposite of its intended target. The infection process involves transfer of *P. parasitica* cytoplasm to the host cell, and parasite-host gene transfer has been observed during infection of *Absidia glauca* (Kellner et al. 1993). Although "mating type" appropriately evokes specific connotations, these parasitic interactions highlight the most basic role of these loci: self- and nonself-recognition.

Another process that involves distinguishing between self and nonself is the fusion of hyphae during vegetative growth. In contrast with fusion of cells for sexual reproduction, many fungi can also fuse to form a hybrid mycelium, or heterokaryon, with different genotypes. In contrast with the mating types that encourage reproduction between genetically different parents, **vegetative fusion** is unsuccessful if the partners are not genetically similar, termed **vegetative** or **heterokaryon incompatibility** (Glass and Kaneko 2003). Cells have the potential to benefit from mycelial fusion by sharing of nutrients, increasing the genetic diversity of the individual, enabling an individual to cover more area in the search for nutrients, and having a better chance of outcompeting rival mycelia (Aanen et al. 2009; Richard et al. 2012). This is especially true of newly germinated cells. A new colony can more rapidly establish if spores germinate and fuse with related cells to share resources and spread, as has been shown for *N. crassa* and *F. oxysporum* (Roca et al. 2010; Ruiz-Roldan et al. 2010). Additionally, hyphae from the same cell can fuse with each other, facilitating rapid movement of nuclei and nutrients throughout the cell. This is especially important in the case of heterokaryons, where nuclei containing different genetic materials are actively distributed throughout a hybrid mycelium to allow sharing of gene products between different genotypes (Pitchaimani and Maheshwari 2003; Roper et al. 2013).

Despite the potential benefits, many different loci participate in the heterokaryon incompatibility response, making it highly unlikely that two cells that find each other in the wild will be compatible. In the event that two incompatible cells fuse, the heterokaryon incompatibility response is triggered, nearby septal pores close, and the fusion compartment undergoes **programmed cell death** (Glass and Kaneko 2003). Vegetative incompatibility is thought to prevent parasitism by selfish genotypes such as the spore killer variants in *N. crassa*, *Fusarium moniliforme*, and *Podospora anserina*, which prevent formation of spores without the spore killer gene (Padieu and Bernet 1967; Kathariou and Spieth 1982; Turner and Perkins 1991;

Hammond et al. 2012). In other examples, certain genotypes may "cheat" and use common resources to produce extra spores at the expense of their heterokaryon partners. Additionally, nonnuclear elements such as viruses or parasitic mtDNA can have negative consequences for fusion partners (Caten 1972; Anagnostakis 1982; Debets et al. 1994; Cortesi and Milgroom 2001). Heterokaryon incompatibility seeks to prevent these scenarios by ensuring that only closely related mycelia can fuse into a single cell (Debets and Griffiths 1998). In most species, the incidence of **selfish genotypes** such as spore killer are low in wild populations, despite the potential for competition between nuclei in a common cell, especially during spore production and dissemination into new environments. Various modeling studies have sought to investigate why these genotypes are not more prevalent. These have shown that the threat of negative fusion consequences can result in the rapid development of many heterokaryon incompatibility loci (Muirhead et al. 2002; Czaran et al. 2014). This suggests that in the presence of heterokaryon incompatibility responses, selfish nuclei do not have the opportunity to compete with nuclei of differing genotypes. In this situation, selfish genotypes and nuclei are unable to become prevalent in the population. Multinucleate fungal cells, therefore, have evolved elaborate mechanisms to limit competition between syncytial nuclei.

B. Intra-organism Interactions

Within a single multinucleate cell, different genotypes are potentially in competition with each other, while the combination of different alleles can confer an advantage to the cell as a whole. The individual must maintain a delicate balance between nuclear **competition** and **cooperation** in order to grow, reproduce, and be competitive in the environment (Fig. 2.3). Despite the evolution of heterokaryon incompatibility loci discussed above, cells containing nuclei with variable genotypes are common. For example, in one study, 26 % of wild *F. moniliforme* isolates contained multiple genetically distinct nuclear populations (SIDHU 1983). One possible reason

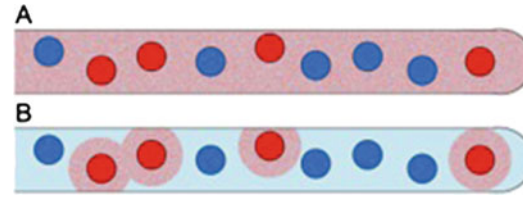


Fig. 2.3 Nuclear competition and cooperation in a syncytium. (**a**) Cooperation—A subset of nuclei that have a different genotype or level of activity (*red*) may share their gene products with all other nuclei in the cytoplasm. (**b**) Competition and autonomy—A subset of genetically different or differentially active nuclei may sequester gene products and limit dissemination to other nuclei in the same cytoplasm

for the prevalence of heterokaryosis is the potential for complementation of different genotypes and the ability to rapidly adapt to different environmental conditions. In the case of the *senescent Neurospora* gene, the wild-type gene product promotes stabilization of the mitochondrial genome. Homokaryons harboring the mutation undergo rapid changes in their mitochondrial genome and do not survive (Navaraj 2000). This phenotype is masked by coexistence with wild-type nuclei in a heterokaryon and therefore is able to persist in wild populations (Fig. 2.3a). Similar senescent phenotypes, some also the result of mitochondrial dysfunction, have been identified in wild isolates of other species as well, indicating that masked deleterious alleles may be a common feature of heterokaryotic fungi (D'Souza et al. 2005). Mutations or ploidy changes during vegetative growth can result in heterokaryosis. Alternatively, it can arise from fusion of cells with matching heterokaryon incompatibility loci but differences elsewhere in the genome. While much of the research on heterokaryons has been completed using auxotrophic markers and was limited to identifying two separate genotypes, it has been demonstrated that *Heterobasidion parviporum* cells can stably contain at least three distinct genotypes, and more examples of such "polykaryotic" cells are sure to follow (James et al. 2008).

The ratio of different genotypes within an individual has been shown to change over time in response to different environmental conditions or developmental stage. These changes offer the potential to rapidly produce a specific

response in the organism, without having to wait for mutation and evolutionary change (Jinks 1952; James et al. 2008). Consistent with these observations, heterokaryosis has been proposed as a mechanism to promote variability and adaptability of fungi in different environmental conditions and stresses.

An additional mechanism for rapid adaptation fungal cells is exemplified in the response of the dimorphic opportunistic pathogen *C. albicans* to clinical treatment using the antifungal **fluconazole**. This organism undergoes changes in **ploidy** resulting in severe genome instability to rapidly generate genome changes in its search for a way to overcome this stressor (Selmecki et al. 2009). Other human pathogens including *Cryptococcus neoformans* and *Candida glabrata* have also been found to rapidly rearrange the genome in response to host immune system stress (Fries and Casadevall 1998; Shin et al. 2007). Though these ploidy alterations have been examined in the context of **pathogenesis**, large-scale genome rearrangements are likely involved in environmental response and evolution in additional fungal species. For example, many industrial *Saccharomyces* strains are polyploid, despite experiments showing that under both stressed and unstressed conditions laboratory strains converge on diploidy (Gerstein et al. 2006; Querol and Bond 2009). Ploidy variation has also been detected in wild isolates of the plant pathogen *Botrytis cinerea*, supporting the hypothesis that this is a widespread fungal adaptation strategy (Büttner et al. 1994).

One of the most rapid strategies for responding to different environmental and developmental needs is alteration of **gene expression**. Large, multinucleate cells are uniquely adapted to exquisitely fine-tune the expression of genes over varying spatial scales. In *N. crassa*, for example, an mRNA profiling study demonstrated considerable differences in the transcripts present in different regions of the same colony, particularly between regions of differing ages (Kasuga and Glass 2008). These transcriptional changes likely promote and respond to functional and physical differences between different areas, as, for example, newer portions of the cell scavenge for nutrients and some older regions develop conidia. In the examination of laboratory-created *N. crassa* strains containing a subset of *his*-nuclei, the amount of enzyme produced was unrelated to the proportion of nuclei within the mycelium, indicating advanced mechanisms for regulating gene expression (Pitchaimani and Maheshwari 2003). Splice variants may also play an important role in gene expression changes and may be more common in fungi than previously appreciated (Grutzmann et al. 2014).

In one example on a smaller spatial scale, only a subset (~12 %) of *A. gossypii* nuclei transcribe the G1 cyclin *CLN3* at any given time, based on a single molecule (sm)FISH study (Lee et al. 2013). This behavior, coupled with membrane-free cytoplasmic compartmentalization strategies, promotes the asynchronous mitotic events characteristic of this fungus (Lee et al. 2013). Mechanisms for generating nuclear transcriptional autonomy in this species when neighboring nuclei are typically only ~5 μm apart remain unclear (Grava et al. 2011).

Another potential method of controlling the expression of different genotypes is **degradation** of a subset of nuclei. In a number of multinucleate species, nuclear degradation has been observed. All nuclei within the fusion compartment during the heterokaryon incompatibility response are degraded, along with everything else within this compartment, to protect the remainder of the cell from potential parasitic elements (Marek et al. 2003). In other species vegetative fusion and nuclear migration can result in degradation of a nucleus in the recipient cell. This was first observed in several basidiomycetes including *S. commune* (Todd and Caylmore 1985), *Coriolus versicolor* (Aylmore and Todd 1984), *Coprinus cinereus* (Bensaude 1918), and *Typhula trifolii* (Noble 1937). Only recently, however, has similar behavior been observed in an ascomycete *F. oxysporum* (Ruiz-Roldan et al. 2010). In contrast to the heterokaryon incompatibility response, this nuclear degradation can occur even between compatible mating partners, and the mechanisms by which nuclei are identified for degradation require further investigation (Ruiz-Roldan et al. 2010).

Nuclei may also be disassembled in order to recycle components. It has been proposed that

an advantage of multinucleate cells is that nuclei can serve as nitrogen and phosphorus storage for later use. During **starvation** in *S. cerevisiae*, nuclear material may be scavenged by a process termed piecemeal microautophagy of the nucleus (Krick et al. 2009). In older regions of *Neurospora* cells, nuclei appear to dissolve, staining poorly with DNA dyes, but evidence for similar degradation of nuclei in subapical hyphal compartments is lacking in other species (Maheshwari 2005). In *P. anserina*, perithecia (fruiting bodies) can only be produced using nutrients scavenged from hyphae, which are degraded upon nutrient exhaustion in order to produce spores (Bernet 1992). In the case of **appressorium** development in *Magnaporthe oryzae*, degradation of nuclei by a separate macroautophagy pathway in the germ tube is required for pathogenesis (Veneault-Fourrey 2006; He et al. 2012). It is thought that the resources in the germ tube are necessary for establishing infection (Solomon et al. 2003). Interestingly, this is not true for all appressorium-forming plant pathogens, as *Colletotrichum gloeosporioides* germ tubes do not undergo autophagy and remain viable after establishment of infection (Nesher et al. 2008). Why cellular degradation is necessary for cellular processes such as perithecium or appressorium formation in some species and not others remains to be investigated.

IV. Open Questions

Though the behavior of nuclei in single cells and the exchange of genetic material between individuals have fascinated mycologists for a century and considerable advances have been made in our understanding of these complex organisms, there still remains a substantial amount of work to do in understanding the biology of these systems. A few topics of current and future interest are presented here, but this is by no means an exhaustive list.

A. Nucleus-Cytoplasm Communication

Many questions still remain in the field with respect to how nuclei organize the cytoplasm around them. Asynchronous nuclear division despite continuous cytoplasm in some species presents fertile ground for the research of membrane-free **cytoplasmic compartmentalization** as well as nuclear intrinsic behaviors. It is likely that an elaborate combination of cytoplasmic organization, genetic differences, epigenetic modification, nuclear import disparities, and other factors is required to produce functionally different regions of complex fungal cells over a vast range of scales from microns to meters and even kilometers in some cases. Nuclei in different areas of the cell must respond to nutrients, light sources, other fungi, and many more stimuli in their local environment while sharing resources and contributing to the success of an extremely large organism. How various conditions are transmitted to specific nuclei, and how those nuclei produce a coordinated or autonomous response, is a very open question.

In some cases, it has been demonstrated that nucleocytoplasmic transport is dependent upon the same processes responsible for large-scale nuclear mobility. For example, in *U. maydis* NPC positioning in the nuclear envelope is mediated by microtubules (Steinberg et al. 2012). It is possible that this increases nuclear sampling of different environments and limits activity of less functional nuclei. Different species employ variable mechanisms for nuclear-cytoplasmic communication. For example, while many multinucleate fungi undergo a completely closed mitosis, *A. nidulans* partially disassembles NPCs during nuclear division (Osmani et al. 2006). This disassembly surely facilitates communication of **mitotic signals** in the parasynchronous mitotic waves observed in this species. Evidence from *A. gossypii* indicates that heterogeneous localization of proteins containing disordered regions is important for generating nuclear asynchrony (Lee et al. 2013). Similar mechanisms have been proposed in various systems for segregating **age-dependent damage**, and uncontrolled aggregation of proteins is linked to a variety of neurodegenerative diseases. The normal functions of aggregation-prone proteins in mammalian neurons have proven difficult to dissect, though oil-and-water-like phase separations of such proteins have been proposed as an organizing principle of cells (Weber and Brangwynne 2012)

{Li:2013fx} {Banjade:2014cz}. The large hyphal networks of filamentous fungal cells require highly advanced cytoplasmic organization and offer an excellent model system in which to study these processes.

Effective cytoplasmic organization likely depends upon regulation of the **nucleocytoplasmic ratio**. The ratio of nuclear to cytoplasmic volume has been demonstrated to be tightly controlled in the size control model *S. pombe* (Schmidt and Schibler 1995; Jovtchev et al. 2006; Jorgensen et al. 2007; Neumann and Nurse 2007). In multinucleate *A. gossypii* cells, mitosis can be inhibited by microtubule-destabilizing drugs, but actin-dependent polarized growth continues. Under these conditions, the nuclear density decreases, and upon release nuclei will progress through the cell cycle more rapidly until they achieve a wild-type nuclear density (Gladfelter et al. 2006). Achievement of an optimal nucleocytoplasmic ratio is an interesting challenge all cells face, and how this ratio is sensed and can regulate cell cycle progression is an area of active research.

In addition to communication with the cytoplasm, nuclei must coordinate their activity with organelles. The endoplasmic reticulum is likely an important hub for cytoplasmic organization; being contiguous with the nuclear envelope, it is in perfect position to mediate interactions between the nucleus and the cytoplasm. Its well-documented interactions with mitochondria in different cell types also render it a prime candidate for a mediator of nucleocytoplasmic and **intra-organelle communication**. Mitochondrial behavior has been shown in *S. cerevisiae* to regulate nuclear activity (Rodley et al. 2012). These interactions are almost certainly essential for adaptation to different metabolic states. Precisely how these communications are mediated and how these signals are coordinated in large cells with multiple nuclei, complex geometries, as they simultaneously experience different environmental conditions are all topics requiring further investigation.

B. Nucleus-Nucleus Communication

In multinucleate cells, nuclei with deleterious alleles must rely on their neighbors for gene products and expression must be regulated so that the appropriate gene dosage is achieved. Changes in expression in response to different environmental cues and developmental needs must be coordinated across nuclei over very different spatial scales. Within syncytia, the mechanisms by which genes are regulated in a **nuclear autonomous** manner and the decision made to share or sequester specific gene products remain unclear (Fig. 2.3). Nuclei within the same cell cooperate to contribute to the maintenance of the larger organism, but are potential units of selection and therefore potentially are in conflict with each other, not unlike how single cells become cancerous in our own bodies. How cells navigate the delicate balance between benefits of **heterokaryosis** and detriments of **nuclear competition** is a fascinating topic, and further research may help us understand this potential driving force of fungal evolution.

Monitoring of the genetic identity of nuclei has most clearly been observed in **dikaryons** of *Basidiomycota*. Limitation of each cell to only two genotypes requires poorly understood monitoring of the state of each nucleus, though there is evidence from *P. anserina* that mating-type-specific presentation of nuclear envelope proteins helps ensure hyphal compartments contain nuclei of different mating types (Zickler et al. 1995). This may be controlled by gene dosage sensing mechanisms related to the mating-type loci, but many questions remain: How is nuclear migration in dikaryon-monokaryon matings inhibited? How is the more genetically different nucleus of the heterokaryon selected to fertilize the monokaryon? After hyphal fusion in *U. maydis*, the host nucleus may be degraded, but how is the "host cell" nucleus specified, and what keeps the donor cell nucleus safe from degradation machinery?

An obvious benefit to the multinucleate strategy is the ability to share resources and products between different nuclei. Though autonomous mitotic and transcriptional behavior in some fungi indicates differences in local concentrations of protein or susceptibility of nuclei to these signals, it is clear that in some situations gene products can be shared between different parts of the cell and different nuclei. In

A. gossypii, when the mitotic cyclin *CLB1/2* was deleted and reintroduced on a plasmid, all nuclei were able to accumulate the protein even if they did not harbor the plasmid (Gladfelter et al. 2006). Using smFISH it was demonstrated that only a subset of nuclei are transcriptionally active for the G1 cyclin *CLN3* in these cells, but sharing of these gene products (at least at the transcript level) increased synchronous mitosis between neighboring nuclei (Lee et al. 2013). Similar phenomena in other filamentous fungi, especially those that undergo asynchronous mitosis, are likely waiting to be observed. The mechanisms controlling nuclear activity to ensure proper stoichiometry of products within the cell, the decision to limit the range of activity of specific gene products to one or only a subset of nuclei, and the maintenance of nuclear activity "identity" as nuclei continuously move throughout these cells are all open questions that apply to many different fungal species and cells in general.

C. Coupling of Cell Growth, Size Control, and Nuclear Division

Though the microtubule cytoskeleton is essential for proper nuclear transport in all fungal systems examined thus far, it is of variable importance in **polarized growth**. In the yeast *S. cerevisiae* and the closely related filamentous *A. gossypii*, microtubules are dispensable for polarized growth, which is instead completely dependent upon actin-dependent transport (Gladfelter et al. 2006). In both *A. nidulans* and *N. crassa*, however, evidence suggests both actin and microtubules are important for regulating polarized growth (Riquelme et al. 2002; Zhang et al. 2011). In *U. maydis*, microtubules are not involved in the growth of the yeast form or germ tube and initiation of pathogenesis, but later stages of invasive hyphal growth require them (Fuchs et al. 2005). This spectrum of microtubule involvement in polarized growth may reflect the development of systems in hyphal cells to link nuclear dynamics with cell growth, but further investigation into the coupling of these systems is needed.

In uninucleate cells, nuclear division is elegantly coordinated with the cytokinetic machinery during cell cycle progression. In multinucleate cells, however, these processes have been uncoupled and alternate mechanisms exist to specify sites for formation of open septal pores, and subsequent signals can trigger complete separation of hyphal compartments, e.g., during heterokaryon incompatibility or environmental stress. Some organisms even are capable of both coupled and uncoupled nucleokinesis and cytokinesis. During germ tube formation in *M. oryzae*, mitosis is linked to septation, but these processes become uncoupled during appressorium formation and subsequent infection (Saunders et al. 2010).

V. Concluding Remarks

Fungal systems have provided excellent models over the past century for understanding the fundamentals of nuclear behavior in all cells. These studies have demonstrated that nuclei are not passive DNA repositories, but highly dynamic organelles that have sophisticated interactions with growth, reproduction, and a variety of other cellular processes. In multinucleate cells, nuclei maintain a delicate balance between competition and cooperation to create elaborate, sometimes gigantic, cellular structures that allow them to survive and thrive in their various environments. Understanding the mechanisms underlying nuclear movement and genetic dynamics within fungal cells has provided insight into analogous processes in our own cells. Studies of mitosis in *S. cerevisiae* paved the way for similar investigations in mammalian cells, and the delicate balance between nuclear competition and cooperation in multinucleate cells can help us understand challenges in the analogous state of multicellularity. Despite the rich history of research into fungal nuclear dynamics, it is clear that we still have much to learn and there are numerous avenues of investigation that have yet to be thoroughly explored.

References

Aanen DK, de Fine Licht HH, Debets AJM et al (2009) High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites. Science 326:1103–1106. doi:10.1126/science.1173462

Abadeh A, Lew RR (2013) Mass flow and velocity profiles in *Neurospora* hyphae: partial plug flow dominates intra-hyphal transport. Microbiology 159:2386–2394. doi:10.1099/mic.0.071191-0

Anagnostakis SL (1982) Biological control of chestnut blight. Science 215:466–471

Ananthanarayanan V, Schattat M, Vogel SK et al (2013) Dynein motion switches from diffusive to directed upon cortical anchoring. Cell 153:1526–1536. doi:10.1016/j.cell.2013.05.020

Anderson CA, Eser U, Korndorf T et al (2013) Nuclear repulsion enables division autonomy in a single cytoplasm. Curr Biol 23:1999–2010. doi:10.1016/j.cub.2013.07.076

Araujo-Palomares CL, Castro-Longoria E, Riquelme M (2007) Ontogeny of the Spitzenkörper in germlings of *Neurospora crassa*. Fungal Genet Biol 44:492–503. doi:10.1016/j.fgb.2006.10.004

Aylmore RC, Todd NK (1984) Hyphal fusion in *Coriolus versicolor*. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge

Becard G, Pfeffer PE (1993) Status of nuclear division in arbuscular mycorrhizal fungi during in vitro development. Protoplasma 174:62–68

Bensaude M (1918) Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes. (PhD Thesis). Imprimerie Nemourienne, Nemours, France, Advisor: Henri Bouloy

Berger L, Hyatt AD, Speare R, Longcore JE (2005) Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. Dis Aquat Org 68:51–63. doi:10.3354/dao068051

Bernet J (1992) In *Podospora anserina*, protoplasmic incompatibility genes are involved in cell-death control via multiple gene interactions. Heredity 68:79–87

Brangwynne CP, Koenderink GH, MacKintosh FC, Weitz DA (2008) Cytoplasmic diffusion: molecular motors mix it up. J Cell Biol 183:583–587. doi:10.1083/jcb.200806149

Brown AJ, Casselton LA (2001) Mating in mushrooms: increasing the chances but prolonging the affair. Trends Genet 17:393–400

Burgeff H (1924) Untersuchungen über Sexualitat und Parasitismus bei Mucorineen. In: Goebel K (ed) Botanische Abhandlungen. Fischer, Jena, Germany, pp 1–135

Burggraaf JP, Beringer JE (1989) Absence of nuclear DNA synthesis in vesicular-arbuscular mycorrhizal fungi during in vitro development. New Phytol 1:25–33

Buss LW (1982) Somatic cell parasitism and the evolution of somatic tissue compatibility. Proc Natl Acad Sci USA 79:5337–5341

Buss L (1987) The evolution of individuality. Princeton University Press, Princeton, NJ

Büttner P, Koch F, Voigt K et al (1994) Variations in ploidy among isolates of *Botrytis cinerea*: implications for genetic and molecular analyses. Curr Genet 25:445–450

Byers B, Goetsch L (1975) Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. J Bacteriol 124:511–523

Casselton LA, Challen MP (2006) The mating type genes of the basidiomycetes. In: Kües U, Fischer R (eds) The mycota. Springer, Berlin, pp 357–374

Castiglioni Pascon R, Pizzirani-Kleiner AA, Miller BL (2001) The *Aspergillus nidulans bncA1* mutation causes defects in the cell division cycle, nuclear movement and developmental morphogenesis. Mol Gen Genet 264:546–554

Caten CE (1972) Vegetative incompatibility and cytoplasmic infection in fungi. J Gen Microbiol 72:221–229

Clutterbuck AJ (1970) Synchronous nuclear division and septation in *Aspergillus nidulans*. J Gen Microbiol 60:133–135

Cortesi P, Milgroom MG (2001) Outcrossing and diversity of vegetative compatibility types in populations of *Eutypa lata* from grapevines. J Plant Pathol 83:79–86

Czaran T, Hoekstra RF, Aanen DK (2014) Selection against somatic parasitism can maintain allorecognition in fungi. Fungal Genet Biol 73:128–137. doi:10.1016/j.fgb.2014.09.010

D'Souza AD, Sultana S, Maheshwari R (2005) Characterization and prevalence of a circular mitochondrial plasmid in senescence-prone isolates of *Neurospora intermedia*. Curr Genet 47:182–193. doi:10.1007/s00294-004-0558-9

Daga RR, Yonetani A, Chang F (2006) Asymmetric microtubule pushing forces in nuclear centering. Curr Biol 16:1544–1550. doi:10.1016/j.cub.2006.06.026

de Queiroz MV, de Azevedo JL (1998) Characterization of an *Aspergillus nidulans* mutant with abnormal distribution of nuclei in hyphae, metulae, phialides and conidia. FEMS Microbiol Lett 166:49–55

Debets A, Griffiths A (1998) Polymorphism of hetgenes prevents resource plundering in *Neurospora crassa*. Mycol Res 102:1343–1349

Debets F, Yang X, Griffiths AJF (1994) Vegetative incompatibility in *Neurospora*: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. Curr Genet 26:113–119. doi:10.1007/BF00313797

Dietrich FS, Voegeli S, Brachat S et al (2004) The *Ashbya gossypii* genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome. Science 304:304–307. doi:10.1126/science.1095781

Ding R, West RR, Morphew DM et al (1997) The spindle pole body of *Schizosaccharomyces pombe* enters and leaves the nuclear envelope as the cell cycle proceeds. Mol Biol Cell 8:1461–1479

Ellingboe AH, Raper JR (1962) The Buller phenomenon in *Schizophyllum commune*: nuclear selection in fully compatible dikaryotic-homokaryotic matings. Am J Bot 49:454–459

Endow SA, Kang SJ, Satterwhite LL et al (1994) Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends. EMBO J 13:2708–2713

Fantes P, Nurse P (1977) Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. Exp Cell Res 107:377–386

Farkasovsky M, Küntzel H (1995) Yeast Num1p associates with the mother cell cortex during S/G2 phase and affects microtubular functions. J Cell Biol 131:1003–1014

Farkasovsky M, Küntzel H (2001) Cortical Num1p interacts with the dynein intermediate chain pac11p and cytoplasmic microtubules in budding yeast. J Cell Biol 152:251–262. doi:10.1083/jcb.152.2.251

Finley KR, Berman J (2005) Microtubules in *Candida albicans* hyphae drive nuclear dynamics and connect cell cycle progression to morphogenesis. Eukaryot Cell 4:1697–1711. doi:10.1128/EC.4.10.1697-1711.2005

Finley KR, Bouchonville KJ, Quick A, Berman J (2008) Dynein-dependent nuclear dynamics affect morphogenesis in *Candida albicans* by means of the Bub2p spindle checkpoint. J Cell Sci 121:724–724. doi:10.1242/jcs.03498

Fischer R, Timberlake WE (1995) *Aspergillus nidulans* Apsa (Anucleate Primary Sterigmata) encodes a coiled-coil protein required for nuclear positioning and completion of asexual development. J Cell Biol 128:485–498

Fries BC, Casadevall A (1998) Serial isolates of *Cryptococcus neoformans* from patients with AIDS differ in virulence for mice. J Infect Dis 178:1761–1766

Fuchs U, Manns I, Steinberg G (2005) Microtubules are dispensable for the initial pathogenic development but required for long-distance hyphal growth in the corn smut fungus *Ustilago maydis*. Mol Biol Cell 16:2746–2758. doi:10.1091/mbc.E05-03-0176

Gerstein AC, Chun HE, Grant A, Otto SP (2006) Genomic convergence toward diploidy in *Saccharomyces cerevisiae*. PLoS Genet 2:1396–1401. doi:10.1371/journal

Gibeaux R, Knop M (2014) When yeast cells meet, karyogamy! Nucleus 4:182–188. doi:10.4161/nucl.25021

Gibeaux R, Politi AZ, Nedelec F et al (2013) Spindle pole body-anchored Kar3 drives the nucleus along microtubules from another nucleus in preparation for nuclear fusion during yeast karyogamy. Genes Dev 27:335–349. doi:10.1101/gad.206318.112

Gladfelter AS, Hungerbuehler AK, Philippsen P (2006) Asynchronous nuclear division cycles in multinucleated cells. J Cell Biol 172:347–362. doi:10.1083/jcb.2050703

Glass NL, Kaneko I (2003) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 2:1–8. doi:10.1128/EC.2.1.1-8.2003

Glass NL, Kuldau GA (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. Annu Rev Phytopathol 30:201–224. doi:10.1146/annurev.py.30.090192.001221

Goldstein RE, Tuval I, van de Meent J-W (2008) From the cover: microfluidics of cytoplasmic streaming and its implications for intracellular transport. Proc Natl Acad Sci USA 105:3663–3667. doi:10.1073/pnas.0707223105

Grava S, Philippsen P (2010) Dynamics of multiple nuclei in *Ashbya gossypii* hyphae depend on control of cytoplasmic microtubules length by Bik1, Kip2, Kip3, and not on a capture/shrinkage mechanism. Mol Biol Cell 21:3680–3692

Grava S, Keller M, Voegeli S et al (2011) Clustering of nuclei in multinucleated hyphae is prevented by dynein-driven bidirectional nuclear movements and microtubule growth control in *Ashbya gossypii*. Eukaryot Cell 10:902–915. doi:10.1128/EC.05095-11

Grutzmann K, Szafranski K, Pohl M et al (2014) Fungal alternative splicing is associated with multicellular complexity and virulence: a genome-wide multispecies study. DNA Res 21:27–39. doi:10.1093/dnares/dst038

Hammond TM, Rehard DG, Xiao H, Shiu PKT (2012) Molecular dissection of *Neurospora* Spore killer meiotic drive elements. Proc Natl Acad Sci USA 109:12093–12098. doi:10.1073/pnas.1203267109

He M, Kershaw MJ, Soanes DM et al (2012) Infection-associated nuclear degeneration in the rice blast fungus *Magnaporthe oryzae* requires non-selective macro-autophagy. PLoS ONE 7, e33270. doi:10.1371/journal.pone.0033270.s009

Heath IB (1980) Variant mitoses in lower eukaryotes: indicators of the evolution of mitosis. Int Rev Cytol 64:1–80

Heaton LLM, Lopez E, Maini PK et al (2010) Growth-induced mass flows in fungal networks. Proc R Soc B Biol Sci 277:3265–3274. doi:10.1093/jxb/38.5.889

Helfer H, Gladfelter AS (2006) AgSwe1p regulates mitosis in response to morphogenesis and nutrients in multinucleated *Ashbya gossypii* cells. Mol Biol Cell 17:4494–4512. doi:10.1091/mbc.E06

Hijri M, Hosny M, van Tuinen D, Dulieu H (1999) Intraspecific ITS polymorphism in *Scutellospora castanea* (Glomales, Zygomycota) is structured within multinucleate spores. Fungal Genet Biol 26:141–151

Hong CI, Zamborszky J, Baek M et al (2014) Circadian rhythms synchronize mitosis in *Neurospora*

crassa. Proc Natl Acad Sci USA 111:1397–1402. doi:10.1073/pnas.1319399111

Horio T, Oakley BR (2005) The role of microtubules in rapid hyphal tip growth of *Aspergillus nidulans*. Mol Biol Cell 16:918–926. doi:10.1091/mbc.E04-09-0798

Inoue S, Turgeon BG, Yoder OC, Aist JR (1998) Role of fungal dynein in hyphal growth, microtubule organization, spindle pole body motility and nuclear migration. J Cell Sci 111:1555–1566

James TY, Stenlid J, Olson Å, Johannesson H (2008) Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus *Heterobasidion parviporum*. Evolution 62:2279–2296. doi:10.1111/j.1558-5646.2008.00462.x

Jedd G, Chua NH (2000) A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat Cell Biol 2:226–231. doi:10.1038/35008652

Jinks JL (1952) Heterokaryosis - a system of adaptation in wild fungi. Proc R Soc Lond B Biol Sci 140:83–99

Johnston GC, Pringle JR, Hartwell LH (1977) Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. Exp Cell Res 105:79–98

Jorgensen P, Edgington NP, Schneider BL et al (2007) The size of the nucleus increases as yeast cells grow. Mol Biol Cell 18:3523–3532. doi:10.1091/mbc.E06-10-0973

Jovtchev G, Schubert V, Meister A et al (2006) Nuclear DNA content and nuclear and cell volume are positively correlated in angiosperms. Cytogenet Genome Res 114:77–82. doi:10.1159/000091932

Kasuga T, Glass NL (2008) Dissecting colony development of *Neurospora crassa* using mRNA profiling and comparative genomics approaches. Eukaryot Cell 7:1549–1564. doi:10.1128/EC.00195-08

Kathariou S, Spieth PT (1982) Spore killer polymorphism in *Fusarium moniliforme*. Genetics 102:19–24

Kellner M, Burmester A, Wöstemeyer A, Wöstemeyer J (1993) Transfer of genetic information from the mycoparasite *Parasitella parasitica* to its host *Absidia glauca*. Curr Genet 23:334–337

Kemper M, Mohlzahn L, Lickfeld M et al (2011) A Bnr-like formin links actin to the spindle pole body during sporulation in the filamentous fungus *Ashbya gossypii*. Mol Microbiol 80:1276–1295. doi:10.1111/j.1365-2958.2011.07644.x

King SB, Alexander LJ (1969) Nuclear behavior septation and hyphal growth of *Alternaria solani*. Am J Bot 56:249–253

Kohli M, Galati V, Boudier K et al (2008) Growth-speed-correlated localization of exocyst and polarisome components in growth zones of *Ashbya gossypii* hyphal tips. J Cell Sci 121:3878–3889. doi:10.1242/jcs.033852

Krick R, Muhe Y, Prick T et al (2009) Piecemeal microautophagy of the nucleus: Genetic and morphological traits. Autophagy 5:270–272

Lang C, Grava S, van den Hoorn T et al (2010) Mobility, microtubule nucleation and structure of microtubule-organizing centers in multinucleated hyphae of *Ashbya gossypii*. Mol Biol Cell 21:18–28. doi:10.1091/mbc.E09-01-0063

Lee SB, Taylor JW (1993) Uniparental inheritance and replacement of mitochondrial DNA in *Neurospora tetrasperma*. Genetics 134:1063–1075

Lee C, Zhang H, Baker AE et al (2013) Protein aggregation behavior regulates cyclin transcript localization and cell-cycle control. Dev Cell 25:572–584. doi:10.1016/j.devcel.2013.05.007

Letcher PM, Powell MJ (2014) Hypothesized evolutionary trends in zoospore ultrastructural characters in Chytridiales (Chytridiomycota). Mycologia 106:379–396. doi:10.3852/13-219

Letcher PM, Vélez CG, Barrantes ME et al (2008) Ultrastructural and molecular analyses of Rhizophydiales (Chytridiomycota) isolates from North America and Argentina. Mycol Res 112:759–782. doi:10.1016/j.mycres.2008.01.025

Lew RR (2005) Mass flow and pressure-driven hyphal extension in *Neurospora crassa*. Microbiology 151:2685–2692. doi:10.1099/mic.0.27947-0

Maheshwari R (2005) Nuclear behavior in fungal hyphae. FEMS Microbiol Lett 249:7–14. doi:10.1016/j.femsle.2005.06.031

Marcinko-Kuehn MM, Yang XX, Debets FF et al (1994) A kalilo-like linear plasmid in Louisiana field isolates of the pseudohomothallic fungus *Neurospora tetrasperma*. Curr Genet 26:336–343. doi:10.1007/BF00310498

Marek SM, Wu J, Louise Glass N et al (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 40:126–137. doi:10.1016/S1087-1845(03)00086-0

Maruyama J-I, Nakajima H, Kitamoto K (2003) Novel role of cytoplasmic dynein motor in maintenance of the nuclear number in conidia through organized conidiation in *Aspergillus oryzae*. Biochem Biophys Res Commun 307:900–906. doi:10.1016/S0006-291X(03)01267-1

Melloy P, Shen S, White E et al (2007) Nuclear fusion during yeast mating occurs by a three-step pathway. J Cell Biol 179:659–670. doi:10.1083/jcb.200706151

Meluh PB, Rose MD (1990) KAR3, a kinesin-related gene required for yeast nuclear fusion. Cell 60:1029–1041

Miller RK, Rose MD (1998) Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. J Cell Biol 140:377–390

Morris NR, Xiang X, Beckwith SM (1995) Nuclear migration advances in fungi. Trends Cell Biol 5:278–282

Muirhead CA, Glass NL, Slatkin M (2002) Multilocus self-recognition systems in fungi as a cause of trans-species polymorphism. Genetics 161:633–641

Navaraj A (2000) Senescent: a new *Neurospora crassa* nuclear gene mutant derived from nature exhibits mitochondrial abnormalities and a "death" phenotype. Fungal Genet Biol 29:165–173. doi:10.1006/fgbi.2000.1193

Nesher I, Barhoom S, Sharon A (2008) Cell cycle and cell death are not necessary for appressorium formation and plant infection in the fungal plant pathogen *Colletotrichum gloeosporioides*. BMC Biol 6:9. doi:10.1186/1741-7007-6-9

Neumann FR, Nurse P (2007) Nuclear size control in fission yeast. J Cell Biol 179:593–600. doi:10.1083/jcb.200708054

Niederpruem DJ (1980) Direct studies of dikaryotization in *Schizophyllum commune*. I. Live intercellular nuclear migration patterns. Arch Microbiol 128:162–171

Noble M (1937) The morphology and cytology of *Typhula trifolii* Rostr. Ann Bot 1:67–98

Nobre T, Koopmanschap B, Baars JJ et al (2014) The scope for nuclear selection within Termitomyces fungi associated with fungus-growing termites is limited. BMC Evol Biol 14:1–12. doi:10.1186/1471-2148-14-121

Nygaard OF, Güttes S, Rusch HP (1960) Nucleic acid metabolism in a slime mold with synchronous mitosis. Biochim Biophys Acta 38:298–306. doi:10.1016/0006-3002(60)91245-2

Oakley BR, Morris NR (1980) Nuclear movement is beta-tubulin-dependent in *Aspergillus nidulans*. Cell 19:255–262

Osmani AH, Davies J, Liu H-L et al (2006) Systematic deletion and mitotic localization of the nuclear pore complex proteins of *Aspergillus nidulans*. Mol Biol Cell 17:4946–4961. doi:10.1091/mbc.E06-07-0657

Padieu E, Bernet J (1967) Mode of action of the genes responsible for abortion of certain products of meiosis in the ascomycete, *Podospora anserina*. C R Acad Sci Hebd Seances Acad Sci D 264:2300–2303

Paoletti A, Chang F (2000) Analysis of Mid1p, a protein required for placement of the cell division site, reveals a link between the nucleus and the cell surface in fission yeast. Mol Biol Cell 11:2757–2773

Pitchaimani K, Maheshwari R (2003) Extreme nuclear disproportion and constancy of enzyme activity in a heterokaryon of *Neurospora crassa*. J Genet 82:1–6

Plamann M, Minke PF, Tinsley JH, Bruno KS (1994) Cytoplasmic dynein and actin-related protein Arp1 are required for normal nuclear distribution in filamentous fungi. J Cell Biol 127:139–149

Querol A, Bond U (2009) The complex and dynamic genomes of industrial yeasts. FEMS Microbiol Lett 293:1–10. doi:10.1111/j.1574-6968.2008.01480.x

Ramos-Garcia SL, Roberson RW, Freitag M et al (2009) Cytoplasmic bulk flow propels nuclei in mature hyphae of *Neurospora crassa*. Eukaryot Cell 8:1880–1890. doi:10.1128/EC.00062-09

Raper JR (1966) Genetics of sexuality in higher fungi. Ronald Press, New York, NY

Raper CA (1985) B-Mating-type genes influence survival of nuclei separated from heterokaryons of *Schizophyllum*. Exp Mycol 9:149–160

Requena N, Alberti-Segui C, Winzenburg E et al (2001) Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. Mol Microbiol 42:121–132

Richard F, Glass NL, Pringle A (2012) Cooperation among germinating spores facilitates the growth of the fungus, *Neurospora crassa*. Biol Lett 8:419–422. doi:10.1098/rsbl.2011.1141

Riquelme M, Roberson RW, McDaniel DP, Bartnicki-Garcia S (2002) The effects of ropy-1 mutation on cytoplasmic organization and intracellular motility in mature hyphae of *Neurospora crassa*. Fungal Genet Biol 37:171–179

Roca MG, Kuo H-C, Lichius A et al (2010) Nuclear dynamics, mitosis, and the cytoskeleton during the early stages of colony initiation in *Neurospora crassa*. Eukaryot Cell 9:1171–1183. doi:10.1128/EC.00329-09

Rodley CDM, Grand RS, Gehlen LR et al (2012) Mitochondrial-nuclear dna interactions contribute to the regulation of nuclear transcript levels as part of the inter-organelle communication system. PLoS ONE 7, e30943. doi:10.1371/journal.pone.0030943.s023

Roper M, Simonin A, Hickey PC et al (2013) Nuclear dynamics in a fungal chimera. Proc Natl Acad Sci USA 110:12875–12880. doi:10.1073/pnas.1220842110

Rose MD, Fink GR (1987) KAR1, a gene required for function of both intranuclear and extranuclear microtubules in yeast. Cell 48:1047–1060

Ross IK (1976) Nuclear migration rates in *Coprinus congregatus*: a new record? Mycologia 68:418–422

Ruiz-Roldan MC, Kohli M, Roncero MIG et al (2010) Nuclear dynamics during germination, conidiation, and hyphal fusion of *Fusarium oxysporum*. Eukaryot Cell 9:1216–1224. doi:10.1128/EC.00040-10

Ruiz-Trillo I, Burger G, Holland PWH et al (2007) The origins of multicellularity: a multi-taxon genome initiative. Trends Genet 23:113–118. doi:10.1016/j.tig.2007.01.005

Ryan FJ, Beadle GW, Tatum EL (1943) The tube method of measuring the growth rate of *Neurospora*. Am J Bot 30:784–799

Samils N, Oliva J, Johannesson H (2014) Nuclear interactions in a heterokaryon: insight from the model *Neurospora tetrasperma*. Proc R Soc B Biol Sci 281:20140084–20140084. doi:10.1371/journal.pgen.1002820

Saunders DG, Dagdas YF, Talbot NJ (2010) Spatial uncoupling of mitosis and cytokinesis during appressorium-mediated plant infection by the

rice blast fungus *Magnaporthe oryzae*. Plant Cell 22:2417–2428. doi:10.1105/tpc.110.074492

Schmidt EE, Schibler U (1995) Cell size regulation, a mechanism that controls cellular RNA accumulation: consequences on regulation of the ubiquitous transcription factors Oct1 and NF-Y and the liver-enriched transcription factor DBP. J Cell Biol 128:467–483

Schultze K, Schimek C, Wöstemeyer J, Burmester A (2005) Sexuality and parasitism share common regulatory pathways in the fungus *Parasitella parasitica*. Gene 348:33–44. doi:10.1016/j.gene.2005.01.007

Schuurs TA, Dalstra HJ, Scheer JM, Wessels JG (1998) Positioning of nuclei in the secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. Fungal Genet Biol 23:150–161

Selmecki AM, Dulmage K, Cowen LE et al (2009) Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. PLoS Genet 5, e1000705. doi:10.1371/journal.pgen.1000705.s009

Shatkin AJ, Tatum EL (1959) Electron microscopy of *Neurospora crassa* mycelia. J Biophys Biochem Cytol 6:423–426

Shin JH, Chae MJ, Song JW et al (2007) Changes in karyotype and azole susceptibility of sequential bloodstream isolates from patients with *Candida glabrata* candidemia. J Clin Microbiol 45:2385–2391. doi:10.1128/JCM.00381-07

Sidhu GS (1983) Genetics of *Gibberella fujikuroi*.3. Significance of heterokaryosis in naturally-occurring corn isolates. Can J Bot-Rev Can Bot 61:3320–3325

Solomon PS, Tan K, Oliver RP (2003) The nutrient supply of pathogenic fungi; a fertile field for study. Mol Plant Pathol 4:203–210

Sparrow FK (1935) Recent contributions to our knowledge of the aquatic phycomycetes. Biol Rev 10:152–186

Steinberg G, Schuster M, Theisen U et al (2012) Motor-driven motility of fungal nuclear pores organizes chromosomes and fosters nucleocytoplasmic transport. J Cell Biol 198:343–355. doi:10.1083/jcb.201201087

Straube A, Weber I, Steinberg G (2005) A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. EMBO J 24:1674–1685. doi:10.1038/sj.emboj.7600644

Suelmann R, Sievers N, Fischer R (1997) Nuclear traffic in fungal hyphae: in vivo study of nuclear migration and positioning in *Aspergillus nidulans*. Mol Microbiol 25:757–769

Swiezynski KM, Day PR (1960) Migration of nuclei in *Coprinus lagopus*. Genet Res 1:129–139

ten Hoopen R, Cepeda-Garcia C, Fernandez-Arruti R et al (2012) Mechanism for astral microtubule capture by cortical bud6p priming spindle polarity in *S. cerevisiae*. Curr Biol 22:1075–1083. doi:10.1016/j.cub.2012.04.059

Tenney K, Hunt I, Sweigard J et al (2000) hex-1, a gene unique to filamentous fungi, encodes the major protein of the Woronin body and functions as a plug for septal pores. Fungal Genet Biol 31:205–217. doi:10.1006/fgbi.2000.1230

Tinsley JH, Minke PF, Bruno KS, Plamann M (1996) p150 (Glued), the largest subunit of the dynactin complex, is nonessential in *Neurospora* but required for nuclear distribution. Mol Biol Cell 7:731–742

Todd NK, Caylmore RC (1985) Cytology of hyphal interactions and reactions in *Schizophyllum commune*. In: Developmental biology of higher fungi

Tran PT, Marsh L, Doye V et al (2001) A mechanism for nuclear positioning in fission yeast based on microtubule pushing. J Cell Biol 153:397–411

Turner BC, Perkins DD (1991) Meiotic drive in *Neurospora* and other fungi. Am Nat 137:416–429

Unger MW, Hartwell LH (1976) Control of cell division in *Saccharomyces cerevisiae* by methionyl-tRNA. Proc Natl Acad Sci USA 73:1664–1668

Veneault-Fourrey C (2006) Autophagic fungal cell death is necessary for infection by the rice blast fungus. Science 312:580–583. doi:10.1126/science.1124550

Vogel SK, Pavin N, Maghelli N et al (2009) Self-organization of dynein motors generates meiotic nuclear oscillations. PLoS Biol 7, e87. doi:10.1371/journal.pbio.1000087.sv007

Weber SC, Brangwynne CP (2012) Getting RNA and protein in phase. Cell 149:1188–1191. doi:10.1016/j.cell.2012.05.022

Wendland J, Walther A (2005) *Ashbya gossypii*: a model for fungal developmental biology. Nat Rev Microbiol 3:421–429. doi:10.1038/nrmicro1148

Wu Q, Sandrock TM, Turgeon BG et al (1998) A fungal kinesin required for organelle motility, hyphal growth, and morphogenesis. Mol Biol Cell 9:89–101

Zamborszky J, Csikász-Nagy A, Hong CI (2014) *Neurospora crassa* as a model organism to explore the interconnected network of the cell cycle and the circadian clock. Fungal Genet Biol 71:52–57. doi:10.1016/j.fgb.2014.08.014

Zhang J, Li S, Fischer R, Xiang X (2003) Accumulation of cytoplasmic dynein and dynactin at microtubule plus ends in *Aspergillus nidulans* is kinesin dependent. Mol Biol Cell 14:1479–1488. doi:10.1091/mbc.E02-08-0516

Zhang J, Tan K, Wu X et al (2011) *Aspergillus* myosin-V supports polarized growth in the absence of microtubule-based transport. PLoS ONE 6, e28575. doi:10.1371/journal.pone.0028575

Zickler D, Arnaise S, Coppin E et al (1995) Altered mating-type identity in the fungus *Podospora anserina* leads to selfish nuclei, uniparental progeny, and haploid meiosis. Genetics 140:493–502

3 Hyphal Tip Growth in Filamentous Fungi

M. Riquelme[1], R.W. Roberson[2], E. Sánchez-León[1]

CONTENTS

I. Introduction

All cells, including fungal hyphae, grow through a balanced and regulated set of complex biochemical and biophysical interactions. The components of the hyphal cytoplasm are similar to those of other heterotrophic eukaryotic organisms; however, because of the polarized mode of growth and diverse ecological interactions of filamentous fungi, hyphae also contain organelles and inclusions that are unique to the Mycota. Though superficially discrete and independent, the cytoplasmic components are in fact interconnected directly or indirectly to maintain the highly ordered and polarized organization of the hypha. Fungal cellular and molecular biologists often concentrate their attention toward narrow questions due to the complexity of the total systems being investigated. The growing number of fungal genomes sequenced, the availability of mutants, and the progress made in live imaging techniques and biochemical analysis have greatly contributed to gain a vast amount of knowledge regarding components and mechanisms involved in hyphal polarity and morphogenesis, cytoskeleton function, vesicle trafficking, and nuclear division. Yet there is not a unified understanding on how these elements act in a cooperative manner to build a hypha. Toward this goal, the ability to resolve, analyze, and understand three-dimensional cytoplasmic order and dynamics over time would provide powerful insights into the fundamental operations of hyphal biology. Equipped with this knowledge, and interfacing it with the strength of "omics" approaches, the fungal scientific community is poised to make significant advancements toward a more complete understanding of the mechanisms that determine hyphal tip growth and morphogenesis.

[1]Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ctra. Ensenada-Tijuana No. 3918, Ensenada, Baja California 22860, Mexico; e-mail: riquelmemeritxell@gmail.com
[2]School of Life Sciences, Arizona State University, Tempe, AZ, USA

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

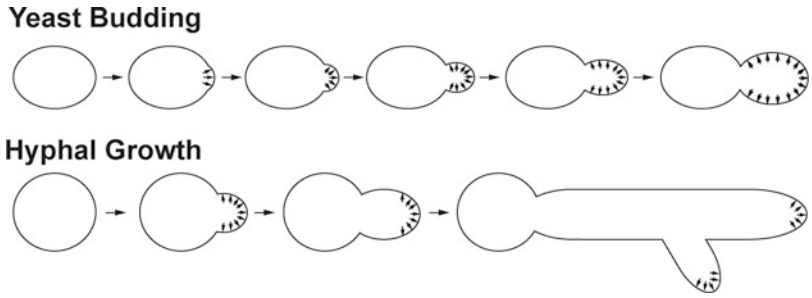


Fig. 3.1 Apical polar growth in budding yeast and filamentous fungi. In yeast, apical growth in the mother cell establishes the initial expansion of the daughter cell, which grows isotropically soon after. In filamentous fungi, apical growth starts by emergence of a germ tube from a spore and is sustained at the apex of an extending hypha and its branches

Table 3.1 Growth rate values reported for some of the species most extensively analyzed in fungal cell biology

Species	Growth rate μm min^{-1} ($mm\ hr^{-1}$)	Temperature °C	Notes	References
Neurospora crassa (mature hyphae)	38.3 (2.3)	28	Cells grown on agar plates	Riquelme et al. (2007)
Ashbya gossypii (mature hyphae)	2.1 (0.12)	30	Cells grown on complete agar plates	Ayad-Durieux et al. (2000)
Candida albicans	0.31 (0.02)	37	Cells grown on serum agar plates	Gow and Gooday (1982)
Aspergillus nidulans (mature hyphae)	5 (0.3)	37	Cells grown on agar plates	Trinci (1969)
Magnaporthe oryzae	1.2 (0.07)	25	Cells grown on agar plates	Kong et al. (2012)
Ustilago maydis	1.5 (0.09)	31	Cells grown on charcoal-agar plates	Steinberg et al. (1998)

II. Hyphae and Other Forms of Tip Growth

The formation of hyphae is one of the characteristic features of filamentous fungi. Hyphae are tubelike cells that elongate and branch through cytoplasmic mechanisms that sustain polarized transport of secretory vesicles to the apex, where they fuse with the plasma membrane and provide the necessary enzymes for cell wall synthesis and the material for plasma membrane expansion. This process of cell growth in filamentous fungi differs significantly from that studied in the unicellular budding yeasts (Fig. 3.1). In *Saccharomyces cerevisiae*, cell growth is polarized during budding of the mother cell but switches to isotropic growth during enlargement of the daughter cell. In contrast, in filamentous fungi once a spore germinates there is a sustained polarized expansion of the cell at the growing apices (Bartnicki-García and Lippman 1969; Momany 2005; Harris 2006; Heath 1994). This process of tip growth occurs at distinct rates for different fungal species (Table 3.1). Information on the triggers that induce symmetry breakage in *S. cerevisiae* during budding or spore germination in filamentous fungi can be found elsewhere (Arkowitz and Bassilana 2011). Hyphal tip growth provides the means for fungi to colonize a substrate, secrete hydrolytic enzymes, assimilate nutrients, and regulate morphogenesis. With the formation of many lateral branches that in turn give rise to additional branches, a complex interconnected network of hyphae, the mycelium, is formed. This represents the body of the fungus, which in some soil-inhabiting

fungi can span many acres (Smith et al. 1992). In addition, fungi can produce many other differentiated cellular forms, which involve reorientation or modification of polar tip growth; these forms include gametes and gametangia during sexual reproduction, conidiophores and sporangiophores during asexual reproduction, paraphyses in ascomycete fruiting bodies, clamp connections in the dikaryotic hyphae of basidiomycetes, cystidia and setae in basidiomycete fruiting bodies, and infectious structures such as appressoria and penetrating pegs. The biological processes leading to the formation of these specialized structures are poorly understood.

A great deal of our knowledge has come from bio-imaging studies of hyphal structure, growth, and behavior (e.g., Girbardt 1957, 1969; Grove and Bracker 1970; Hoch and Howard 1980; Howard 1981; Freitag et al. 2004; McDaniel and Roberson 1998; Suelmann et al. 1997). While a growing number of studies have identified molecular markers at hyphal apices (polarisome components, cell end markers, etc.), little is known about the complex interacting processes that regulate hyphal tip growth and morphogenesis. Very likely, all cell types that exhibit polarized tip growth, whether they are hyphae, neurons, root hairs, or pollen tubes, contain comparable signaling pathways and carry out parallel molecular interactions to sustain this mode of growth (Borkovich et al. 2004; Harris 2006; Palanivelu and Preuss 2000). It is the unique and sometimes subtle differences in the regulatory mechanisms of their interactomes that remain poorly understood.

III. Cell Wall: Growth and Remodeling

The discovery that fungal hyphae extend at the tip occurred more than a century ago (Reinhardt 1892). In the 1950s and the 1960s, a link was made between hyphal apical extension and localized cell wall synthesis (Bartnicki-García and Lippman 1969; Robertson 1965). Fungal cell walls contain primarily polysaccharides and glycoproteins (Aronson 1965; Bartnicki-García 1968; Latgé and Calderone 2006). The percentage of each cell wall component varies among fungal species (reviewed by de Groot et al. 2016; Bartnicki-García 1968). Although not extensively analyzed, the composition of the lateral cell wall is different than that of the septal cell wall (Hunsley and Gooday 1974). This suggests a differential localization or regulation of activity of the cell wall-building enzymes. Microfibrils of chitin and β-1,3-β-1,6-glucans (alkali-insoluble fraction) are embedded in an amorphous gel-like matrix constituted of alkali-soluble polysaccharides [α-1,3-glucans] and glycoproteins (mainly galactomannoproteins) (Hunsley and Burnett 1970). The enzymes responsible for the synthesis of the polysaccharides contain several transmembrane domains and are presumably inserted into the plasma membrane at sites of new cell wall assembly (Fig. 3.2). The transport of the polysaccharide synthesizing enzymes in an inactive form until they get inserted into the plasma membrane by exocytosis of the corresponding vesicular carriers ensures the in situ synthesis of chitin and β-1,3-glucan at the cell surface. The glycoproteins are synthesized in the cytosol via the secretory endoplasmic reticulum (ER)-to-Golgi pathway, where they undergo posttranslational modifications such as N- and O-glycosylations or glypiation (addition of a glycosyl-phosphoinositol (GPI) anchor) and are then transported to sites of cell wall growth. To understand hyphal morphogenesis, it is therefore of crucial importance to understand the basis of the cellular machinery involved in the process of cell wall synthesis.

Chitin is synthesized by chitin synthase (CHS). The enzyme is likely positioned at the plasma membrane receiving its substrate, UDP-*N*-acetylglucosamine (UDP-GlcNAc), from the cytoplasmic side of the membrane and assembling linear chains of β-1,4-GlcNAc toward the external side (Roncero 2002; Sietsma and Wessels 2006; Latgé and Calderone 2006). All biochemical evidence for CHS in fungi has been gathered from partially purified cell-free extracts under the assumption that there is one catalytic protein (Glaser and Brown 1957; McMurrough et al. 1971). However, genomic studies have identified a surprising multiplicity

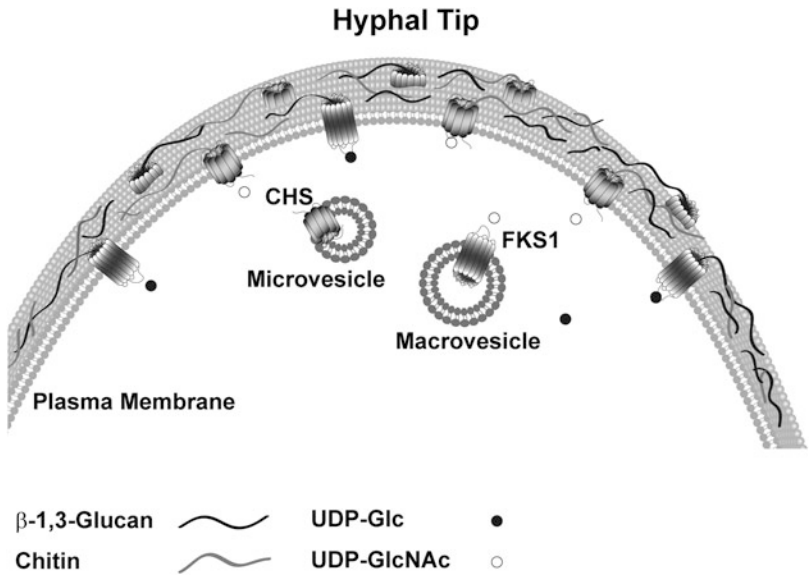


Fig. 3.2 Representation of a hyphal dome and the main components participating in cell wall assembly. Membrane-bound chitin synthases (CHS) and β-1,3-glucan synthase (FKS1) are transported within microvesicles and macrovesicles, respectively, to the hyphal apex. After vesicles fuse with the apical plasma membrane, inserted polysaccharide synthases catalyze the transfer of substrate monomers from the cytosol to the growing chains of chitin and β-1,3-glucan of the cell wall

of sequences with putative CHS activity. Up to seven different classes of CHS have been recognized (Bulawa 1993; Choquer et al. 2004; Riquelme and Bartnicki-García 2008). Filamentous fungi have more *chs* genes than yeasts, which might correlate with the higher content of chitin in their cell wall. However, the role of each class of CHS in growth and development does not seem to be conserved among different fungal species studied (Fajardo-Somera et al. 2015; Riquelme and Bartnicki-García 2008). A phylogenetic study taking into account 54 fungal genomes has indicated conserved patterns of distribution of *chs* genes within the genomes and has identified as well putative cell wall metabolism clusters (Pacheco-Arjona and Ramírez-Prado 2014). The fate and activity of CHS is highly regulated (Choi et al. 1994a). Some of the CHS are zymogenic and are presumably activated when they reach the plasma membrane (Bracker et al. 1976; Choi et al. 1994b). For example, in *S. cerevisiae* Chs3 requires to interact with chaperone Chs7 at the ER and the exomer complex at Golgi for proper transport to the plasma membrane, where it is activated by chitin synthase activator Chs4 (Trautwein et al. 2006; Sanchatjate and Schekman 2006). In *Candida albicans* Chs3 function and localization at the growing tips is regulated by phosphorylation (Lenardon et al. 2010). In *Mucor rouxii*, the isolation of 16 S particles with zymogenic CHS demonstrated the existence of this enzyme as a complex of ~500 kDa (Lending et al. 1991).

β-1,3-glucans are synthesized by a glucan synthase complex (GSC) that contains at least a catalytic subunit (Fks) and a regulatory subunit (Rho1 GTPase). Filamentous fungi have only one *fks* gene, in contrast to yeast, where as many as four *fks* genes have been identified (Latgé and Calderone 2006; Lesage et al. 2004). Fks has several transmembrane domains. It presumably accepts UDP-glucose from the cytoplasmic site and assembles the β-1,3-glucans outside the plasma membrane. Rho1 is synthesized in the ER and undergoes geranylgeranylation, which enables its insertion into the plasma membrane (Inoue et al. 1999). In *S. cerevisiae*, Rho1 and Fks1 are transported to the plasma membrane in an inactive form, until

reaching the plasma membrane, where Rom2 (Rho1 GDP-GTP exchange factor or GEF) activates Rho1, which in turn activates Fks1 (Abe et al. 2003).

As mentioned above, α-1,3-glucans constitute the amorphous matrix of the fungal cell wall and are synthesized by α-1,3-glucan synthases, which are also integral membrane proteins that appear to contain multiple functional domains (Hochstenbach et al. 1998). However, little is known about the synthesis of this polysaccharide.

The unitary model for fungal growth proposed the simultaneous action of cell wall-loosening enzymes and cell wall-synthesizing enzymes at growing apices (Bartnicki-García 1973). A few years later, the steady-state model for apical wall growth favored the idea of an expanding plastic wall that becomes rigid in the subapex by the action of cross-linking enzymes (Wessels 1986); this was based on the fact that *Schizophyllum commune* hyphae did not present chitin microfibrils at the very apex, only in the subapical regions. Until present, there are very few reports of localization of cell wall remodeling enzymes in filamentous fungi. Although scarce, the current evidence reinforces the notion that cell wall remodeling might occur at hyphal tips. In *Aspergillus nidulans*, the endochitinase ChiA tagged with GFP localized at the apex of germ tubes, at hyphal branching sites, and at hyphal tips (Yamazaki et al. 2008). In *S. cerevisiae* endo β-1,3-glucanase Eng1 is asymmetrically located at one side of the septum of the daughter cell during cell separation (Baladrón et al. 2002). In *Neurospora crassa* the predicted endo β-1,3-glucanases BGT-1 and BGT-2 have also putative glycosyl transferase activity and were localized not only at hyphal septa and interconidial septa but also at hyphal apical plasma membranes (Martínez-Núñez and Riquelme 2015). Cross-linking between β-1,3-glucans and chitin was shown to be essential for the formation of a resistant cell wall skeletal component in *Aspergillus fumigatus* (Fontaine et al. 2000). Nonetheless, localization of cross-linking enzymes has not yet been attained in filamentous fungi. In *S. cerevisiae*, the cross-linking enzymes Gas1, Crh1, and Crh2 have been found at the cell surface at sites of budding, bud neck, and bud scars (Rolli et al. 2009; Rodríguez-Peña et al. 2000). A reconciliation of the unitary and steady-state models would imply that cell wall-loosening enzymes, such as non-secreted chitinases and glucanases, participate in the breakage of preexisting or newly formed polysaccharide chains, allowing the addition of newly arrived material and generating free ends, which would serve as substrate for the cross-linking enzymes that rigidify the cell wall (Bartnicki-García 1999).

Combined biochemical and phylogenomic analyses have recently allowed to characterize the composition of the cell wall and identify putative genes involved in cell wall metabolic pathways in *Rhizopus oryzae* and *Phycomyces blakesleanus*, two fungi belonging to the Mucoromycotina (Mélida et al. 2014).

IV. The Hyphal Tip

A. Spitzenkörper or Apical Vesicle Crescent

1. Organization, Ultrastructure, Composition

The movement of secretory vesicles from their sites of origin to the growing hyphal tip takes place in a cytoplasm that maintains a high level of polarity. Cytoplasmic polarity is not unique to the fungal hyphae; indeed, it is, to one degree or another, a fundamental characteristic of most cells. A clear example of cytoplasmic polarity in growing hyphae is the maintenance of a cluster of secretory vesicles that accumulate just beneath the apical plasma membrane prior to exocytosis. In filamentous Basidiomycota and Ascomycota, this aggregation of vesicles and associated proteins is known as the Spitzenkörper (Spk) (reviewed by Riquelme and Sánchez-León 2014). The Spk when imaged with phase-contrast light microscopy (LM) appears as a phase-dark body at the apex of actively growing hyphae (Girbardt 1969; Grove and Bracker 1970; López-Franco and Bracker 1996) (Fig. 3.3). It was first identified in fixed hyphae stained with iron hematoxylin, a common acidic histological dye, in two species of *Coprinus* (Brunswik 1924), and later revealed in

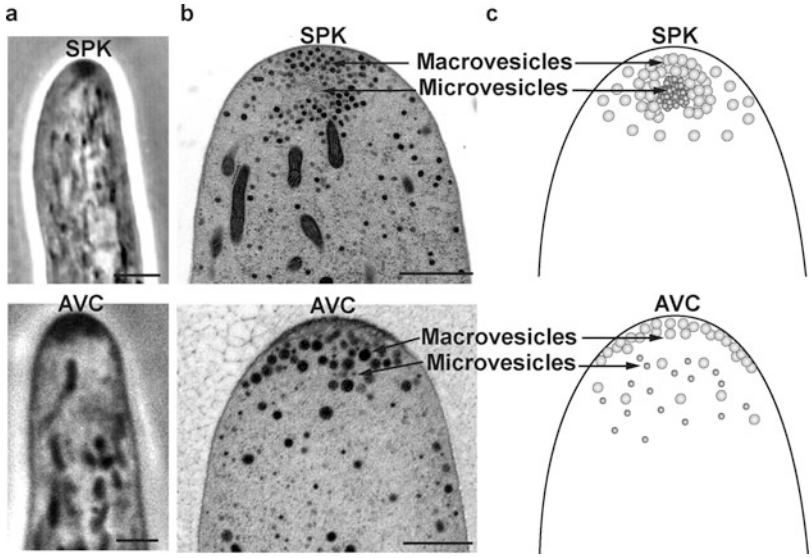


Fig. 3.3 Hyphal dome organization in *Neurospora crassa* (*top panels*) and *Mucor indicus* (*bottom panels*). (**a**) Phase-contrast microscopy; scale bar, *top*: 3 μm; *bottom*: 1 μm; (**b**) transmission electron microscopy; scale bars, 1 μm; (**c**) apical vesicular organization models of fungal hyphae: the Spitzenkörper (Spk) and the apical vesicle crescent (AVC)

living cells through staining with the vital fluorescent stain FM4-64 (Fischer-Parton et al. 2000). The Spk is an ephemeral structure with its presence tightly correlated with hyphal tip growth (Girbardt 1957).

By transmission electron microscopy (TEM), the dark-phase Spk observed with LM corresponds to a dense accumulation of secretory vesicles or macrovesicles (70–100 nm in diameter), a core enriched with microvesicles (25–40 nm in diameter), and a network of actin microfilaments (MFs) and amorphous or granular material of undefined nature (Howard 1981; Grove and Bracker 1970; Girbardt 1969; Mcclure et al. 1968; Bourett and Howard 1991). Subtending the Spk of ascomycetous fungi, there is a cluster of cytosolic ribosomes (i.e., not associated with rough ER) (Howard 1981; Riquelme et al. 2002), likely involved in the localized synthesis of proteins with a Spk function. In *N. crassa* the microvesicles at the core of the Spk have been shown to carry CHS and correspond to the previously described chitosomes (Fajardo-Somera et al. 2015; Riquelme et al. 2007; Verdín et al. 2009; Sánchez-León et al. 2011), while the macrovesicles of the outer layer carry components of the glucan synthase complex (Verdín et al. 2009). The Spk is a macromolecular structure that is not bound by a membrane and shows variability in size, shape, position, and behavior between hyphae of different fungal species and also between different hyphae of the same fungal species in response to endogenous or environmental signals and perturbations (Roberson et al. 1989; López-Franco and Bracker 1996; Bracker et al. 1997).

A typical Spk is not present in hyphae of most zygomycetous fungi or of other early fungal lineages. Indeed, in most of these fungi, secretory vesicles aggregate as a crescent just beneath the apical plasma membrane of growing hyphae and have a less complex organization than the conventional Spk (Fig. 3.3). For example, in *Gilbertella persicaria* (Mucoromycotina; Grove and Bracker 1970), *Phycomyces blakesleeanus* (Mucoromycotina; Girbardt 1969), *Rhizopus oryzae*, *Coemansia reversa*, *Mucor indicus* (Mucoromycotina; Roberson unpublished; Fig. 3.3), and three different species of *Gigaspora* (Glomeromycota; Bentivenga et al. 2013), the accumulation of vesicles near the hyphal apex appears by LM as a thin crescent-shaped band, referred to as an apical

vesicle crescent (AVC). It seems reasonable to assume that the AVC, like the Spk, is involved in polarized cell growth, but the degree to which it is correlated to growth direction is unclear. Thus far, Spk within the non-Dikarya fungi have been confirmed in only two genera: *Basidiobolus* (Entomophthoromycotina; Roberson et al. 2011) and *Allomyces* (Blastocladiomycota; Vargas et al. 1993). The Spk of *Basidiobolus* is similar in structure and apparent function to those that are present in the Ascomycota and Basidiomycota (Roberson et al. 2011). The *Allomyces* Spk is structurally distinct, with a large phase-light core. The ultrastructural equivalent of the phase-light area contains a granular to fibrous matrix with an irregular boundary in which microvesicles are embedded within a cytoskeletal matrix (Roberson and Vargas 1994). Though not common outside the Mycota, a Spk has been identified in rhizoids of the alga *Chara globularis* (Braun 2001). In *S. cerevisiae* while no Spk had been recognized during budding, a Spk-like structure that corresponded to an accumulation of vesicles was reported at tips of mating projections (Chapa-Y-Lazo et al. 2011; Baba et al. 1989).

The cryo-methods for preserving cells for ultrastructural investigations significantly improved the preservation of subcellular structures compared to traditional preparation protocols (Howard and Aist 1980), and as its usage became more common, the knowledge of fungal cellular structure increased (e.g., Howard 1981; Roberson and Fuller 1988; Vargas et al. 1993). High-resolution and three-dimensional analysis of hyphal organization and organelles has now been revealed using electron tomographic methods (Hohmann-Marriott et al. 2006; Müller et al. 2000; Nicastro et al. 2000). Live-cell imaging using LM methods coupled with advances such as high-resolution cameras, computer technology, and fluorescently conjugated molecular probes has also contributed greatly to the understanding of hyphal subcellular behavior and organization and strongly supported the ultrastructural data (Freitag et al. 2004; Mouriño-Pérez et al. 2006; Uchida et al. 2008; Suelmann et al. 1997; Spellig et al. 1996; Hickey et al. 2004; Marschall and Tudzynski 2014). Our challenge in the future, as it has been in the past, is the correlation of structures described at the LM level with those at the EM level (i.e., correlative light and electron microscopy (CLEM)). For example, combining fluorescent protein (FP) live-cell imaging with immuno-EM using anti-FP antibodies or photooxidation technology holds great promise (Asakawa et al. 2014; Mari et al. 2014).

2. Diversity Across Fungal Taxa

Using phase-contrast LM to analyze 32 species of filamentous fungi within the Ascomycota and Basidiomycota, nine distinct Spk morphological patterns were identified (López-Franco and Bracker 1996; Fig. 3.4). It is unclear, however, how or if the diversity of Spk organization influences Spk function.

The first pattern was observed as a spheroidal phase-dark vesicle cluster lacking a visible phase-bright core. This pattern was noted primarily in hyphae of Basidiomycota fungi. Pattern two contained a dark spheroidal vesicle cluster with a phase-bright narrow core appearing a cleft or slit that opened at the posterior surface of the Spk. A similar pattern has been reported in *Ashbya gossypii* (Ascomycota; Köhli et al. 2008) and *Basidiobolus* (Entomophthoromycotina; Roberson et al. 2011). Pattern two was also characteristic of *Athelia* (formerly *Sclerotium*) *rolfsii*, in which a Spk had been previously described with differential interference contrast LM optics (Roberson et al. 1989). The third Spk morphological pattern had a dense vesicle cluster with a spherical phase-bright core positioned near the anterior region of the Spk. Pattern three was characteristic of members of the Ascomycota such as *Bipolaris*, *Nectria*, *Trichoderma*, and *Magnaporthe*. Pattern four was illustrated as having a less phase-dark vesicle cluster that surrounded a spherical phase-dark core that was positioned in the center or toward the posterior side of the surrounding vesicle cluster. This was a common pattern observed in *Aspergillus* and *Sclerotinia*, both members of the Ascomycota. Pattern five was described as composed of a gray vesicle cloud with light core positioned near the apical pole and a dark core near the back of the vesicle cloud. This morphological pattern was characteristic of *Morchella* and *Galactomyces* (Ascomycota). The sixth Spk pattern was seen as a phase-dark vesicle aggregation reminiscent of a cup partially surrounding a prominent phase-light core positioned near the back of the Spk. This pattern was noted in the basidiomycete *Rhizoctonia*. The organization of pattern seven is described as a less dense cloud

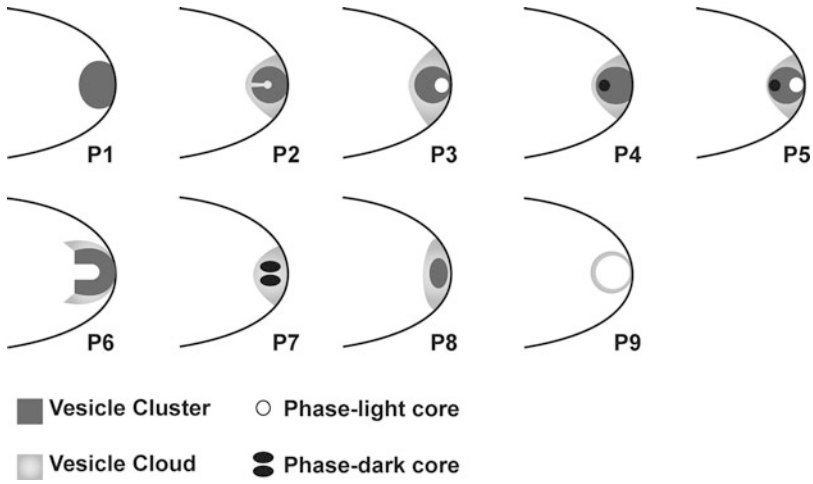


Fig. 3.4 Illustration showing the diversity of morphological Spk patterns (P) displayed by different fungal species; based on observations by phase-contrast light microscopy (based on López-Franco and Bracker 1996). P1: *Dark spheroid* spot representing a vesicle cluster without a visible core (*Agaricus bisporus*, *A. brunnescens*, *Gaeumannomyces graminis*, *Trametes versicolor*); P2: An *open dark spheroid* vesicle cluster, with a crevice-like light core localized toward the rear of the cluster (*Sclerotium rolfsii*); P3: *Dark vesicle cluster* with an eccentric light core oriented toward the apex (*Alternaria solani*, *Bipolaris maydis*, *B. zeicola*, *Exserohilum turcicum*, *Fusarium culmorum*, *F. oxysporum* f. sp. *lycopersici*, *Gibberella fujikuroi*, *Magnaporthe grisea*, and *Trichoderma viride*); P4: *Gray cluster of vesicles* with an eccentric dark core located near the back of the cluster (*Aspergillus niger* and *Sclerotinia sclerotiorum*); P5: *Gray vesicle* cloud containing a light and a dark core, oriented toward the apex and the back of the vesicle cluster, respectively (*Galactomyces citri-aurantii* and *Morchella deliciosa*); P6: Polarized cup-shaped dark vesicle cluster partially enclosing a large phase-light core (*Gelasinospora* spp., *Rhizoctonia solani*, and *R. cereaiis*); P7: *Gray cloud of vesicles* containing two or more dark apical "granules" (*Colletotrichum graminicola*, *C. magna*, and *Leptosphaerulina briosana*); P8: Highly pleomorphic Spk, often appearing as a *thick dark band* within a vesicle cloud, with a light core behind the dark cluster (*Neurospora crassa* and *Sordaria fimicola*); P9: *Large phase-light* core encompassed by a thin layer of apical vesicles (*Allomyces macrogynus*)

of apical vesicles containing multiple phase-dark bodies, which appear crystalline in nature when viewed with transmission electron microscopy (TEM). No phase-bright core region was identified. This pattern is seen in the ascomycete fungi *Colletotrichum* and *Leptosphaerulina*. The eighth pattern was described as highly pleomorphic and difficult to characterize. In general it was seen as a dark vesicle cluster surrounded by a less dense cloud of vesicles. A phase-bright core was present behind the dark vesicle cluster. This pattern was observed in hyphae of *Neurospora* and *Sordaria* (Ascomycota). The final Spk pattern described is unique to the *Allomyces* (Blastocladiomycota), and it is characterized by having a large phase-bright core, surrounded by a small region of vesicles (Vargas et al. 1993).

3. Spk Behavior and Direction of Growth: Tip Orienteering

Hyphae have the ability to respond to environmental cues and reorient tip growth to penetrate diverse host tissues or to form mating structures. It has been suggested that although the response to environmental cues (galvanotropic, thigmotropic, etc.) may be fungus-specific, tip reorientation of the hyphal tip may be achieved by modulation of the conserved machinery that sustains polarized hyphal tip growth (Brand and Gow 2009).

Hyphal growth direction is positively correlated with Spk position and movements within the apex, and it plays a fundamental role in regulation of morphogenesis (Girbardt 1957; Bartnicki-García et al. 1995; López-Franco and Bracker 1996; Reynaga-Peña et al. 1997; Riquelme et al. 1998; Roberson et al. 2011). In most analyzed fungi, the Spk is a highly dynamic and pleomorphic complex. Although hyphae grow in a rather fixed direction, they possess the ability to meander intrin-

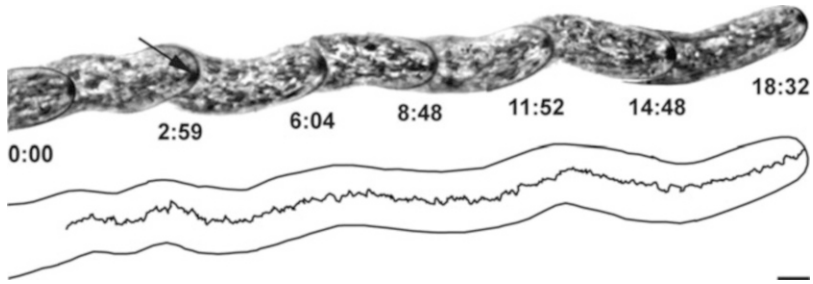


Fig. 3.5 A wild-type hypha of *N. crassa* displaying meandering growth (digital assembly of several frames obtained by phase-contrast microscopy). Time in min: s. Overall profile shown in the bottom along with Spk path (position mapped every 2 s). Scale bar, 5 μm (From Riquelme et al. (1998) with permission)

sically, without responding to any obvious external stimuli (Fig. 3.5). Studies in *N. crassa* wild type (wt) showed that in straight-growing hyphae, the Spk produces dominant forward movements accompanied by frequent but short transverse oscillations, while in naturally meandering hyphae the Spk suffers additional sustained directional shifts (Riquelme et al. 1998). In hyphae of *N. crassa ropy* mutants, with impaired microtubular traffic, or in hyphae treated with microtubule inhibitors, the Spk showed a remarkable erratic trajectory that resulted in a distorted hyphal morphology, suggesting the importance of the microtubular cytoskeleton in maintaining Spk positioning at the hyphal dome and ultimately in regulating hyphal morphogenesis (Riquelme et al. 2000). Further proof that the position of the Spk determines hyphal growth directionality and morphogenesis was obtained by manipulating hyphae of *Trichoderma viride* with a laser beam, which caused the displacement of the Spk and altered the direction of elongation and overall profile of the hyphae (Bracker et al. 1997).

4. Function

Several functions have been attributed to the Spk according to its main constituents. The Spk was proposed to operate as a vesicle supply center (VSC), serving as (1) a collection site for secretory (wall-building) cytoplasmic vesicles arriving from the subapical region and (2) the point of departure for these vesicles as they migrate to and fuse with the cell membrane to produce new cell wall and extend the plasma membrane (Bartnicki-García 1990). In essence, the Spk is a manifestation of the secretory pathway, and its primary role can likely be considered as an exocytic regulatory apparatus that receives and distributes secretory vesicles and signaling molecules (Bartnicki-García et al. 1989; Riquelme et al. 2014). The localization of the cell wall-synthesizing enzymes at the Spk (see above) provided definite evidence of the direct involvement of the Spk in the cell wall growth process (Riquelme et al. 2007; Verdín et al. 2009; Sánchez-León et al. 2011; Fajardo-Somera et al. 2015). The localized fusion of vesicles at the apex is presumably assisted by positional landmarks, which define polarization sites for the sequential recruitment and activation of Rho GTPases such as Cdc42, Rac1, etc., implicated in the formation of the polarisome complex. The polarisome participates in the assembly of actin microfilaments through the nucleation of actin and may have a role in recruiting vesicles at the Spk and their subsequent delivery to the plasma membrane (Harris et al. 2005). Components of the polarisome have been found at hyphal tips of *A. nidulans* (Sharpless and Harris 2002), *A. gossypii* (Köhli et al. 2008), *C. albicans* (Crampin et al. 2005), and *N. crassa* (Araujo-Palomares et al. 2009). Given the partial co-localization of the Spk with some of the polarisome components and actin microfilaments (see below), a role as a microfilament-organizing center (MFOC) has also been attributed to the Spk (Harris et al. 2005). As mentioned above, only the *Allomyces* Spk has thus far been also shown

to function as an MTOC (Roberson and Vargas 1994; McDaniel and Roberson 1998). This is of particular interest because as other members of the Blastocladiomycota, *Allomyces* is a zoosporic fungus. Unlike strictly terrestrial fungi that possess spindle pole bodies, *Allomyces* and other zoospore-producing fungi have centrosomes. Finally, it has also been proposed that the Spk acts as a signaling hub that coordinates developmental transitions (Garzia et al. 2009; Etxebeste et al. 2009).

5. Ontogeny

The Spk assembles de novo as lateral or apical branches emerge (Riquelme and Bartnicki-García 2004; Reynaga-Peña and Bartnicki-arcía 1997) and as germ tubes mature into hyphae (Araujo-Palomares et al. 2007). In hyphae of both *N. crassa* wt and *A. niger ramosa-1 ts* mutant grown at the restrictive temperature, apical branching was preceded by a cytoplasmic contraction, a retraction and subsequent disappearance of the Spk, arrest of apical growth, and temporary isotropic enlargement of the hyphal tip (Riquelme and Bartnicki-García 2004; Reynaga-Peña and Bartnicki-García 1997). Emergence of the apical branches coincided with the gradual formation of new Spk. Similarly, the formation of a mature hypha in *N. crassa* wt coincided with the transition from an immature phase-light gray amorphous Spk to a phase-dark spherical Spk (Araujo-Palomares et al. 2007). When imaged by video-enhanced phase-contrast microscopy, sometimes, in addition to the main Spk, smaller Spks were observed arising next to the lateral plasma membrane a few micrometers behind the apical pole (López-Franco et al. 1995). These smaller Spks were named satellite Spk and were observed by TEM to correspond to packages of vesicles (López-Franco et al. 1995). Satellite Spks have been reported for 14 different fungal species, including *Fusarium culmorum*, *T. viride*, *N. crassa*, *Magnaporthe grisea*, and *Rhizoctonia solani*. Satellite Spks are transient, move at faster rates than the hyphae growth rates, and most often merge with the main Spk (López-Franco et al. 1995). It remains to be determined whether the small change in size in the main Spk immediately after satellite Spks merge results in changes in hyphal growth.

B. Cytoskeleton

Microtubules (MTs), actin MFs, septins, and a plethora of associated proteins such as molecular motors perform specific and shared duties in the cell to maintain polarity and cytoplasmic order and regulate intracellular motility. Intermediate filaments are an additional cytoskeletal component in eukaryotic cells, but their presence in fungi remains unverified.

1. Microtubules

MTs are constructed from αβ-tubulin heterodimer subunits that assemble end to end via non-covalent bonds to form 13 parallel protofilaments that join laterally to construct a small tubular structure (~25 nm diameter) of variable lengths. Like the αβ-tubulin dimers, the MT is a structural polar unit with the plus, fast-growing end extending into the cytoplasm, while the minus end is often anchored at the MTOC. MT nucleation is regulated by the MTOC (e.g., SPB, centrosome) through the interactions of the MT minus end with the γ-tubulin ring complex (Oakley and Oakley 1989) that is embedded in the MTOC. Interphase MTs are primarily solitary, though can occur in bundles of two to four. MT bundles are transient structures providing increased cytoplasmic support and the potential for generating contractile forces when MTs are arranged with opposite polarities. MTs can occupy the entire cytoplasm and are generally oriented in parallel to the growing axis of the hypha. In large hyphae of *N. crassa*, MTs can loosely associate together forming a meshwork in the central cytoplasm (Mouriño-Pérez et al. 2006). Oblique or transverse MT orientations are observed during branch formation, nuclear division, and occasionally within the apical dome.

The dynamic instability of the MTs is the primary behavior of these cytoskeletal structures, which is characterized by the rapid

assembly and disassembly of the MT plus end. In *N. crassa* strain expressing GFP-tagged tubulin dynamic instability is visualized as rapid anterograde growth of MTs often reaching into the apical dome and interacting with the Spk and apical plasma membrane at their plus ends (Mouriño-Pérez et al. 2006; Uchida et al. 2008). This is followed by catastrophic disassembly and shrinkage of the MTs until rescued and the cycle repeats (Uchida et al. 2008). Small MT fragments have been observed in *N. crassa* moving through the hyphal cytoplasm in anterograde and retrograde directions (Uchida et al. 2008). MTs tend to converge within the apical dome (Mouriño-Pérez et al. 2006; Freitag et al. 2004), which is most pronounced in hyphae of *A. macrogynus*, where the Spk contains γ-tubulin and may function as a microtubule-organizing center (MTOC; McDaniel and Roberson 1998; Roberson and Vargas 1994). The idea that the Spk might be an MTOC in all fungi is a provocative one, but repeated attempts to localize γ-tubulin to the Spk of septate fungi have failed. Interestingly, apical MT nucleation was reported in germ tubes of *Uromyces phaseoli* (Hoch and Staples 1985) and *N. crassa* (Mouriño-Pérez et al. 2006), though not in association with the Spk, suggesting that the cytosolic surface of the apical plasma membrane may serve as a site for MT nucleation. There is also evidence that septal regions act as MTOCs in *A. nidulans* (Veith et al. 2005).

While the direct functions of cytoplasmic MTs in the growth of hyphae are not completely understood, it is clear that together with their associated proteins and the actin cytoskeleton, they play fundamental roles in maintaining cytoplasmic order, apical transport of secretory vesicles, mRNAs and associated factors, cell morphology, growth direction, and nuclear division (see Riquelme 2013; Takeshita et al. 2014, and references therein).

2. Actin Microfilaments

MFs are composed of globular actin proteins that assemble into two protofilaments forming a single helical filament (~7 nm diameter). These filaments appear as thin electron-opaque fibers when viewed with TEM. MFs and actin monomers (i.e., G-actin), like MTs, are polar structures that are regulated through the interactions of many associated proteins. Actin concentrates at the core of the Spk within the hyphal apex (Delgado-Álvarez et al. 2010). Actin is also abundant in subapical hyphal regions and appears as small spots or patches in the subapical cortex of growing hyphae associated with an endocytic collar and as rings in association with septum formation (Hoch and Staples 1983; Roberson 1992; Araujo-Bazán et al. 2008; Upadhyay and Shaw 2008; Srinivasan et al. 1996; Berepiki et al. 2010; Delgado-Álvarez et al. 2010; Echauri-Espinosa et al. 2012). Like MTs, actin plays essential roles in hyphal biology, but it is primarily required for maintenance of tip shape, exocytosis and endocytosis, and septation (Takeshita et al. 2014).

3. Septins

Septins are a family of cytoskeletal proteins first discovered in *S. cerevisiae* (Hartwell 1971) and highly conserved in all eukaryotes (Fung et al. 2014; Mostowy and Cossart 2012). They bind GTP and contain a phosphoinositide-binding domain. Septins polymerize into hetero-oligomer complexes that form nonpolar filamentous bundles or rings. They function primarily as cytoplasmic scaffolding proteins that associate with cellular membranes and serve as platforms for other functional proteins to associate (McMurray and Thorner 2009). In hyphae, septins contribute to cell shape and formation of septa, though the mechanistic details are not completely understood (Riquelme 2013). One of the suggested roles of the subapical septins is to act as a barrier to prevent the diffusion of polarity factors to distal areas beyond the subapical endocytic collar (Gladfelter 2006).

4. Molecular Motors

As in other eukaryotes, fungal MTs have two associated molecular motors: dyneins (minus-end-directed) and kinesins (plus-end-directed). Both play fundamental roles in supporting cell morphology, cytoplasmic order, intracellular

motility, nuclear division, MT dynamics, and organization (Riquelme et al. 2000, 2002; Steinberg 2007; Fischer et al. 2008; Seiler et al. 1999; Konzack et al. 2005). While there appears to be one form of dynein in filamentous fungi, multiple forms of kinesins have been identified in the Ascomycota (Schoch et al. 2003). In regard to specific roles performed by these motors, kinesin-1 influences Spk stability, exocytosis, and pathogenicity (Takeshita et al. 2014, and references therein). Kinesin-3 and dynein have been shown to transport early endosomes (Seidel et al. 2013; Egan et al. 2012). Furthermore, studies of kinesin-3 indicate the possible presence of posttranslationally modified MTs (Zekert and Fischer 2009).

Myosins are a superfamily of molecular motors that associate with MFs to generate force and movement through plus-end-directed momentum. The unconventional myosin VI is a minus-end-directed motor and is not found in fungi (Nishikawa et al. 2002; Odronitz and Kollmar 2007). Myosins are involved in exocytosis and hyphal morphogenesis (myosin V), endocytosis (myosin I), and cytokinesis (myosin II) (Taheri-Talesh et al. 2012; Steinberg 2007).

V. The Secretory Machinery

In filamentous fungi the exocytic zone is delimited at the extreme hyphal apex, while the endocytic zone occupies the subapical hyphal region (Araujo-Bazán et al. 2008). Hyphal tip growth is sustained by the continuous fusion of secretory vesicles with the apical plasma membrane. Vesicular transport was proposed to exist 40 years ago as a mean to move newly synthesized proteins through a series of membrane-enclosed organelles (ER, Golgi, etc.) to the extracellular space (Palade 1975). In fast-growing fungi such as *N. crassa*, 38,000 vesicles/min were predicted to fuse with the plasma membrane (Collinge and Trinci 1974), whereas in slower-growing fungi, such as *A. nidulans*, predictions estimated 500 vesicles/min (Prosser 1979). These predictions were based on the differences in vesicle numbers encountered in apices of fixed hyphae of *N. crassa* and *A. nidulans* analyzed by TEM (Roberson unpublished). The regulation of the traffic of secretory vesicles has been attributed in great part to the activity of three Rab GTPases: Rab1, Rab8, and Rab11. In *A. nidulans*, RabO, the Rab1 orthologue, was identified at the Spk of hyphal tips and the corresponding *ts* mutant presented hyphal tip morphogenetic defects, which correlated with Golgi cisterna disorganization (Pinar et al. 2013). In the same species, RabE, orthologue of Rab11, was associated to post-Golgi carriers that accumulated at the hyphal apex (Pantazopoulou et al. 2014). Remarkably, in *N. crassa*, distinct Rab GTPases have been found at each vesicular layer of the Spk; YPT-1/Rab1 concentrates at the microvesicular layer, while SEC-4/Rab8 and YPT-31/Rab11 were identified at the outer macrovesicular layer of the Spk (Sánchez-León et al. 2015). This spatial organization of Rabs at distinct layers of the Spk suggested that they might differentially regulate the traffic of the subpopulations of vesicles of the Spk.

The latest stages of the secretory pathway include a tethering step (initial interaction between a vesicle and its target membrane) and a fusion step (mixing of lipid bilayers). In the budding yeast, the tethering step is mediated by the exocyst, an octameric complex, which exerts as effector of Sec4; this Rab protein in its GTP-bound form and together with the exocyst subunit Sec15 is involved in tethering events at specific growing sites (Salminen and Novick 1989). Sec4 has also been identified at hyphal tips of *A. gossypii*, *C. albicans*, *A. fumigatus*, and *N. crassa* (Schmitz et al. 2006; Jones and Sudbery 2010; Powers-Fletcher et al. 2013; Sánchez-León et al. 2015). In slow-growing fungi, exocyst subunits accumulate as an apical crescent immediately anterior to the Spk (Köhli et al. 2008; Taheri-Talesh et al. 2008; Jones and Sudbery 2010; Hayakawa et al. 2011). In hyphae of the fast-growing *N. crassa*, the exocyst subunits EXO-70 and EXO-84 were found at the macrovesicular layer of the Spk, while the other subunits accumulated at the apical plasma membrane (Riquelme et al. 2014). An intact exocyst is required not only for the formation of a functional Spk but also for maintaining hyphal tip growth and morphogenesis (Riquelme et al. 2014). The fusion

step of the vesicles with their target membrane is mediated by soluble N-ethylmaleimide-sensitive factor-attachment protein receptors (SNAREs). Genome analysis of >70 fungal species revealed the existence of nearly 20 different SNAREs (Kienle et al. 2009). In *S. cerevisiae* a small set of SNAREs have been implicated in late secretory fusion steps, the t-SNAREs (Sso1, Sso2, Sec9, and Spo20) and the v-SNAREs (Snc1 and Snc2) (Burri and Lithgow 2004). In growing hyphae of *A. nidulans* and *N. crassa*, SynA and SYN-1, the orthologues of the yeast synaptobrevin Snc1, respectively, have been identified at the Spk, suggesting their putative association with secretory vesicles (Taheri-Talesh et al. 2008; Sánchez-León et al. 2015). The polarized accumulation of secretory machinery components at hyphal tips suggests the high secretory activity that takes place at the apical region. Further studies are still needed to unravel the concerted molecular events that sustain the polarized organization of the secretory components involved in tip growth.

Phosphoinositides are important lipid signaling molecules with roles in actin machinery assembly and secretory vesicle exocytosis (Yakir-Tamang and Gerst 2009; Martin 2014). Phosphatidylinositol 4-phosphate (PI4P) and phosphatidylinositol (4,5)-bi-phosphate (PI(4,5)P_2) have been found to be crucial for polarized growth in *S. cerevisiae* and *C. albicans*, where these lipids adopt a polarized distribution (Arkowitz and Bassilana 2014). However, in *N. crassa* and *A. nidulans* hyphae, PI(4,5)P_2 appeared more evenly distributed along the plasma membrane (Mähs et al. 2012; Pantazopoulou and Peñalva 2009), and it remains to be proven whether they have a direct role in the polarization of the exocytic processes.

VI. The Role of "Omics" Studies Toward Understanding Tip Growth

As mentioned above, hyphal tip growth implies the coordinated interaction of many structural cellular components. Although a lot of information has been obtained on some key elements needed for tip growth, essential questions in order to understand this complex process are still unresolved: How many genes are devoted to fungal tip growth? Are there any differences in the expression of these genes within the diverse fungal taxa? What are the molecular determinants that regulate tip growth under different environmental cues? To date, more than 250 fungal genomes have been sequenced and are publicly available at MycoCosm, an integrated fungal genomics resourceful portal developed by the US Department of Energy Joint Genome Institute (JGI; http://genome.jgi-psf.org/programs/index.jsf). Notably, the 1000 Fungal Genomes Project initiative of JGI aims to sequence 1000 fungal genomes taking into account nominations from the research community (Grigoriev et al. 2014). The comparative analysis of genomic data with other "omics" information (i.e., transcriptomics, proteomics, lipidomics, metabolomics, etc.) will provide useful data to address the underlying mechanism of hyphal tip growth. For example, recently, interesting differences on cell wall structural components in the Mucoromycotina and the Dikarya were revealed (Mélida et al. 2014). Phylogenomic profiling of genes putatively involved in formation or modification of cell wall polysaccharides allowed comparisons of the predicted biosynthetic machinery in these different fungal taxa for which no cell biology information is yet available (Mélida et al. 2014).

The transcriptomic analysis of the *A. niger ramosa-1 ts* mutant shed light on multiple genes belonging to signaling networks possibly involved in tip growth regulation (Meyer et al. 2009). At the restrictive temperature, this mutant suffered a retraction of the Spk, loss of hyphal polarity, formation of new Spk, and apical branching, all of which was thought to be the consequence of disturbance of the actin cytoskeleton (Reynaga-Peña and Bartnicki-García 1997). The *A. niger rmsA* gene, obtained by complementation of the mutation, was found to be the functional equivalent of the *S. cerevisiae* TORC2 component Avo1, required

for actin polarization and cell wall integrity (Meyer et al. 2009).

VII. Conclusions and Perspectives

- There are clear ultrastructural differences at the level of organization of vesicles and organelles at the hyphal apex across different fungal taxa. From the hundreds of fungal species with genomes sequenced, only a handful of them have been subject of hyphal morphogenesis studies. More fungal species within each taxa need to be analyzed, ideally those with available sequenced genomes and amenable to molecular manipulations. This would allow us to identify conserved or diverse patterns of hyphal apical organization and analyze whether a correlation between tip growth machinery and cell wall composition exists.
- Most of the fungal species analyzed by TEM have macrovesicles and microvesicles at their hyphal apices. However, a scarce record for fusion events of these vesicles at the tips exists. What remains to be uncovered is the exocytic pattern, both at the spatial and temporal levels, of discharge of the different vesicles at the hyphal tip surface.
- Structural data of apices of germlings is very scarce. This information is key to understand the ontogeny of the mature hyphae and to correlate the differences found between the two cell types by transcriptomic profiling.
- There is a total lack of understanding on how the in situ synthesis of the cell wall occurs. This process, beyond being relevant to fungal cell biology by itself, could be a true revelation to design targeted antimycotic drugs against fungal pathogens of plants and humans.
- High-resolution ultrastructural and protein mapping (electron tomography, serial section reconstruction, immuno-EM) of the apical dome is needed to understand (1) distribution and orientations of cytoskeletal elements and their associations with vesicles and the plasma membrane, (2) the number and position of vesicles that make up the Spk, (3) the character of the endocytic collar, and (4) precise distribution of important tip growth-related proteins.
- Several secretory machinery components have been identified at hyphal apices, but little is known about the particular contribution of each of them in the numerous molecular events during tip growth. Functional dissection of the distinct components of the secretory apparatus through the development of molecular tools including generation of *ts* mutants, and use of regulatable promoters and more selectable markers, will enable to reveal mechanistic details of hyphal tip secretion.
- Revised models for hyphal morphogenesis will allow re-evaluating the roles of the Spk in light of its absence in most zygomycetous and zoosporic fungi and elucidate the evolutionary advantage of having a Spk.

Acknowledgments Work in the MR and RWR labs was supported by grants from Consejo Nacional de Ciencia y Tecnología CONACYT CB-2008-105-600-Q and CONACYT-DFG I0110/193/10 FON.INST.-30-10 and National Science Foundation Collaborative Research: AFTOL: Resolving the Evolutional History of the Fungi (NSFDEB-0732503), respectively.

References

Abe M, Qadota H, Hirata A, Ohya Y (2003) Lack of GTP-bound Rho1p in secretory vesicles of *Saccharomyces cerevisiae*. J Cell Biol 162:85–97

Araujo-Bazán L, Peñalva MA, Espeso EA (2008) Preferential localization of the endocytic internalization machinery to hyphal tips underlies polarization of the actin cytoskeleton in *Aspergillus nidulans*. Mol Microbiol 67:891–905

Araujo-Palomares CL, Castro-Longoria E, Riquelme M (2007) Ontogeny of the Spitzenkörper in germlings of *Neurospora crassa*. Fungal Genet Biol 44:492–503

Araujo-Palomares CL, Riquelme M, Castro-Longoria E (2009) The polarisome component SPA-2 localizes at the apex of *Neurospora crassa* and partially colocalizes with the Spitzenkörper. Fungal Genet Biol 46:551–563

Arkowitz RA, Bassilana M (2011) Polarized growth in fungi: symmetry breaking and hyphal formation. Semin Cell Dev Biol 22:806–815

Arkowitz RA, Bassilana M (2014) Rho GTPase-phosphatidylinositol phosphate interplay in fungal cell polarity. Biochem Soc Trans 42:206–211

Aronson JM (1965) The cell wall. In: Sussman AS, Ainsworth GC (eds) The fungi, vol 1. Academic, London/ New York, pp 49–76

Asakawa H, Hiraoka Y, Haraguchi T (2014) A method of correlative light and electron microscopy for yeast cells. Micron 61:53–61

Ayad-Durieux Y, Knechtle P, Goff S, Dietrich F, Philippsen P (2000) A PAK-like protein kinase is required for maturation of young hyphae and septation in the filamentous ascomycete *Ashbya gossypii*. J Cell Sci 113(24):4563–4575

Baba M, Baba N, Ohsumi Y, Kanaya K, Osumi M (1989) Three-dimensional analysis of morphogenesis induced by mating pheromone-alpha factor in *Saccharomyces cerevisiae*. J Cell Sci 94:207–216

Baladrón V, Ufano S, Dueñas E, Martín-Cuadrado AB, del Rey F, de Aldana CRV (2002) Eng1p, an endo-1,3-beta-glucanase localized at the daughter side of the septum, is involved in cell separation in *Saccharomyces cerevisiae*. Eukaryot Cell 1:774–786

Bartnicki-García S (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu Rev Microbiol 22:87–108

Bartnicki-García S (1973) Fundamental aspects of hyphal morphogenesis. In: Ashworth JM, Smith E (eds) Microbial differentiation, Symposium of the society for general microbiology. Cambridge University Press, Cambridge, pp 245–267

Bartnicki-García S (1990) Role of vesicles in apical growth and a new mathematical model of hyphal morphogenesis. In: Heath IB (ed) Tip growth in plants and fungal cells. Academic, San Diego, pp 211–232

Bartnicki-García S (1999) Glucans, walls, and morphogenesis: on the contributions of J. G. H. Wessels to the golden decades of fungal physiology and beyond. Fungal Genet Biol 27:119–127

Bartnicki-García S, Lippman E (1969) Fungal morphogenesis: cell wall construction in *Mucor rouxii*. Science 165:302–304

Bartnicki-García S, Hergert F, Gierz G (1989) Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. Protoplasma 153:46–57

Bartnicki-García S, Bartnicki DD, Gierz G, López-Franco R, Bracker CE (1995) Evidence that Spitzenkörper behavior determines the shape of a fungal hypha: a test of the hyphoid model. Exp Mycol 19:153–159

Bentivenga SP, Kumar TK, Kumar L, Roberson RW, McLaughlin DJ (2013) Cellular organization in germ tube tips of *Gigaspora* and its phylogenetic implications. Mycologia 105:1087–1099

Berepiki A, Lichius A, Shoji JY, Tilsner J, Read ND (2010) F-actin dynamics in *Neurospora crassa*. Eukaryot Cell 9:547–557

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N, Plamann M, Goodrich-Tanrikulu M, Schulte U, Mannhaupt G, Nargang FE, Radford A, Selitrennikoff C, Galagan JE, Dunlap JC, Loros JJ, Catcheside D, Inoue H, Aramayo R, Polymenis M, Selker EU, Sachs MS, Marzluf GA, Paulsen I, Davis R, Ebbole DJ, Zelter A, Kalkman ER, O'Rourke R, Bowring F, Yeadon J, Ishii C, Suzuki K, Sakai W, Pratt R (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Bourett TM, Howard RJ (1991) Ultrastructural immunolocalization of actin in a fungus. Protoplasma 163:199–202

Bracker CE, Ruiz-Herrera J, Bartnicki-García S (1976) Structure and transformation of chitin synthetase particles (chitosomes) during microfibril synthesis in vitro. Proc Natl Acad Sci USA 73:4570–4574

Bracker CE, Murphy DJ, López-Franco R (1997) Laser microbeam manipulation of cell morphogenesis in growing fungal hyphae. In: Farkas DL, Tromberg BJ (eds) Functional imaging of optical manipulation of living cells, vol 2983. International Society for Optical Engineering, Bellingham, WA, pp 67–80

Brand A, Gow NAR (2009) Mechanisms of hypha orientation of fungi. Curr Opin Microbiol 12:350–357

Braun M (2001) Association of spectrin-like proteins with the actin-organized aggregate of endoplasmic reticulum in the Spitzenkörper of gravitropically tip-growing plant cells. Plant Physiol 125:1611–1619

Brunswik H (1924) Untersuchugen uber die Geslechts und Kernverhaltnisse bei der Hymenomyzetengattung Coprinus, vol 5, Botanische Abhadlung. Gustav Fisher, Jena

Bulawa CE (1993) Genetics and molecular biology of chitin synthesis in fungi. Annu Rev Microbiol 47:505–534

Burri L, Lithgow T (2004) A complete set of SNAREs in yeast. Traffic 5:45–52

Chapa-Y-Lazo B, Lee S, Regan H, Sudbery P (2011) The mating projections of *Saccharomyces cerevisiae* and *Candida albicans* show key characteristics of hyphal growth. Fungal Biol 115:547–556

Choi W-J, Santos B, Duran A, Cabib E (1994a) Are yeast chitin synthases regulated at the transcriptional or the posttranslational level? Mol Cell Biol 14:7685–7694

Choi W-J, Sburlati A, Cabib E (1994b) Chitin synthase 3 from yeast has zymogenic properties that depend on both the CALI and the CAL3 genes. Proc Natl Acad Sci USA 91:4727–4730

Choquer M, Boccara M, Goncalves IR, Soulie MC, Vidal-Cros A (2004) Survey of the *Botrytis cinerea* chitin synthase multigenic family through the analysis of six euascomycetes genomes. Eur J Biochem 271:2153–2164

Collinge AJ, Trinci APJ (1974) Hyphal tips of wild-type and spreading colonial mutants of *Neurospora crassa*. Arch Microbiol 99:353–368

Crampin H, Finley K, Gerami-Nejad M, Court H, Gale C, Berman J, Sudbery P (2005) *Candida albicans*

hyphae have a Spitzenkörper that is distinct from the polarisome found in yeast and pseudohyphae. J Cell Sci 118:2935–2947

de Groot PWJ, de Boer AD, Brandt BW, Valentín E (2016) The ascomycetous cell wall, from a proteomic perspective. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 81–101

Delgado-Álvarez DL, Callejas-Negrete OA, Gómez N, Freitag M, Roberson RW, Smith LG, Mouriño-Pérez RR (2010) Visualization of F-actin localization and dynamics with live cell markers in *Neurospora crassa*. Fungal Genet Biol 47:573–586

Echauri-Espinosa RO, Callejas-Negrete OA, Roberson RW, Bartnicki-García S, Mouriño-Pérez RR (2012) Coronin is a component of the endocytic collar of hyphae of *Neurospora crassa* and is necessary for normal growth and morphogenesis. PLoS ONE 7, e38237

Egan MJ, McClintock MA, Reck-Peterson SL (2012) Microtubule-based transport in filamentous fungi. Curr Opin Microbiol 15:637–645

Etxebeste O, Herrero-García E, Araujo-Bazán L, Rodriguez-Urra AB, Garzia A, Ugalde U, Espeso EA (2009) The bZIP-type transcription factor FlbB regulates distinct morphogenetic stages of colony formation in *Aspergillus nidulans*. Mol Microbiol 73:775–789

Fajardo-Somera RA, Jöhnk B, Bayram O, Valerius O, Braus GH, Riquelme M (2015) Dissecting the function of the different chitin synthases in vegetative growth and sexual development in *Neurospora crassa*. Fungal Genet Biol 75:30–45

Fischer R, Zekert N, Takeshita N (2008) Polarized growth in fungi-interplay between the cytoskeleton, positional markers and membrane domains. Mol Microbiol 68:813–826

Fischer-Parton S, Parton RM, Hickey PC, Dijksterhuis J, Atkinson HA, Read ND (2000) Confocal microscopy of FM4-64 as a tool for analysing endocytosis and vesicle trafficking in living fungal hyphae. J Microsc 198:246–259

Fontaine T, Simenel C, Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M, Latgé JP (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. J Biol Chem 275:27594–27607

Freitag M, Hickey PC, Raju NB, Selker EU, Read ND (2004) GFP as a tool to analyze the organization, dynamics and function of nuclei and microtubules in *Neurospora crassa*. Fungal Genet Biol 41:897–910

Fung KYY, Dai L, Trimble WS (2014) Cell and molecular biology of septins. Int Rev Cell Mol Biol 310:289–339

Garzia A, Etxebeste O, Herrero-García E, Fischer R, Espeso EA, Ugalde U (2009) *Aspergillus nidulans* FlbE is an upstream developmental activator of conidiation functionally associated with the putative transcription factor FlbB. Mol Microbiol 71:172–184

Girbardt M (1957) Der Spitzenkörper von *Polystictus versicolor* (L). Planta 50:47–59

Girbardt M (1969) Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma 67:413–441

Gladfelter AS (2006) Control of filamentous fungal cell shape by septins and formins. Nat Rev Microbiol 4:223–229

Glaser L, Brown DH (1957) The enzymic synthesis of chitin by extracts of *Neurospora crassa*. Biochim Biophys Acta 23:449–450

Gow NA, Gooday GW (1982) Growth kinetics and morphology of colonies of the filamentous form of *Candida albicans*. J Gen Microbiol 128(9):2187–2194

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao XL, Korzeniewski F, Smirnova T, Nordberg H, Dubchak I, Shabalov I (2014) MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res 42:D699–D704

Grove SN, Bracker CE (1970) Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. J Bacteriol 104:989–1009

Harris SD (2006) Cell polarity in filamentous fungi: shaping the mold. Int Rev Cytol 251:41–77

Harris SD, Read ND, Roberson RW, Shaw BD, Seiler S, Plamann M, Momany M (2005) Polarisome meets Spitzenkörper: microscopy, genetics, and genomics converge. Eukaryot Cell 4:225–229

Hartwell LH (1971) Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Exp Cell Res 69:265–276

Hayakawa K, Tatsumi H, Sokabe M (2011) Actin filaments function as a tension sensor by tension dependent binding of cofilin to the filament. J Cell Biol 195:721–727

Heath IB (1994) The cytoskeleton in hyphal growth, organelle movements, and mitosis. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol 1. Springer, Berlin, pp 43–65

Hickey PC, Swift SR, Roca MG, Read ND (2004) Live-cell imaging of filamentous fungi using vital fluorescent dyes and confocal microscopy. Methods Microbiol 34:63–87

Hoch HC, Howard RJ (1980) Ultrastructure of freeze-substituted hyphae of the basidiomycete *Laetisaria arvalis*. Protoplasma 103:281–297

Hoch HC, Staples RC (1983) Visualization of actin in situ by rhodamine-conjugated phalloin in the fungus *Uromyces phaseoli*. Eur J Cell Biol 32:52–58

Hoch HC, Staples RC (1985) The microtubule cytoskeleton in hyphae of *Uromyces phaseoli* germlings: its relationship to the region of nucleation and to the F-actin cytoskeleton. Protoplasma 124:112–122

Hochstenbach F, Klis FM, van den Ende H, van Donselaar E, Peters PJ, Klausner RD (1998) Identification of a putative alpha-glucan synthase essential for cell wall construction and morphogenesis in fission yeast. Proc Natl Acad Sci USA 95:9161–9166

Hohmann-Marriott MF, Uchida M, van de Meene AM, Garret M, Hjelm BE, Kokoori S, Roberson RW (2006) Application of electron tomography to fungal ultrastructure studies. New Phytol 172:208–220

Howard RJ (1981) Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. J Cell Sci 48:89–103

Howard RJ, Aist JR (1980) Cytoplasmic microtubules and fungal morphogenesis: ultrastructural effects of methyl benzimidazole-2-ylcarbamate determined by freeze-substitution of hyphal tip cells. J Cell Biol 87:55–64

Hunsley D, Burnett JH (1970) The ultrastructural architecture of the walls of some hyphal fungi. J Gen Microbiol 62:203–218

Hunsley D, Gooday GW (1974) The structure and development of septa in *Neurospora crassa*. Protoplasma 82:125–146

Inoue SB, Qadota H, Arisawa M, Watanabe T, Ohya Y (1999) Prenylation of Rho1p is required for activation of yeast 1,3-β-glucan synthase. J Biol Chem 274:38119–38124

Jones LA, Sudbery PE (2010) Spitzenkörper, exocyst, and polarisome components in *Candida albicans* hyphae show different patterns of localization and have distinct dynamic properties. Eukaryot Cell 9:1455–1465

Kienle N, Kloepper TH, Fasshauer D (2009) Phylogeny of the SNARE vesicle fusion machinery yields insights into the conservation of the secretory pathway in fungi. BMC Evol Biol 9:19

Köhli M, Galati V, Boudier K, Roberson RW, Philippsen P (2008) Growth-speed-correlated localization of exocyst and polarisome components in growth zones of *Ashbya gossypii* hyphal tips. J Cell Sci 121:3878–3889

Kong LA, Yang J, Li GT, Qi LL, Zhang YJ, Wang CF, Zhao WS, Xu JR, Peng YL (2012) Different chitin synthase genes are required for various developmental and plant infection processes in the rice blast fungus *Magnaporthe oryzae*. PLoS Pathog 8, e1002526

Konzack S, Rischitor PE, Enke C, Fischer R (2005) The role of the kinesin motor KipA in microtubule organization and polarized growth of *Aspergillus nidulans*. Mol Biol Cell 16:497–506

Latgé JP, Calderone R (2006) The fungal cell wall. In: Kues U, Fischer R (eds) The mycota, vol 1. Springer, Berlin, pp 73–104

Lenardon MD, Milne SA, Mora-Montes HM, Kaffarnik FA, Peck SC, Brown AJ, Munro CA, Gow NA (2010) Phosphorylation regulates polarisation of chitin synthesis in *Candida albicans*. J Cell Sci 123:2199–2206

Lending CR, Leal-Morales CA, Flores-Martínez A, Bracker CE, Bartnicki-García S (1991) Purification and characterization of 16 S chitin synthetase particles from cell walls of *Mucor rouxii*. Exp Mycol 15:11–25

Lesage G, Sdicu AM, Menard P, Shapiro B, Hussein S, Bussey H (2004) Analysis of β-1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. Genetics 167:35–49

López-Franco R, Bracker CE (1996) Diversity and dynamics of the Spitzenkörper in growing hyphal tips of higher fungi. Protoplasma 195:90–111

López-Franco R, Howard RJ, Bracker CE (1995) Satellite Spitzenkörper in growing hyphal tips. Protoplasma 188:85–103

Mähs A, Ischebeck T, Heilig Y, Stenzel I, Seiler S, Heilmann I (2012) The essential phosphoinositide kinase MSS-4 is required for polar hyphal morphogenesis, localizing to sites of growth and cell fusion in *Neurospora crassa*. PLoS ONE 7, e51454

Mari M, Geerts WJ, Reggiori F (2014) Immuno- and correlative light microscopy-electron tomography methods for 3D protein localization in yeast. Traffic 15:1164–1178

Marschall R, Tudzynski P (2014) A new and reliable method for live imaging and quantification of reactive oxygen species in *Botrytis cinerea*. Fungal Genet Biol 71:68–75

Martin TF (2014) PI(4,5)P-binding effector proteins for vesicle exocytosis. Biochim Biophys Acta 1851:785–793

Martínez-Núñez LE, Riquelme M (2015) Role and subcellular localization of EGL-1 and EGL-2, two putative GPI anchored cell wall β (1–3) endoglucanases, in *Neurospora crassa*. Fungal Genet Rep 61(Suppl), Abstract 156

Mcclure WK, Park D, Robinson PM (1968) Apical organization in the somatic hyphae of fungi. J Gen Microbiol 50:177–182

McDaniel DP, Roberson RW (1998) γ-Tubulin is a component of the Spitzenkörper and centrosomes in hyphal-tip cells of *Allomyces macrogynus*. Protoplasma 203:118–123

McMurray MA, Thorner J (2009) Septins: molecular partitioning and the generation of cellular asymmetry. Cell Div 4:18

McMurrough I, Flores-Carreón A, Bartnicki-García S (1971) Pathway of chitin synthesis and cellular localization of chitin synthetase in *Mucor rouxii*. J Biol Chem 246:3999–4007

Mélida H, Sain D, Stajich JE, Bulone V (2014) Deciphering the uniqueness of Mucoromycotina cell walls by combining biochemical and phylogenomic approaches. Environ Microbiol 17:1649–1662

Meyer V, Arentshorst M, Flitter SJ, Nitsche BM, Kwon MJ, Reynaga-Peña CG, Bartnicki-García S, van den Hondel CA, Ram AF (2009) Reconstruction of signaling networks regulating fungal morphogenesis by transcriptomics. Eukaryot Cell 8:1677–1691

Momany M (2005) Growth control and polarization. Med Mycol 43:23–25

Mostowy S, Cossart P (2012) Septins: the fourth component of the cytoskeleton. Nat Rev Mol Cell Biol 13:183–194

Mouriño-Pérez RR, Roberson RW, Bartnicki-García S (2006) Microtubule dynamics and organization during hyphal growth and branching in *Neurospora crassa*. Fungal Genet Biol 43:389–400

Müller WH, Koster AJ, Humbel BM, Ziese U, Verkleij AJ, van Aelst AC, van der Krift TP, Montijn RC, Boekhout T (2000) Automated electron tomography of the septal pore cap in *Rhizoctonia solani*. J Struct Biol 131:10–18

Nicastro D, Frangakis AS, Typke D, Baumeister W (2000) Cryo-electron tomography of *Neurospora* mitochondria. J Struct Biol 129:48–56

Nishikawa S, Homma K, Komori Y, Iwaki M, Wazawa T, Hikikoshi Iwane A, Saito J, Ikebe R, Katayama E, Yanagida T, Ikebe M (2002) Class VI myosin moves processively along actin filaments backward with large steps. Biochem Biophys Res Commun 290:311–317

Oakley CE, Oakley BR (1989) Identification of γ-tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*. Nature 338:662–664

Odronitz F, Kollmar M (2007) Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species. Genome Biol 8:R196

Pacheco-Arjona JR, Ramírez-Prado JH (2014) Large-Scale phylogenetic classification of fungal chitin synthases and identification of a putative cell-wall metabolism gene cluster in Aspergillus genomes. PLoS ONE 9, e108920

Palade G (1975) Intracellular aspects of the process of protein synthesis. Science 89:347–358

Palanivelu R, Preuss D (2000) Pollen tube targeting and axon guidance: parallels in tip growth mechanisms. Trends Cell Biol 10:517–524

Pantazopoulou A, Peñalva MA (2009) Organization and dynamics of the *Aspergillus nidulans* Golgi during apical extension and mitosis. Mol Biol Cell 20:4335–4347

Pantazopoulou A, Pinar M, Xiang X, Peñalva MA (2014) Maturation of late Golgi cisternae into RabE (RAB11) exocytic post-Golgi carriers visualized in vivo. Mol Biol Cell 25:2428–2443

Pinar M, Pantazopoulou A, Arst HN Jr, Peñalva MA (2013) Acute inactivation of the *Aspergillus nidulans* Golgi membrane fusion machinery: correlation of apical extension arrest and tip swelling with cisternal disorganization. Mol Microbiol 89:228–248

Powers-Fletcher MV, Feng X, Krishnan K, Askew DS (2013) Deletion of the sec4 homolog srgA from *Aspergillus fumigatus* is associated with an impaired stress response, attenuated virulence and phenotypic heterogeneity. PLoS ONE 8, e66741

Prosser JI (1979) Mathematical modelling of mycelial growth. In: Burnett JH, Trinci APJ (eds) Fungal walls and hyphal growth. Cambridge University Press, Cambridge, pp 359–384

Reinhardt MO (1892) Das Wachsthum der Pilzhyphen. Jahrbucher fur wissenschafliche Botanik 23:479–566

Reynaga-Peña CG, Bartnicki-García S (1997) Apical branching in a temperature sensitive mutant of *Aspergillus niger*. Fungal Genet Biol 22:153–167

Reynaga-Peña CG, Gierz G, Bartnicki-García S (1997) Analysis of the role of the Spitzenkörper in fungal morphogenesis by computer simulation of apical branching in *Aspergillus niger*. Proc Natl Acad Sci USA 94:9096–9101

Riquelme M (2013) Tip growth in filamentous fungi: a road trip to the apex. Annu Rev Microbiol 67:587–609

Riquelme M, Bartnicki-García S (2004) Key differences between lateral and apical branching in hyphae of *Neurospora crassa*. Fungal Genet Biol 41:842–851

Riquelme M, Bartnicki-García S (2008) Advances in understanding hyphal morphogenesis: ontogeny, phylogeny and cellular localization of chitin synthases. Fungal Biol Rev 22:56–70

Riquelme M, Sánchez-León E (2014) The Spitzenkörper: a choreographer of fungal growth and morphogenesis. Curr Opin Microbiol 20:27–33

Riquelme M, Reynaga-Peña CG, Gierz G, Bartnicki-García S (1998) What determines growth direction in fungal hyphae? Fungal Genet Biol 24:101–109

Riquelme M, Gierz G, Bartnicki-García S (2000) Dynein and dynactin deficiencies affect the formation and function of the Spitzenkörper and distort hyphal morphogenesis of *Neurospora crassa*. Microbiology 146:1743–1752

Riquelme M, Roberson RW, McDaniel DP, Bartnicki-García S (2002) The effects of ropy-1 mutation on cytoplasmic organization and intracellular motility in mature hyphae of *Neurospora crassa*. Fungal Genet Biol 37:171–179

Riquelme M, Bartnicki-García S, González-Prieto JM, Sánchez-León E, Verdín-Ramos JA, Beltrán-Aguilar A, Freitag M (2007) Spitzenkörper localization and intracellular traffic of green fluorescent protein-labeled CHS-3 and CHS-6 chitin synthases in living hyphae of *Neurospora crassa*. Eukaryot Cell 6:1853–1864

Riquelme M, Bredeweg EL, Callejas-Negrete O, Roberson RW, Ludwig S, Beltrán-Aguilar A, Seiler S, Novick P, Freitag M (2014) The *Neurospora crassa* exocyst complex tethers Spitzenkörper vesicles to the apical plasma membrane during polarized growth. Mol Biol Cell 25:1312–1326

Roberson RW (1992) The actin cytoskeleton in hyphal cells of *Sclerotium rolfsii*. Mycologia 84:41–51

Roberson RW, Fuller MS (1988) Ultrastructural aspects of the hyphal tip of Sclerotium rolfsii preserved by freeze substitution. Protoplasma 146:143–149

Roberson RW, Vargas MM (1994) The tubulin cytoskeleton and its sites of nucleation in hyphal tips of *Allomyces macrogynus*. Protoplasma 182:19–31

Roberson RW, Fuller MS, Grabski C (1989) Effects of the demethylase inhibitor, cyproconazole, on hyphal tip cells of *Sclerotium rolfsii*: I. A light microscope study. Pestic Biochem Physiol 34:130–142

Roberson RW, Saucedo E, Maclean D, Propster J, Unger B, Oneil TA, Parvanehgohar K, Cavanaugh C, Lowry D (2011) The hyphal tip structure of *Basidiobolus* sp.: a zygomycete fungus of uncertain phylogeny. Fungal Biol 115:485–492

Robertson NF (1965) The fungal hypha. Trans Br Mycol Soc 48:1–8

Rodríguez-Peña JM, Cid VJ, Arroyo J, Nombela C (2000) A novel family of cell wall-related proteins regulated differently during the yeast life cycle. Mol Cell Biol 20:3245–3255

Rolli E, Ragni E, Calderon J, Porello S, Fascio U, Popolo L (2009) Immobilization of the glycosylphosphatidylinositol-anchored Gas1 protein into the chitin ring and septum is required for proper morphogenesis in yeast. Mol Biol Cell 20:4856–4870

Roncero C (2002) The genetic complexity of chitin synthesis in fungi. Curr Genet 41:367–378

Salminen A, Novick PJ (1989) The Sec15 protein responds to the function of the GTP binding protein, Sec4, to control vesicular traffic in yeast. J Cell Biol 109:1023–1036

Sanchatjate S, Schekman R (2006) Chs5/6 complex: a multiprotein complex that interacts with and conveys chitin synthase III from the trans-Golgi network to the cell surface. Mol Biol Cell 17:4157–4166

Sánchez-León E, Verdín J, Freitag M, Roberson RW, Bartnicki-García S, Riquelme M (2011) Traffic of chitin synthase 1 (CHS-1) to the Spitzenkörper and developing septa in hyphae of *Neurospora crassa*: actin dependence and evidence of distinct microvesicle populations. Eukaryot Cell 10:683–695

Sánchez-León E, Bowman B, Seidel C, Fischer R, Novick P, Riquelme M (2015) The Rab GTPase YPT-1 associates with Golgi cisternae and Spitzenkörper microvesicles in *Neurospora crassa*. Mol Microbiol 95:472–490

Schmitz HP, Kaufmann A, Kohli M, Laissue PP, Philippsen P (2006) From function to shape: a novel role of a formin in morphogenesis of the fungus *Ashbya gossypii*. Mol Biol Cell 17:130–145

Schoch CL, Aist JR, Yoder OC, Turgeon BG (2003) A complete inventory of fungal kinesins in representative filamentous ascomycetes. Fungal Genet Biol 39:1–15

Seidel C, Moreno-Velásquez SD, Riquelme M, Fischer R (2013) *Neurospora crassa* NKIN2, a Kinesin-3 motor, transports early endosomes and is required for polarized growth. Eukaryot Cell 12:1020–1032

Seiler S, Plamann M, Schliwa M (1999) Kinesin and dynein mutants provide novel insights into the roles of vesicle traffic during cell morphogenesis in *Neurospora*. Curr Biol 9:779–785

Sharpless KE, Harris SD (2002) Functional characterization and localization of the *Aspergillus nidulans* formin SEPA. Mol Biol Cell 13:469–479

Sietsma JH, Wessels JGH (2006) Apical wall biogenesis. In: Kues U, Fischer R (eds) The mycota, vol 1. Springer, Berlin, pp 53–72

Smith ML, Bruhn JN, Anderson JB (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428–431

Spellig T, Bottin A, Kahmann R (1996) Green fluorescent protein (GFP) as a new vital marker in the phytopathogenic fungus *Ustilago maydis*. Mol Gen Genet 252:503–509

Srinivasan S, Vargas MM, Roberson RW (1996) Functional, organizational, and biochemical analysis of actin in hyphal tip cells of *Allomyces macrogynus*. Mycologia 88:57–70

Steinberg G (2007) Preparing the way: fungal motors in microtubule organization. Trends Microbiol 15:14–21

Steinberg G, Schliwa M, Lehmler C, Bolker M, Kahmann R, McIntosh JR (1998) Kinesin from the plant pathogenic fungus *Ustilago maydis* is involved in vacuole formation and cytoplasmic migration. J Cell Sci 111:2235–2246

Suelmann R, Sievers N, Fischer R (1997) Nuclear traffic in fungal hyphae: in vivo study of nuclear migration and positioning in *Aspergillus nidulans*. Mol Microbiol 25:757–769

Taheri-Talesh N, Horio T, Araujo-Bazán L, Dou X, Espeso EA, Peñalva MA, Osmani SA, Oakley BR (2008) The tip growth apparatus of *Aspergillus nidulans*. Mol Biol Cell 19:1439–1449

Taheri-Talesh N, Xiong Y, Oakley BR (2012) The functions of myosin II and myosin V homologs in tip growth and septation in *Aspergillus nidulans*. PLoS ONE 7, e31218

Takeshita N, Manck R, Grun N, de Vega SH, Fischer R (2014) Interdependence of the actin and the microtubule cytoskeleton during fungal growth. Curr Opin Microbiol 20:34–41

Trautwein M, Schindler C, Gauss R, Dengjel J, Hartmann E, Spang A (2006) Arf1p, Chs5p and the ChAPs are required for export of specialized cargo from the Golgi. EMBO J 25:943–954

Trinci AP (1969) A kinetic study of the growth of *Aspergillus nidulans* and other fungi. J Gen Microbiol 57(1):11–24

Uchida M, Mouriño-Pérez RR, Freitag M, Bartnicki-García S, Roberson RW (2008) Microtubule dynamics and the role of molecular motors in *Neurospora crassa*. Fungal Genet Biol 45:683–692

Upadhyay S, Shaw BD (2008) The role of actin, fimbrin and endocytosis in growth of hyphae in *Aspergillus nidulans*. Mol Microbiol 68:690–705

Vargas M, Aronson JM, Roberson RW (1993) Cytological organization of hyphal tip cells of *Allomyces macrogynus*. Protoplasma 176:43–52

Veith D, Scherr N, Efimov VP, Fischer R (2005) Role of the spindle-pole-body protein ApsB and the cortex protein ApsA in microtubule organization and nuclear migration in *Aspergillus nidulans*. J Cell Sci 118:3705–3716

Verdín J, Bartnicki-García S, Riquelme M (2009) Functional stratification of the Spitzenkörper of *Neurospora crassa*. Mol Microbiol 74:1044–1053

Wessels JGH (1986) Cell wall synthesis in apical hyphal growth. In: Bourne GH, Jeon KW, Friedlander M (eds) International review of cytology, vol 104. Academic, Orlando, pp 37–79

Yakir-Tamang L, Gerst JE (2009) Phosphoinositides, exocytosis and polarity in yeast: all about actin? Trends Cell Biol 19:677–684

Yamazaki H, Tanaka A, Kaneko J, Ohta A, Horiuchi H (2008) *Aspergillus nidulans* ChiA is a glycosylphosphatidylinositol (GPI)-anchored chitinase specifically localized at polarized growth sites. Fungal Genet Biol 45:963–972

Zekert N, Fischer R (2009) The *Aspergillus nidulans* kinesin-3 UncA motor moves vesicles along a subpopulation of microtubules. Mol Biol Cell 20:673–684

4 Septation and Cytokinesis in Pathogenic Fungi

Míriam Osés-Ruiz[1], Wasin Sakulkoo[1], Nicholas J. Talbot[1]

CONTENTS

I. Introduction to Septation and Cytokinesis

Cell division in all organisms requires the coordination of all cellular processes to allow the formation of two new individual cells. During the eukaryotic cell cycle, correct duplication of genetic material and its subsequent even division, together with the contents of the cytoplasm, are necessary for successful generation of daughter cells. In order to ensure this outcome, the cell possesses cell cycle checkpoints at crucial stages to ensure fidelity and temporal coordination of cell division. In eukaryotes the mechanism that allows final coordination of cell division and compartmentalisation of new cellular components is called cytokinesis. In fungi, cytokinesis adopts different forms. In *Saccharomyces cerevisiae* the process of cytokinesis is called budding, resulting in a new daughter cell from a bud formed by the mother, while in the other major model yeast species *Schizosaccharomyces pombe*, it is called fission and results in two daughter cells of equal size. In filamentous fungi, cytokinesis within a hypha is known as septation and results in discrete hyphal compartments within the multicellular fungal hyphal filament. The processes of budding and fission lead to separation of a daughter cell from its mother cell, allowing formation of new individuals. However, the equivalent process in filamentous fungi, septation, results in new hyphal compartments in which daughter cells remain attached to form a continuous, multicellular organism. This important principle means there are major mechanistic differences in cytokinesis among fungi. In both budding and fission yeast, for example, when a septum is being formed, septum-related proteins are recruited to the site creating a septum and establishing the plane of cell division. It is only after cytokinesis that the septum is degraded and cell separation begins. However, in filamentous fungi, cell separation does not occur, and the septum remains intact, and instead, cell wall material is deposited to the site to contribute to compartmentalisation of the organism (Fig. 4.1), although in the majority of cases, septa remain open, to allow movement of organelles and cytoplasm between fungal cells, which is important for long-distance trafficking and communication, both of which are pivotal to growth and development of filamentous fungi.

The life cycles of pathogenic fungal species are often characterised by morphogenetic

[1]Biosciences, College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK; e-mail: N.J.Talbot@exeter.ac.uk

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

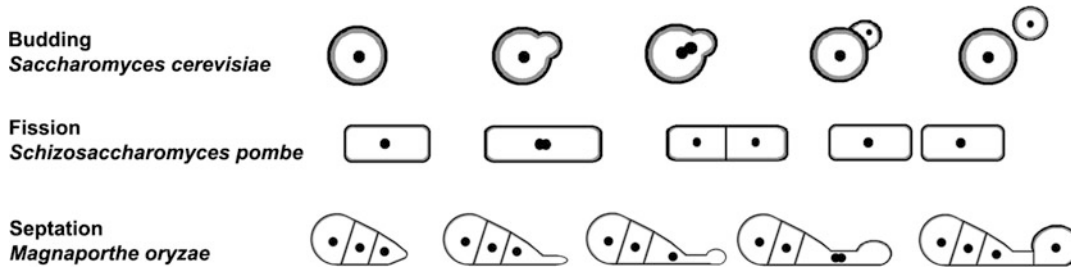


Fig. 4.1 Septation and cytokinesis in filamentous fungi and yeast model organisms. Diagram to show the patterns of nuclear division and septum formation that occur in the budding yeast *S. cerevisiae,* the fission yeast *Sch. pombe* and the filamentous, ascomycete, rice blast fungus *M. oryzae* during infection-associated development

changes, with different fungal growth forms being associated with primary host infection, invasive growth, propagation and dispersal. Many of these developmental transitions have been shown to be essential for the ability to cause disease. For example, the opportunistic pathogen of humans, *Candida albicans*, the cotton pathogen *Ashbya gossypii* and the corn smut fungus *Ustilago maydis* all display pleomorphism with yeast-hyphal transitions and formation of other differentiated cellular forms such as resting spores. These are essential to their ability to cause disease. On many instances, a discrete septation event is necessary for developmental transitions required to cause disease. For example in *Candida albicans*, the core septin GTPase, Cdc12, is associated with cytokinesis and required for virulence (Li et al. 2012). This is part of an emerging body of evidence which suggests that septation and cytokinesis are often key processes in conditioning fungal pathogenicity, due to their important role in infection-related morphogenesis and the changes in cellular form that accompany host invasion (Bridges and Gladfelter 2014). In this chapter, we evaluate emerging evidence that points to a vital role for septation in plant pathogenic fungi, which undergo appressorium development to facilitate entry into their plant hosts. We specifically focus on the filamentous ascomycete fungus *Magnaporthe oryzae*, which is the causal agent of rice blast disease. We have focused on rice blast because of rapid progress in this appressorium-forming fungus compared to other species, which has largely resulted from the size of its research community, driven by the economic importance of the disease. We draw conclusions, however, which we believe may be of more general significance in plant pathogens and, indeed, in other pathogenic fungal species. We also describe parallel studies in other experimental systems that point to more general principles. Finally, we look ahead to the research that will be needed to translate recent advances in understanding the cell biology of infection into a set of more general concepts about fungal biology.

II. The Rice Blast Fungus *Magnaporthe oryzae*

Rice blast disease represents a worldwide threat to food security, causing severe crop losses every year (Talbot 2003). In order to feed the growing world population, which is estimated to reach 9 billion by 2050, global food production will need to increase by at least threefold over the next three decades (Godfray et al. 2010). It is estimated that at least 800 million of the world's population currently live with insufficient nutrition and at least 10 % of the world's food crop production is lost due to plant diseases and infection (Strange and Scott 2005). One of the world's most important crops is rice (*Oryza sativa*), upon which half of the world's population relies (Strange and Scott 2005) and which supplies 23 % of the total calorific intake of humankind. Global rice production is 874 million tonnes per annum (FAO Figures 2012–2013), but as the world population grows, it is predicted that rice yields will

have to double by 2050 to meet increasing demand and projected population growth (Godfray et al. 2010).

Rice blast disease is caused by the heterothallic ascomycete fungus *Magnaporthe oryzae* and is responsible for up to 30 % annual losses to the rice harvest (Talbot 2003; Thinlay et al. 2000) (Talbot 2003; Thinlay et al. 2000). The name 'rice blast' is due to rapid development of the disease in the field, causing 'blasting' of entire crops (Ou 1985). *M. oryzae* can, however, also infect other important grass crops, such as barley (*Hordeum vulgare*), finger millet (*Eleusine coracana*) and wheat (*Triticum aestivum*) (Talbot 2003). Furthermore, blast disease of wheat, known as 'brusone' disease, which was first reported in 1985, is an emerging problem across South America, and, due to the lack of effective disease control by either resistant cultivars or fungicide treatment, serious outbreaks of brusone have occurred since 2009, especially across Brazil, Paraguay and Bolivia (Maciel et al. 2014).

Rice blast disease is currently controlled by fungicide treatment or disease resistant cultivars, developed by plant breeding. These methods have had limited success, however, because of the rapid emergence of new pathotypes, fungicide-resistant strains and the high genetic variability of *M. oryzae* in the field (Latterell and Rossi 1986). As a consequence of its importance and difficulty to control, *M. oryzae* has emerged as a model pathogenic fungus over the last 30 years for understanding fungal-pathogen interactions (Dean et al. 2005; Perez-Nadales et al. 2014).

III. Septation and Cytokinesis in *Magnaporthe oryzae*

In filamentous fungi, septation is required for fungal growth, differentiation and cell division and thereby guarantees correct compartmentalisation and development. Septation can be divided into four main steps. These are (1) selection of a future division plane, (2) assembly at this site of a contractile actomyosin ring (CAR), (3) constriction of the CAR coupled with plasma membrane invagination and (4) formation and deposition of cross-wall materials (Harris et al. 1994).

The septation site selection systems used in the model yeasts, *S. cerevisiae* and *Sch. pombe*, are fundamentally different. In *S. cerevisiae*, for instance, the site of a future bud is determined by a cortical cue generated by the previous cell division and cell-end-dependent spatial signals (Wu et al. 2013). After the site is selected, the mitotic nucleus is positioned in the selected site, and a nuclear division across the cell division plane occurs (Wu et al. 2013). By contrast, in *Sch. pombe*, the future site of cytokinesis is specified by the pre-mitotic nucleus itself with negative signals originating from both cell ends (Chang and Peter 2003). Interestingly, septation in filamentous fungi is a variable process that has fundamental similarities and differences from that of the model yeast organisms. For example, selection of the future septation site in the two filamentous *Pezizomycotina* species, *Aspergillus nidulans* and *Neurospora crassa*, employs a closely related programme to the one of fission yeast (reviewed by Seiler and Justa-Schuch (2010)). In other filamentous fungi, however, septation is only loosely coordinated with nuclear division, resulting in multinuclear hyphal compartments with daughter cells remaining attached to one another (Gladfelter 2006). For example, during conidial germination in *A. nidulans*, the conidium undergoes a short period of isotropic growth before the emergence of a germ tube. Only once the germ tube has reached a certain size and after the third round of mitosis has occurred is the first septum formed between two nuclei near the base of the germ tube (Harris et al. 1994). Using conditional mutants defective in cell cycle control and nuclear migration, Wolkow et al. (1996) demonstrated that formation of the first septum is triggered by nuclear division, after the cell reaches a certain threshold size. Therefore, in a similar manner to fission yeast, the position of the *A. nidulans* mitotic nucleus specifies the future site of septation (Wolkow et al. 1996). One hypothesis that may explain the multinucleate nature of *A. nidulans* hyphae is that a gradient of an inhibitory factor is formed, which originates

at the hyphal tip, that restricts septum formation until each cell achieves a certain size. However, the exact nature of such an inhibitory gradient controlling septation of *A. nidulans* germling remains unknown.

The septation initiation network (SIN) was first described in *Sch. pombe* and consists of a signal transduction cascade which ensures temporal coordination between nuclear division and cytokinesis. Septation initiation has also been extensively studied in filamentous fungi, and it has been shown to have a number of functions, one of which is to act as a checkpoint to ensure coordination of the cell cycle when there are defects in CAR assembly or integrity. Activation of SIN depends on cyclin-dependent kinase (Cdk) activity and has been shown to activate a guanine nucleotide exchange factor-GTPase-activating protein (GEF-GTPase) module, composed of Bud3 and Rho4, which triggers recruitment of formins, which nucleate actin filaments to initiate CAR assembly (Si et al. 2010). In *A. nidulans*, a homologue of the SIN protein kinase Cdc7 from fission yeast, SepH, for example, is required for formation of contractile actin ring during cytokinesis. When SepH activity is disrupted, it prevents Bud3 from localising as a ring at the incipient septation site, thereby preventing septum formation. At the same time, *A. nidulans* mutants lacking Bud3 also fail to produce septate hyphae (Bruno et al. 2001; Si et al. 2010). In the filamentous ascomycete *Neurospora crassa*, the Rho-type GTPase Rho4, along with its GEFs BudS and Rgf3, activates septation. Rho4 is dependent on an anilin-like landmark protein Bud4 which accumulates as punctate structures at the plasma membrane at the future site of septation, which coalesce into a ring during septation (Justa-Schuch et al. 2010). Rho4 and Bud3 are proposed to recruit and trigger the formin Bni1, which regulates the position and formation of the CAR. Formins are conserved nucleators of actin filaments and contain a formin homology 1 domain (FH1), which serves as a binding site for the profilin-actin complex, and a formin homology 2 domain (FH2), which regulates actin nucleation and cable assembly. Upon binding with activated Rho GTPases, formins become activated and able to catalyse actin nucleation (Evangelista et al. 2002). *A. nidulans* and *N. crassa* possess a single formin, namely SepA and Bni1, respectively, which are essential for viability. A *sepA* conditional mutant causes dramatic defects in hyphal development, and hyphae are morphologically abnormal and aseptate. In both *A. nidulans* and *N. crassa*, the formins localise as a crescent at hyphal tips or at the extreme apex of a hypha and as dot-like structures which colocalise with the lipophilic styryl dye, FM4-64, in the Spitzenkörper and to the septum where they promote CAR formation. Recruitment of SepA and Bni1 to the site of incipient CAR formation can be prevented in *A. nidulans* and *N. crassa* in mutants lacking Bud3 or Bud4 homologues. Therefore, in *A. nidulans* and probably *N. crassa* too, the SIN is likely to operate upstream of the Bud3-Rho4 module controlling selection of future septation sites by recruiting formins to the incipient septum (Si et al. 2010). How the precise position of the CAR is determined in filamentous fungi and how CAR formation is controlled are not well understood and require further investigation.

In vegetative hyphae of *M. oryzae,* the site of septation is consistently associated with the medial position of the spindle during the preceding nuclear division. This pattern also occurs during hyphal branching, defining the position of the subsequent septum, and cellular compartments are, as a consequence, evenly distributed along hyphae with a relatively uniform intercalary length and a single nucleus typically maintained in each hyphal cell. Therefore, mitosis and cytokinesis are spatially coupled during hyphal growth of *M. oryzae.* However, strikingly it has been shown that cytokinesis is regulated asymmetrically from the position of nuclear division during the formation of appressoria for plant infection (Saunders et al. 2010b).

M. oryzae undergoes several morphogenetic transitions before successful formation of the appressorium, which leads to cuticle rupture and entry of the fungus into rice tissue. The infection cycle of *M. oryzae* starts when a three-celled conidium lands on a rice leaf, to which it attaches by secreting spore tip mucilage from an apical compartment at the apex of the

pyriform conidium (Hamer et al. 1988). From the apical cell, a polarised germ tube elongates and, after 4–6 h, changes direction to form a flattened hook at the leaf surface and, ultimately, to form a unicellular dome-shaped appressorium. The appressorium generates a melanin layer in the inner part of the chitin-rich cell wall, which prevents efflux of osmotically active solutes in the appressorium. As a consequence, the appressorium takes up external water against the high osmotic gradient generated inside the cell by accumulation of polyols, such as glycerol (de Jong et al. 1997; Talbot 2003). High turgor inside the appressorium is focused as mechanical force at the appressorium pore to pierce the tough cuticle of the leaf (Howard et al. 1991; Howard and Valent 1996; de Jong et al. 1997; Talbot 2003). At the same time, generation of turgor is achieved by relocation of storage products from the conidium to the incipient appressorium, followed by autophagic cell death of the conidium (Kershaw and Talbot 2009; Thines et al. 2000). To pierce the cuticle of the leaf, the fungus develops a penetration peg, which is a specialised narrow hypha that emerges from the appressorium pore (Bourett 1990). After breaching the cuticle, the peg develops into a primary invasive hypha which at its tip forms a biotrophic interfacial complex (BIC). The BIC is a membrane-rich plant-derived structure, which may be associated with fungal effector delivery into the plant or be a focal immune response by the host plant. The primary invasive hypha develops into bulbous invasive hyphae to invade and colonise the initial epidermal cell and adjacent tissues (Zhang and Xu 2014), and the BIC becomes associated with a subapical invasive cell during further fungal proliferation and appears to be the site of active fungal secretion (Giraldo et al. 2013). After 3 days, small oval lesions appear on the leaf and become necrotic, and after 5 days they develop aerial conidiophores which sporulate profusely and are splash dispersed to adjacent plants (Talbot 2003).

The infection cycle is tightly dependent on cell cycle regulation (Saunders et al. 2010a; Talbot 2003). The switch from anisotropic growth at the germ tube tip to isotropic growth, which leads to formation of the incipient appressorium, depends on successful DNA replication (S phase entry) in *M. oryzae* (Saunders et al. 2010a). After hooking, a single round of mitosis must occur prior to development of a mature appressorium and subsequent infection. Temperature-sensitive mutants in the mitosis-promoting kinase, NimA, which is associated with regulation of the G2 to M transition, prevents maturation of appressoria (Saunders et al. 2010a). By contrast, appressoria still form in a conditional mutant, bimE, which is blocked within mitosis and when a stabilised version of the B cyclin, Cyc1, is expressed preventing mitotic exit. When considered together, these experiments suggest that entry into S phase controls initiation of appressorium development, its maturation requires entry into mitosis and completion of mitosis is a prerequisite for the appressorium to be functional (Saunders et al. 2010a).

A single septation event occurs during appressorium development at the neck of the appressorium, where it is joined to the germ tube tip (Fig. 4.2). The position of this septum is first defined by a hetero-oligomeric septin complex which occurs prior to mitosis. The contractile actomyosin ring (CAR) then forms at the neck of the appressorium after mitosis allowing nuclear migration into the developing appressorium. After this single round of mitosis, one of the daughter nuclei migrates back into the conidial cell from which its mother nucleus originated. This leads to degeneration of the conidium in an autophagy-dependent process leaving a single nucleus in the appressorium at the time of plant penetration (Kershaw and Talbot 2009; Veneault-Fourrey et al. 2006). Penetration of the fungus into plant tissue occurs by formation of a peg emerging from the appressorial pore, which develops into the primary invasive hypha. Therefore, the appressorium pore marks the point of the direct contact between the host and the fungus and is marked by a very thin pore wall overlay at the contact point with the rice leaf (de Jong et al. 1997; Howard et al. 1991; Howard and Valent 1996; Talbot 2003). At the

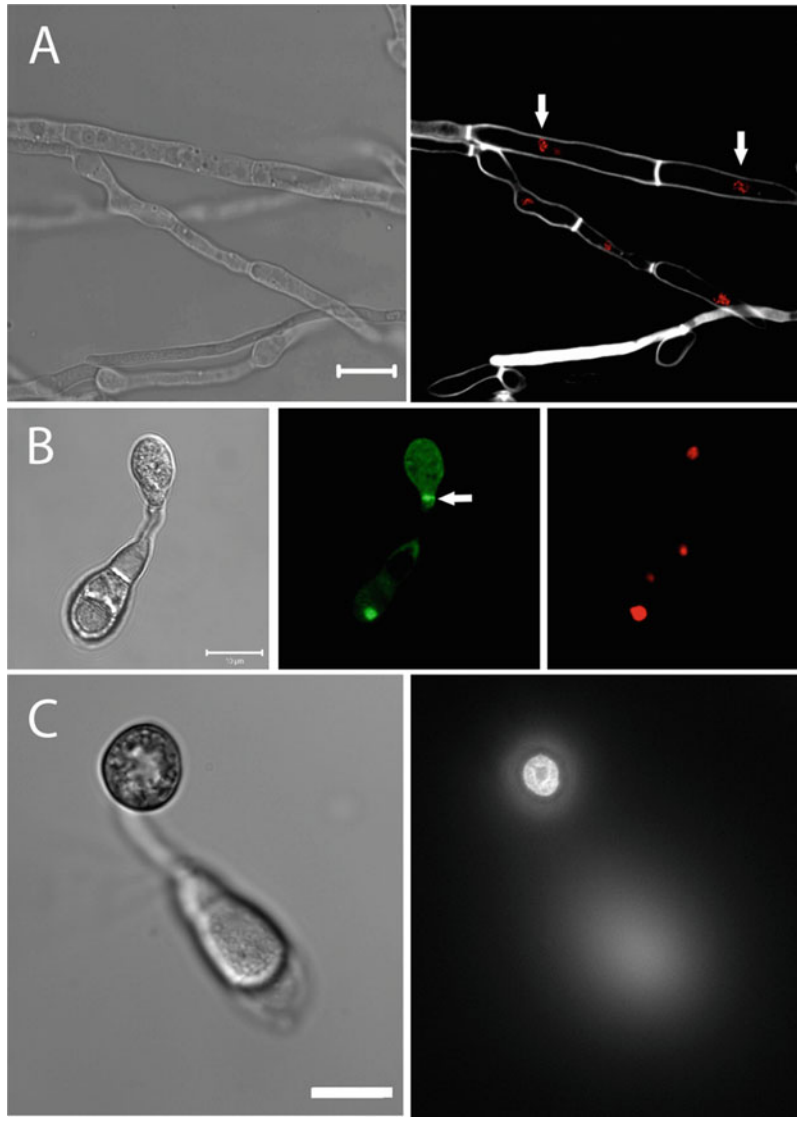


Fig. 4.2 Septation during different developmental stages of the plant pathogenic fungus *M. oryzae*. (**a**) Micrographs showing vegetative hyphae of *M. oryzae* strain expressing nuclear marker, fluorescently labelled histone H1, with cell wall staining by calcofluor white. *White arrows* indicate fungal nuclei. (**b**) Micrographs showing contractile actomyosin ring at the neck of developing appressorium marked by the accumulation of fluorescently tagged tropomyosin (*white arrow*). (**c**) Septin ring formation around appressorium pore in mature appressorium of *M. oryzae* strain expressing Sep4-GFP fusion (Dagdas et al. 2012) (Images (**a**) and (**b**) were taken from Diane G.O. Saunders' PhD thesis. Scale bar = 10 μm)

appressorium pore, the core septin GTPases, Sep3, Sep4, Sep5 and Sep6 (which are homologues of Cdc3, Cdc10, Cdc11 and Cdc12 from *S. cerevisiae*), form a toroidal ring structure at the base of the appressorium. This structure is required for appressorium-mediated plant penetration (Dagdas et al. 2012).

IV. Septin Ring Formation at the Base of the Appressorium

During cytokinesis, the coordinated interaction of cytoskeletal components is required for assembly and progressive constriction of the

Table 4.1 Septin homologues and their biological functions in selected model fungi

Species	Septin homologues	Biological functions	References
Saccharomyces cerevisiae	Cdc3, Cdc10, Cdc11, Cdc12 and Shs1	Scaffold or diffusion barrier during cytokinesis and cell polarity	Oh and Bi (2011)
Ashbya gossypii	Cdc3, Cdc10, Cdc11, Cdc12 and Shs1	Sporulation, limiting hyphal diameter and polarity and branch pattern	Schmitz et al. (2006)
Aspergillus nidulans	AspA, AspB, AspC, AspD and AspE	Chitin deposition, branching pattern and conidiophore development	Momany et al. (2001)
Candida albicans	Cdc3, Cdc10, Cdc11, Cdc12 and Sep7	Scaffold, cell division, cell polarity, chitin deposition, virulence	Warenda and Konopka (2002)
Magnaporthe oryzae	Sep3, Sep4, Sep5, Sep6 and a putative AspE homologue	Plant penetration and morphogenesis of infection structure	Dagdas et al. (2012), Saunders et al. (2010b)
Ustilago maydis	Sep1, Sep2, Sep3 and Sep4	Cellular morphology, cell division of budding haploid cells, minor role in virulence	Alvarez-Tabares and Perez-Martin (2010)

actomyosin ring at the cell cortex, as well as for scaffolding the cross-wall-synthesising machinery during cytokinesis and septation. The CAR consists of actin, myosin II and other actin-binding proteins and is assembled at the septation site followed by actomyosin-mediated constriction, which ultimately leads to membrane invagination and deposition of cell wall components (Wu et al. 2013).

The role of actin during septation in filamentous fungi was first investigated in *A. nidulans* by using the actin-depolymerising agent cytochalasin A and its effect on septum formation and cytokinesis (Harris et al. 1994). The CAR marks the future site of septation and forms a cortical ring which constricts leading to invagination of the plasma membrane. The CAR later broadens, invaginates and forms an hourglass structure at the site of septation (Momany and Hamer 1997). In *N. crassa*, by using live-cell imaging of fluorescently tagged proteins, the accumulation of actin cables was shown as a mass of thick filaments associated with tropomyosin and class II myosin. This 'septal actomyosin tangle' (SAT) was identified as the earliest marker of septum development (Delgado-Alvarez et al. 2014). The SAT then condenses into the F-actin ring of the CAR. After that, the actin ring splits into two rings, and cross-wall materials are deposited (Berepiki et al. 2010; Delgado-Alvarez et al. 2014).

Septins are morphogenetic GTPases that act as cytoskeletal components by forming hetero-oligomers and various higher-order structures, including filaments, rings and gauzes, and serving as platforms for recruitment and organisation of proteins at sites of polarised growth and cytokinesis (Oh and Bi 2011). They were first identified in *S. cerevisiae* through analysis of mutants unable to complete cytokinesis and which produced elongated buds that do not separate from the mother cell (Hartwell 1971). Later studies defined four important groups of septins from metazoans and an extra group, specific to filamentous fungi (Pan et al. 2007), as shown in Table 4.1. *S. cerevisiae* possesses five septins, Cdc3, Cdc10, Cdc11, Cdc12 and Shs1, of which the first four are considered core septins. During budding, the initial septin ring forms an hourglass structure, called the septin collar, linked with the plasma membrane at the bud neck. The septin collar scaffolds proteins associated with cell division and septation, but also acts as a lateral diffusion barrier to spatially regulate morphogenetic factors to the site of cytokinesis (Oh and Bi 2011). Recently, septins have been demonstrated to serve roles in cell signalling, membrane remodelling, cellular morphology and, most importantly, pathogenesis in filamentous fungi (Bridges and Gladfelter 2014). Septins in filamentous fungi appear to be able to form more diverse higher-order structures than those of unicellular yeasts (Hernandez-Rodriguez et al. 2012). For example, the filamentous fungus *A. nidulans* possesses four

core septins, AspA, AspB, AspC and AspD, that form hetero-polymeric complexes, while the additional septin, AspE, plays a minor role in stabilising other core septins (Hernandez-Rodriguez et al. 2014). At the septation site, AspB forms a postmitotic single ring which then splits into two rings at the division plane across the main hypha. After completion of septation, the ring of AspB located at the basal side of the septum is lost, while the one at the apical side persists. AspB also localises into a ring on the side of hypha just before branch emergence, therefore suggesting that the AspB septin is an early marker for hyphal branch formation (Hernandez-Rodriguez et al. 2012; Westfall and Momany 2002).

In *M. oryzae*, four core septins have been identified, Sep3, Sep4, Sep5, Sep6, as well as fungal-specific septin Sep7 (Dagdas et al. 2012; Saunders et al. 2010b). The core septins showed 47 % amino acid identity to Cdc3, 55 % to Cdc10, 45 % to Cdc11 and 57 % to Cdc12 of *S. cerevisiae,* respectively. Sep7 shows 55 % identity to AspE from *Aspergillus nidulans*. During early appressorium formation, the core septins localise to the germ tube tip during its initial apical polarised growth. At 4 h, an incipient appressorium is formed, and core septins localise at the appressorial neck, prior to mitosis, at the site of the septation event that delimits the appressorium from the other pre-penetration structure. From 8 h onwards, until the primary invasive hypha is formed and the cuticle of the rice leaf has been breached, the core septins form a ring at the base of the appressorium (Dagdas et al. 2012). The septins are required for the remodelling of actin to form a toroidal F-actin network at the base of the appressorium. Null mutants in any of the core septins result in a dramatic disorganisation of the F-actin structure leading to impairment of penetration peg formation and failure to cause tissue invasion (Dagdas et al. 2012). The septin structure not only provides rigidity to the cortex around the appressorium pore but also appears to act as a diffusion barrier to restrict proteins to the pore region that are required for generating curvature of the plasma membrane and F-actin polymerisation during polarity re-establishment and penetration peg development. In budding yeast, activation of the master polarity regulator, Cdc42, must occur, to generate apical polarised growth and bud formation (Howell and Lew 2012). Null mutants of *CDC42* in *M. oryzae* are able to form appressoria that have a disrupted cytoskeleton and are therefore unable to cause infection (Zheng et al. 2009). Cdc42 acts through two PAK kinases, Ste20 and Cla4, and two formins, Bni and Bnr, to activate formation of F-actin and septin toroidal ring structures (Howell and Lew 2012). The PAK kinase Cla4 homologue, Chm1, is required for septin ring formation at the base of the appressorium and is therefore necessary for infection (Dagdas et al. 2012; Kadota et al. 2004). Surprisingly, these null mutants are able to form appressoria, suggesting that the activity of septins during earlier stages of development is regulated distinctly than during appressorium function. Moreover, the mechanism involved in the formation and recruitment of the septin ring at the base of the appressorium is still not known. Other cytoskeleton components that are also localised to the appressorium pore include a homologue of the ezrin/radixin/moesin protein Tea1, which in yeast binds at its N-terminal domain to the plasma membrane to connect to F-actin (Turunen et al. 1994). Tea1 has also been reported to be required for septin-mediated scaffolding. At least one of the Bin-amphiphysin-Rvs (BAR) domain proteins in *M. oryzae* also localises to the appressorium pore in a septin-dependent manner. BAR proteins have been extensively studied in budding yeast and are involved in generation of cellular invaginations (F-BAR proteins) or protrusions (inverse or I-BAR proteins) (Dawson et al. 2006). Bar proteins bind to actin and to phosphatidylinositol (4, 5)-bisphosphate (PtdIns(4, 5)P2) (Bompard et al. 2005; Miki et al. 2009). Localisation of the BAR protein, Rvs167, in *M. oryzae* to the appressorium pore has been shown, suggesting localising of endocytic and perhaps exocytic machinery to the appressorium pore (Dagdas et al. 2012). Las17 is part of the actin-related protein 2/3 complex (Arp2/3) involved in F-actin polymerisation (Urbanek et al. 2013). The Arp2/3 complex is necessary for F-actin polymerisation, organisation and recycling (Goley and Welch 2006). In

M. oryzae Las17-GFP was shown to be localised to the appressorial pore, in a septin-dependent manner (Dagdas et al. 2012).

The septin ring in the appressorium pore therefore acts as a means of coordinating processes associated with polarity re-establishment and the rapid F-actin polymerisation and membrane curvature necessary for development and protrusion of a penetration peg at the base of the infection cell. It appears to fulfil this role by acting as a means of scaffolding these proteins at the appropriate point in the cell and spatially focusing proteins involved in polarity to the correct domain, probably by preventing their diffusion from the region. In this way, the penetration peg resembles a bud site in *S. cerevisiae*, where similar concentration of polarity determinants is required to complete budding. The key difference, however, is the generation of enormous invasive forces at the appressorium pore that are sufficient to rupture the cuticle and allow the fungus entry into epidermal cells. This implies a level of controlled application of force generated by the repolarisation process itself and the focusing of the enormous turgor of the cell at this point of contact with the underlying leaf surface. The septin complex itself appears to require regulated synthesis of reactive oxygen species (ROS) by means of the Nox2 NADPH oxidase complex in order to form (Ryder et al. 2013). Deletion of the *NOX2* gene or its cognate regulator *NOXR* prevents penetration peg formation (Egan et al. 2007) and rice blast disease because the septin ring cannot be formed, and, as a consequence, Las17, Tea1, Rvs167, Cdc42 and other components are not localised correctly to the appressorium pore (Ryder et al. 2013). It seems likely that a turgor-sensing mechanism exists in the appressorium which, upon reaching a threshold level of turgor, triggers the Nox2 complex to release ROS which acts directly on actin cytoskeletal components and, through redox signalling, affects a complex of proteins, including Bem1 and Cdc24, that leads to the activation of Chm1 and assembly of the septin complex. Understanding the details of this process and the precise function of Nox2 remains a high priority.

A. Dynamics of Septin Ring Formation in *M. oryzae*

Septin ring assembly at the base of the appressorium also requires activation of the Pmk1 and Mps1 MAPK signalling pathways (Dagdas et al. 2012). The Pmk1 MAPK signalling, homologous to the Fus3 MAP kinase signalling pathway involved in the yeast mating response and invasive growth in yeast, regulates infection-related development in *M. oryzae*. Deletion of *PMK1* results in mutants that are unable to form appressoria and fail to infect rice plant through wounds (Talbot 2003; Xu and Hamer 1996). The Δ*pmk1* phenotype suggests that Pmk1 is required for appressorium morphogenesis, but also for invasive growth in *M. oryzae* (Xu and Hamer 1996). In addition, the Mst12 transcription factor, a homologue of *S. cerevisiae* Ste12, was identified to operate downstream of the Pmk1 MAP kinase cascade and is essential for appressorium-mediated plant penetration. Δ*mst12* null mutants are able to form melanised, pressurised appressoria but cannot develop penetration pegs and, therefore, fail to grow invasively in plant tissue (Park et al. 2002, 2004). Formation of the penetration peg at the base of the appressorium also requires the Mps1 MAPK signalling pathway, homologous to the Slt2 MAP kinase-dependent cell integrity pathway in *S. cerevisiae* (Xu et al. 1998). The role of these pathways in the control of septin ring formation in the appressorium is important. Deletion of genes, encoding either the Mst12 transcription factor or the Mps1 MAP kinase, not only prevents plant penetration but also prevents assembly of the septin and actin network at the base of the appressorium (Dagdas et al. 2012). During initiation of appressorium formation, loss of apical polarised growth occurs leading to isotropic expansion of the germ tube tip and formation of the dome-shaped appressorium. Conversely penetration peg development requires correct assembly and maintenance of the septin and F-actin rings (Dagdas et al. 2012; Ryder et al. 2013). Both the Pmk1 and Mps1 MAP kinase signalling pathways are necessary for repolarisation of the appressorium. Involvement of

these signalling pathways is consistent with the functional similarities between penetration peg formation in *M. oryzae* and budding in *S. cerevisiae* in which a future bud site is specified by septin collar assembly early in the cell cycle, followed by polarised bud growth. In *M. oryzae*, emergence of the penetration peg during plant penetration involves repolarisation and maintenance of polarised cell growth at the base of the appressorium. This leads to further questions such as how appressorium-mediated plant penetration is regulated with the cell division cycle.

B. Cell Cycle Regulation of Septin Ring Formation

Recent evidence in *M. oryzae* suggests that appressorium morphogenesis is tightly linked to cell cycle control (Saunders et al. 2010a), as described above. Correct progression through DNA replication is known, for example, to be required for appressorium formation (Saunders et al. 2010a). An open question is whether the nucleus within the developing appressorium, from which all subsequent genetic information during plant infection is derived, has to be arrested in the cell cycle in order for turgor to be generated correctly prior to repolarisation of the appressorium. Recent evidence suggests that DNA replication is necessary for stabilisation of the appressorium septin ring, as HU treatment leads to breakdown of the septin ring and lack of sustained organisation of F-actin at the appressorium pore (M. Oses-Ruiz, W. Sakulkoo, and N.J.Talbot, unpublished). Furthermore, arresting the cell cycle in G1 phase appears to prevent plant infection by mature appressoria (S. Sakulkoo and N.J. Talbot, unpublished). When considered together, this suggests that progression of the appressorium nucleus into G2 may also be necessary for repolarisation of the appressorium to occur. In such a model, septin ring formation would occur in G1 as soon as a threshold level of appressorium turgor is generated, leading to cell cycle progression into S phase and subsequent extension of the penetration peg. Therefore, coordination of cell cycle regulation with septin-mediated remodelling of the F-actin cytoskeleton in the appressorium is likely to be critical for plant infection.

V. Conclusions

The control of cytokinesis and septation by pathogenic fungi is critical to the developmental changes associated with host invasion and fungal pathogenesis. Pathogenic processes, such as growth within host tissue, often require changes between yeast-like, determinate, isotropic growth and hyphal, polarised, anisotropic growth, which must be appropriately regulated in concert with nuclear division. In plant pathogenic fungi, a body of evidence, primarily generated from studies of the rice blast fungus *M. oryzae*, shows that septin-mediated remodelling of the actin cytoskeleton is fundamental to the action of its specialised infection cells, called appressoria, during plant infection. Future studies will need to ask several questions which logically follow these recent discoveries. First of all, is the role of septin GTPases in appressorium repolarisation regulated distinctly from their role in each round of septation within vegetative and invasive hyphae? For example, is there a turgor-sensing mechanism that regulates septin ring formation only once the required turgor has been generated in the infection structure to breach the plant cuticle? Is the regulated synthesis of reactive oxygen species, which is essential for septin assembly in *M. oryzae* appressoria, a common mechanism by which septin-dependent processes are regulated in fungi? Can this explain why regulated bursts of ROS are often associated with cellular differentiation in fungi, such as fruit body formation and sporulation? Are the processes identified in *M. oryzae* appressorium development highly conserved among appressorium-forming fungi, such as the rusts, anthracnose-causing fungi and the powdery mildews? If so, can we find evidence for common mechanisms that regulate appressorium-mediated plant infection, such as the conserved MAP kinase cascades (Perez-Nadales et al. 2014), that might be targeted to

develop broad spectrum anti-penetrant fungicides? Finally, it is clear that there are important parallels between the way in which yeast budding operates and the operation of fungal appressoria, particularly in the processes leading up to penetration peg formation and repolarisation. Some of the most conserved components probably play similar roles. Clearly, there are important differences as well, not least of all in the enormous invasive forces deployed by appressoria, but the conserved components involved in some of these processes can provide an important roadmap to test hypotheses and define the fundamental mechanisms by which infection structures work in plant pathogenic fungi.

References

Alvarez-Tabares I, Perez-Martin J (2010) Septins from the phytopathogenic fungus Ustilago maydis are required for proper morphogenesis but dispensable for virulence. PLoS ONE 5, e12933

Berepiki A, Lichius A, Shoji J-Y, Tilsner J, Read ND (2010) F-actin dynamics in *Neurospora crassa*. Eukaryot Cell 9:547–557

Bompard G, Sharp SJ, Freiss G, Machesky LM (2005) Involvement of Rac in actin cytoskeleton rearrangements induced by MIM-B. J Cell Sci 118:5393–5403

Bourett TMH (1990) In vitro development of penetration structures in the rice blast fungus *Magnaporthe grisea*. Can J Bot Rev Can Bot 68:329–342

Bridges AA, Gladfelter AS (2014) Fungal pathogens are platforms for discovering novel and conserved septin properties. Curr Opin Microbiol 20:42–48

Bruno KS, Morrell JL, Hamer JE, Staiger CJ (2001) SEPH, a Cdc7p orthologue from *Aspergillus nidulans*, functions upstream of actin ring formation during cytokinesis. Mol Microbiol 42:3–12

Chang F, Peter M (2003) Yeasts make their mark. Nat Cell Biol 5:294–299

Dagdas YF, Yoshino K, Dagdas G, Ryder LS, Bielska E, Steinberg G, Talbot NJ (2012) Septin-mediated plant cell invasion by the rice blast fungus, *Magnaporthe oryzae*. Science 336:1590–1595

Dawson JC, Legg JA, Machesky LM (2006) Bar domain proteins: a role in tubulation, scission and actin assembly in clathrin-mediated endocytosis. Trends Cell Biol 16:493–498

de Jong JC, McCormack BJ, Smirnoff N, Talbot NJ (1997) Glycerol generates turgor in rice blast. Nature 389:244–244

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan H et al (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434:980–986

Delgado-Alvarez DL, Bartnicki-Garcia S, Seiler S, Mourino-Perez RR (2014) Septum development in *Neurospora crassa*: the septal actomyosin tangle. PLoS ONE 9, e96744

Egan MJ, Wang ZY, Jones MA, Smirnoff N, Talbot NJ (2007) Generation of reactive oxygen species by fungal NADPH oxidases is required for rice blast disease. Proc Natl Acad Sci USA 104:11772–11777

Evangelista M, Pruyne D, Amberg DC, Boone C, Bretscher A (2002) Formins direct Arp2/3-independent actin filament assembly to polarize cell growth in yeast. Nat Cell Biol 4:260–269

Giraldo MC, Dagdas YF, Gupta YK, Mentlak TA, Yi M, Martinez-Rocha AL, Saitoh H, Terauchi R, Talbot NJ, Valent B (2013) Two distinct secretion systems facilitate tissue invasion by the rice blast fungus *Magnaporthe oryzae*. Nat Commun 4:1996

Gladfelter AS (2006) Control of filamentous fungal cell shape by septins and formins. Nat Rev Microbiol 4:223–229

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Goley ED, Welch MD (2006) The ARP2/3 complex: an actin nucleator comes of age. Nat Rev Mol Cell Biol 7:713–726

Hamer JE, Howard RJ, Chumley FG, Valent B (1988) A mechanism for surface attachment in spores of a plant pathogenic fungus. Science 239:288–290

Harris SD, Morrell JL, Hamer JE (1994) Identification and characterization of *Aspergillus nidulans* mutants defective in cytokinesis. Genetics 136:517–532

Hartwell LH (1971) Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Exp Cell Res 69:265–276

Hernandez-Rodriguez Y, Hastings S, Momany M (2012) The septin AspB in *Aspergillus nidulans* forms bars and filaments and plays roles in growth emergence and conidiation. Eukaryot Cell 11:311–323

Hernandez-Rodriguez Y, Masuo S, Johnson D, Orlando R, Smith A, Couto-Rodriguez M, Momany M (2014) Distinct septin heteropolymers co-exist during multicellular development in the filamentous fungus *Aspergillus nidulans*. PLoS ONE 9, e92819

Howard RJ, Valent B (1996) Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. Annu Rev Microbiol 50: 491–512

Howard RJ, Ferrari MA, Roach DH, Money NP (1991) Penetration of hard substrates by a fungus employing enormous turgor pressures. Proc Natl Acad Sci USA 88:11281–11284

Howell AS, Lew DJ (2012) Morphogenesis and the cell cycle. Genetics 190:51–77

Justa-Schuch D, Heilig Y, Richthammer C, Seiler S (2010) Septum formation is regulated by the RHO4-specific exchange factors BUD3 and RGF3 and by the landmark protein BUD4 in Neurospora crassa. Mol Microbiol 76:220–235. doi:10.1111/j.1365-2958.2010.07093.x

Kadota J, Yamamoto T, Yoshiuchi S, Bi E, Tanaka K (2004) Septin ring assembly requires concerted action of polarisome components, a PAK kinase Cla4p, and the actin cytoskeleton in *Saccharomyces cerevisiae*. Mol Biol Cell 15:5329–5345

Kershaw MJ, Talbot NJ (2009) Genome-wide functional analysis reveals that infection-associated fungal autophagy is necessary for rice blast disease. Proc Natl Acad Sci USA 106:15967–15972

Latterell FMR, Rossi AE (1986) Longevity and pathogenic stability of *Pyricularia oryzae*. Phytopathology 76:231–235

Li L, Zhang C, Konopka JB (2012) A Candida albicans temperature-sensitive cdc12-6 mutant identifies roles for septins in selection of sites of germ tube formation and hyphal morphogenesis. Eukaryot Cell 11:1210–1218

Maciel JL, Ceresini PC, Castroagudin VL, Zala M, Kema GH, McDonald BA (2014) Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil. Phytopathology 104:95–107

Miki S, Matsui K, Kito H, Otsuka K, Ashizawa T, Yasuda N, Fukiya S, Sato J, Hirayae K, Fujita Y et al (2009) Molecular cloning and characterization of the AVR-Pia locus from a Japanese field isolate of *Magnaporthe oryzae*. Mol Plant Pathol 10:361–374

Momany M, Hamer JE (1997) Relationship of actin, microtubules, and crosswall synthesis during septation in *Aspergillus nidulans*. Cell Motil Cytoskeleton 38:373–384

Momany M, Zhao J, Lindsey R, Westfall PJ (2001) Characterization of the *Aspergillus nidulans* septin (asp) gene family. Genetics 157:969–977

Oh Y, Bi E (2011) Septin structure and function in yeast and beyond. Trends Cell Biol 21:141–148

Ou SH (1985) Rice disease. Commonwealth Mycological Institute, Surrey

Pan F, Malmberg RL, Momany M (2007) Analysis of septins across kingdoms reveals orthology and new motifs. BMC Evol Biol 7:103

Park G, Xue C, Zheng L, Lam S, Xu JR (2002) MST12 regulates infectious growth but not appressorium formation in the rice blast fungus *Magnaporthe grisea*. Mol Plant Microbe Interact 15: 183–192

Park G, Bruno KS, Staiger CJ, Talbot NJ, Xu JR (2004) Independent genetic mechanisms mediate turgor generation and penetration peg formation during plant infection in the rice blast fungus. Mol Microbiol 53:1695–1707

Perez-Nadales E, Almeida Nogueira MF, Baldin C, Castanheira S, El Ghalid M, Grund E, Lengeler K, Marchegiani E, Mehrotra PV, Moretti M et al (2014) Fungal model systems and the elucidation of pathogenicity determinants. Fungal Genet Biol 70:42–67

Ryder LS, Dagdas YF, Mentlak TA, Kershaw MJ, Thornton CR, Schuster M, Chen J, Wang Z, Talbot NJ (2013) NADPH oxidases regulate septin-mediated cytoskeletal remodeling during plant infection by the rice blast fungus. Proc Natl Acad Sci USA 110:3179–3184

Saunders DG, Aves SJ, Talbot NJ (2010a) Cell cycle-mediated regulation of plant infection by the rice blast fungus. Plant Cell 22:497–507

Saunders DG, Dagdas YF, Talbot NJ (2010b) Spatial uncoupling of mitosis and cytokinesis during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*. Plant Cell 22:2417–2428

Schmitz HP, Kaufmann A, Kohli M, Laissue PP, Philippsen P (2006) From function to shape: a novel role of a formin in morphogenesis of the fungus *Ashbya gossypii*. Mol Biol Cell 17: 130–145

Seiler S, Justa-Schuch D (2010) Conserved components, but distinct mechanisms for the placement and assembly of the cell division machinery in unicellular and filamentous ascomycetes. Mol Microbiol 78:1058–1076

Si H, Justa-Schuch D, Seiler S, Harris SD (2010) Regulation of septum formation by the Bud3-Rho4 GTPase module in *Aspergillus nidulans*. Genetics 185:165–176

Strange RN, Scott PR (2005) Plant disease: a threat to global food security. Annu Rev Phytopathol 43: 83–116

Talbot NJ (2003) On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. Annu Rev Microbiol 57:177–202

Thines E, Weber RW, Talbot NJ (2000) MAP kinase and protein kinase A-dependent mobilization of triacylglycerol and glycogen during appressorium turgor generation by *Magnaporthe grisea*. Plant Cell 12:1703–1718

Thinlay X, Finckh MR, Bordeos AC, Zeigler RS (2000) Effects and possible causes of an unprecedented rice blast epidemic on the traditional farming system of Bhutan. Agric Ecosyst Environ 78: 237–248

Turunen O, Wahlstrom T, Vaheri A (1994) Ezrin has a COOH-terminal actin-binding site that is conserved in the ezrin protein family. J Cell Biol 126:1445–1453

Urbanek AN, Smith AP, Allwood EG, Booth WI, Ayscough KR (2013) A novel actin-binding motif in Las17/WASP nucleates actin filaments independently of Arp2/3. Curr Biol 23:196–203

Veneault-Fourrey C, Barooah M, Egan M, Wakley G, Talbot NJ (2006) Autophagic fungal cell death is

necessary for infection by the rice blast fungus. Science 312:580–583

Warenda AJ, Konopka JB (2002) Septin function in *Candida albicans* morphogenesis. Mol Biol Cell 13:2732–2746

Westfall PJ, Momany M (2002) *Aspergillus nidulans* septin AspB plays pre- and postmitotic roles in septum, branch, and conidiophore development. Mol Biol Cell 13:110–118

Wolkow TD, Harris SD, Hamer JE (1996) Cytokinesis in *Aspergillus nidulans* is controlled by cell size, nuclear positioning and mitosis. J Cell Sci 109:2179–2188

Wu CF, Savage NS, Lew DJ (2013) Interaction between bud-site selection and polarity-establishment machineries in budding yeast. Philos Trans R Soc Lond Ser B Biol Sci 368:20130006

Xu JR, Hamer JE (1996) MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. Genes Dev 10:2696–2706

Xu J-R, Staiger CJ, Hamer JE (1998) Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci 95:12713–12718

Zhang S, Xu JR (2014) Effectors and effector delivery in *Magnaporthe oryzae*. PLoS Pathog 10, e1003826

Zheng W, Zhao Z, Chen J, Liu W, Ke H, Zhou J, Lu G, Darvill AG, Albersheim P, Wu S et al (2009) A Cdc42 ortholog is required for penetration and virulence of *Magnaporthe grisea*. Fungal Genet Biol 46:450–460

5 The Ascomycetous Cell Wall: From a Proteomic Perspective

Piet W.J. de Groot[1], Albert D. de Boer[1], Bernd W. Brandt[2], Eulogio Valentín[3]

CONTENTS

I. Introduction

Fungal cell walls are essential organelles that define cell shape and provide osmotic integrity and strength to resist the high internal turgor pressure (Klis et al. 2007b). For pathogenic fungi, the fungal wall is the interface between pathogen and host and therefore plays an important role in the infection process and in host immune recognition (Hall and Gow 2013). The cell wall also provides protection against harmful factors in the external growth environment through its limited porosity and scavenging of oxidative molecules, while at the same time, it also plays a role in acquiring scarce nutrients such as iron from the environment (Klis et al. 2007b; Latgé 2007). Ultrastructural studies using electron microscopy have shown the cell walls in many ascomycetous fungi as a bilayered structure, an internal polysaccharide matrix that is surrounded by a layer mainly packed with secretory glycoproteins; see, for instance, Backhaus et al. (2010), De Groot et al. (2008), Ene et al. (2012), Kurtz et al. (1994), Osumi (2012), Schoffelmeer et al. (1999), and Tokunaga et al. (1986). Based on ample evidence from biochemical and proteomic studies with purified wall extracts, it is generally assumed that the proteins in the outer layer are covalently bound to the internal glycan matrix (Klis et al. 2007b, 2009; Ecker et al. 2006; Fujii et al. 1999). Although long thought to be a static structure, it is now known that the fungal cell wall is dynamic and both the polysaccharide and protein compositions are growth phase dependent and continuously change to adapt to the environment (Smits et al. 1999; Latgé 2007). These modifications also help pathogenic fungi to escape from host immune systems (Latgé and Beauvais 2014). Specific cell wall proteins have also been shown to be involved in biofilm formation and adhesion to host cell tissues or to abiotic compounds, such as the plastics or silicones that are used in medical devices, or have proteolytic activity that may help to degrade and provide access into host tissues (De Groot et al. 2005; Heilmann et al. 2012; Klis et al. 2009).

The fungal cell wall accounts for 15–30 % of the cellular dry weight and therefore represents

[1]Regional Center for Biomedical Research, Albacete Science and Technology Park, University of Castilla–La Mancha, 02008 Albacete, Spain; e-mail: piet.degroot@uclm.es
[2]Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University, Amsterdam, The Netherlands
[3]Departamento de Microbiología y Ecología, Universidad de Valencia, Burjassot, Valencia, Spain

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

a considerable metabolic investment. An estimation for yeast, based on phenotypic analysis of a large number of deletion mutants, indicated that about 20 % of its genes are directly or indirectly involved in cell wall biology (De Groot et al. 2001; Klis et al. 2014). As the fungal cell wall is an essential organelle and is composed of molecules that are mostly absent in humans, it is a very attractive target for the development of safe antifungal drugs to combat fungal infections. Therefore, it is perhaps not surprising that among the newest types of marketed antifungals are the echinocandins caspofungin, micafungin, and anidulafungin that act by inhibiting the synthesis of β-1,3-glucans, a common and abundant wall molecule of the wall matrix in most fungi.

In terms of cell wall biology, the most thoroughly studied fungus is the ascomycetous budding yeast *Saccharomyces cerevisiae*, and several very good reviews are available; see, for instance, Orlean (2012), Lipke and Ovalle (1998), Klis et al. (2006), Lesage and Bussey (2006), and references therein. The paper by P. Orlean (2012) is of special interest as it very comprehensively describes biosynthesis and architecture of the *S. cerevisiae* cell wall and the (putative) roles of the genes involved in great detail. The main components of the *S. cerevisiae* wall are β-1,3-glucan, β-1,6-glucan, chitin, and mannoproteins (Fig. 5.1). Because of the relatively simple lifestyle as unicellular organism and the wealth of information and genetic tools available, the cell wall of *S. cerevisiae* has served as a model for the studies of many other fungal species, budding yeasts, as well as more distantly related species. For instance, biochemical studies in *Candida albicans* and *Candida glabrata*, using experimental approaches and tools that were first applied to *S. cerevisiae*, confirmed that these yeast species have a very similar wall architecture (De Groot et al. 2008; Orlean 2012; Klis et al. 2001). Moreover, in recent years, several publications presenting the genome sequences of new species, also more distantly related ones, have successfully used *S. cerevisiae* genes involved in various aspects of cell wall biosynthesis as queries to find homologous genes (Butler et al. 2009; De Groot et al. 2009; Pel et al. 2007; Gioti et al. 2013; Jackson et al. 2009). In this way, many of the genes and pathways putatively involved in wall synthesis can easily be identified and may lead to a rough tentative description of the cell wall structure. For instance, β-1,3-glucan and chitin, two of the macromolecules in the cell wall of *S. cerevisiae,* are widely distributed across the fungal kingdom, and homologues of the genes encoding proteins involved in their synthesis, regulation, modification, and degradation are consistently identified with this approach. However, cell wall structures of different fungi are diverse. Therefore, when undertaking such a screening approach, it is important to incorporate genes involved in biosynthesis of wall components from other fungi that are lacking in *S. cerevisiae*. For instance, in the fission yeast *Schizosaccharomyces pombe*, an important cell wall molecule is α-1,3-glucan and the gene encoding the α-1,3-glucan synthase has been described in detail (Hochstenbach et al. 1998; Katayama et al. 1999). In *Aspergillus*, a linear β-1,3/1,4-glucan has been identified as well as galactomannan (Fontaine et al. 2000) that is probably bound to protein (Klis et al. 2007b). Thus, making a genomic inventory can provide a wealth of information concerning cell wall biosynthesis, even for very distantly related species; nonetheless, in doing so, one should take care not to draw firm conclusions until the findings are supported by biochemical evidence.

In the previous volume of The Mycota published in 2007, "a molecular and genomic view of the fungal cell wall" based on available biochemical data and genome information was presented by F. Klis and colleagues (2007b). Descriptions of cell wall structures in this chapter included ascomycetous yeasts, dimorphic and filamentous species, as well as some basidiomycetes. Rather than repeating these descriptions, in this chapter, we will focus on new data that has become available since then. As a result of having complete genome sequences available, a line of research that has developed fast during the previous years is identifying cell wall proteins (CWPs), also termed (cell) wall proteomics, using tandem

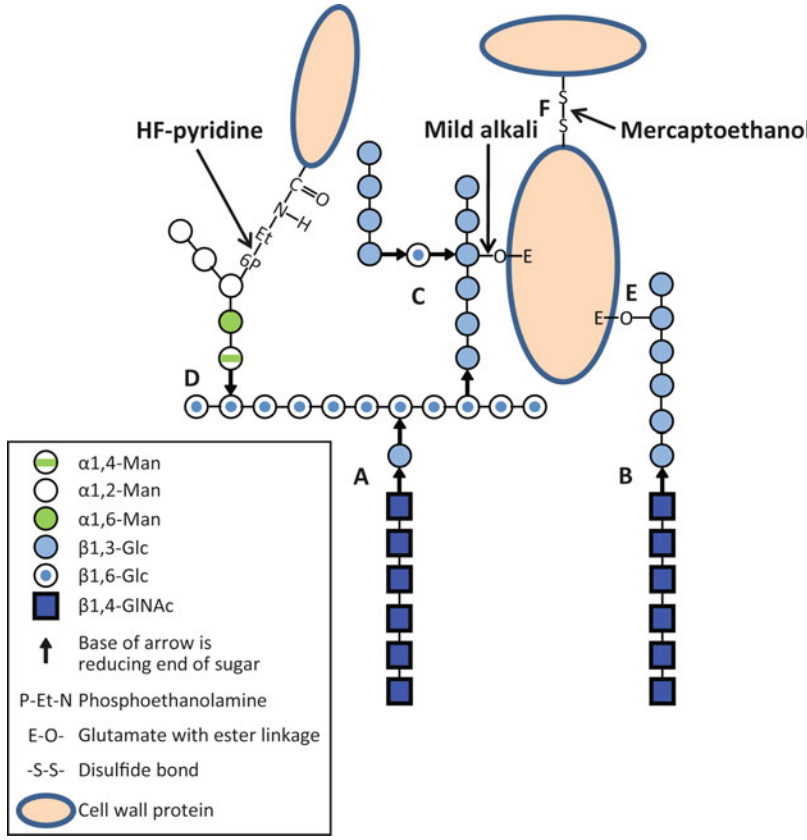


Fig. 5.1 Cell wall components and linkages in *S. cerevisiae*. (**a**) Linkage between β-1,4-N-acetylglucosamine (β1,4-GlcNAc) and a side branch of β-1,3-glucan (β1,3-Glc) that is linked to β-1,6-glucan (β1,6-Glc). The reducing end is from β1,4-GlcNAc. (**b**) Linkage between β1,4-GlcNAc and the nonreducing end of β1,3-Glc. (**c**) Linkage between β1,3-Glc and a side branch of β1,6-Glc that is linked to β1,3-Glc. The reducing end is from β1,3-Glc. (**d**) Linkage between GPI-glycan (possibly the α-1,4-mannose) to an internal glucose residue in β1,6-Glc (linkage to the nonreducing end of β1,6-Glc is also possible). (**e**) Ester linkage between the glutamate in the repeats of ASL proteins and β1,3-Glc. (**f**) Disulfide bond between cell wall proteins. Chemical treatments to release cell wall proteins are indicated [Figure was adapted from Orlean (2012)]

mass spectrometry. To date, wall proteomics research has largely focused on ascomycetes. Therefore, we will update our views on the cell wall structures of ascomycetes by discussing new data that is mostly from wall proteomics studies. In addition, interesting work has been presented in recent years to address the question how different cell wall polymers, which are individually synthesized by membrane-localized enzymes, become modified and covalently connected to each other and assemble into the multifunctional cell wall structures that are essential for fungal existence.

II. Cell Wall Proteins

A. Three Classes of Cell Wall Proteins

Based on the work on the model organism *S. cerevisiae*, three types of covalently bound cell wall proteins (CWPs) have been described (Fig. 5.1). Most of the glycoproteins in the outer layer are glycosylphosphatidylinositol (GPI)-modified proteins. Depending on the presence of a GPI anchor signal peptide in their C-terminal region, secretory proteins are cleaved and the new C-terminus is coupled to a preformed GPI

glycolipid in the luminal leaflet of the endoplasmic reticulum (ER) membrane by a transamidase complex. Most proteins that transit through the secretory pathway, including GPI proteins, are decorated with carbohydrate side chains in the ER and Golgi apparatus by *O*- and/or *N*-glycosylation. For reviews on glycosylation, especially focusing on *S. cerevisiae* and *Candida albicans*, we refer to Cutler (2001), Ernst and Prill (2001), Tanner and Lehle (1987), and Hall and Gow (2013). In *S. cerevisiae* and related species, glycosylations are mainly additions of mannose residues, which generally account for 80–90 % of the mass of the proteins, hence called mannoproteins. In other species, such as filamentous *Aspergillus* and *Trichoderma* spp., *O*-glycans have been found to be more diverse and contain galactosyl in addition to mannosyl residues (Goto 2007). Upon arrival at the cell surface, the GPI-CWPs are cleaved in the glycan part of their GPI anchor and become linked to β-1,6-glucan. Although solid biochemical evidence is still lacking, genetic evidence is emerging that GPI-anchored proteins of the Dfg5 family with similarity to α-1,6-mannanases are involved in this step (Maddi et al. 2012; Kitagaki et al. 2002; Spreghini et al. 2003). Although the actual link between GPI-CWPs and β-1,6-glucan has been demonstrated for only very few proteins (Fujii et al. 1999; Orlean 2012), evidence for this linkage has been presented in many cases either by releasing the proteins enzymatically from the cell wall matrix using β-1,6-glucanase preparations or chemically using HF-pyridine, which breaks the phosphodiester bond between the GPI anchor remnant and the protein. However, only a part of the GPI proteins is significantly incorporated in the cell wall while others are mostly retained at the plasma membrane. The division between cell wall and plasma membrane localization depends on the amino acids immediately upstream of the residue (the ω-site) to which the GPI anchor is coupled (Frieman and Cormack 2003; Hamada et al. 1999; Terashima et al. 2003; Ouyang et al. 2013). Rather than being localized either only in the cell wall or only in the plasma membrane, it is likely that in many cases, part of the molecules of a given GPI protein will be retained at the plasma membrane, while others are incorporated in the cell wall or may end up in the extracellular environment. For example, members of the Gas1 and Ecm33 families are well known to function as abundant plasma membrane proteins (Bruneau et al. 2001; Terashima et al. 2003) but are also part of the core cell wall proteome in ascomycetous budding yeasts (De Groot et al. 2004, 2008; Yin et al. 2005) and filamentous fungi (Prados-Rosales et al. 2009; De Groot et al. 2009) and are also encountered in the secretome (Buerth et al. 2011; Sorgo et al. 2011).

In addition to GPI proteins, the cell wall of *S. cerevisiae* and related *Candida* species contains a minor second class of mannoproteins that are not released by treatment with HF-pyridine or β-1,6-glucanase, but instead can be extracted with mild alkali, therefore named alkali-sensitive linkage (ASL) proteins. Among these are the Pir family of proteins containing internal repeats with the core sequence Q[I/V]XDGQ[I/V]Q. For Pir4 in *S. cerevisiae*, it has been shown that the protein was coupled to the wall by a link between a deamidated side chain of a glutamine residue in the repeat and β-1,3-glucan by virtue of a transglutaminase reaction (Ecker et al. 2006). For some other proteins that also can be extracted by mild alkali and lack GPI anchors, e.g., Scw4, Scw10, and Tos1, the linkage to the glucan matrix is less clear (see also Sect. III.A).

A third class of CWPs can be released with reducing agents (DTT, β-mercaptoethanol) indicating that they are covalently linked to other proteins by disulfide bridges. Examples are Pir1 in *Yarrowia lipolytica* and Pir2, Pir4, Scw4, and Scw10 in *S. cerevisiae* (Moukadiri et al. 1999; Moukadiri and Zueco 2001; Cappellaro et al. 1998; Jaafar et al. 2003); thus, there is overlap between this group of proteins and the group of ASL proteins, at least in baker's yeast.

In addition, in several filamentous ascomycetes and basidiomycetes of the order Agaricales, the presence of hydrophobins, small secretory proteins containing a pattern of eight conserved cysteines, has been shown. These proteins have the unique capacity to self-assemble into amphipathic amyloid monolayers at hydrophobic–hydrophilic interfaces (Morris and Sunde 2013) and can be extracted with trifluoroacetic acid (TFA). Examples are

the rodlet layers surrounding ascospores and aerial hyphae of *Aspergillus* species and the hydrophobic peel of mushroom fruit body caps (Wösten 2001; De Groot et al. 1996). However, until now hydrophobins have not been encountered in proteomics studies using purified walls upon stringent washings with hot solutions containing the reducing agent β-mercaptoethanol and are not further discussed in this chapter.

Several proteomic studies have documented the presence of proteins with known intracellular functions in cell wall preparations, leading to the suggestion that these proteins have moonlighting functions in the cell wall and may reach the cell surface by (an) alternative secretion system(s) (Nombela et al. 2006; Pitarch et al. 2006; Chaffin 2008). However, proteomic extractions of cell walls often include treatments with reducing agents such as β-mercaptoethanol with the undesired side effect that the membrane becomes more permeable to cytosolic proteins (Klis et al. 2007a). Yet, studies in various fungal pathogens including *Cryptococcus neoformans*, *Histoplasma capsulatum*, *C. albicans*, *Candida parapsilosis*, and *Sporothrix schenckii*, as well as the model yeast *S. cerevisiae*, have shown the presence of extracellular vesicles migrating through the cell wall, which perhaps may provide an explanation how these proteins end up in cell wall preparations and influence host–pathogen interactions (Vargas et al. 2015; Gil-Bona et al. 2015; Wolf et al. 2014; Rodrigues et al. 2008).

B. Cell Wall Proteomics

For identification of covalently bound fungal CWPs, a method was developed using so-called cell wall shaving and tandem mass spectrometry (De Groot et al. 2004; Yin et al. 2005). In this approach, cell walls are first purified by stringent washings in NaCl and hot reducing agent solutions to remove intracellular contaminants. As a consequence, proteins that are bound to the cell wall through disulfide bridges to other proteins are also removed, and what is left is a relatively small number of GPI-CWPs and ASL proteins. Since most CWPs are abundantly and heterogeneously glycosylated, they fractionate poorly using classical 1D or 2D gel separation techniques, and in addition, mass fingerprinting will not identify these proteins. A more efficient approach is to treat the purified cell walls directly with an endoprotease (usually trypsin) to release peptide fragments. Samples containing a mix of tryptic peptides derived from different CWPs can be analyzed by a nano HPLC tandem mass spectrometry setup (LC/MS/MS). This will yield a snapshot of the wall proteome and identify proteins that are expressed at detectable levels under the tested growth conditions. However, even when expressed, some proteins may escape identification if the endoproteolytic digestion does not yield unglycosylated peptides that are within the mass range of the used mass spectrometry equipment. Applying this technique to *S. cerevisiae*, *C. albicans*, and *C. glabrata*, and to other species usually identifies a total of 20–30 CWPs under a given condition; see the sections below. Further, it can be combined with stable-isotope labeling to yield absolute quantification of the number of CWP molecules per cell or relative quantitation for monitoring the dynamics of the CWP population (Sorgo et al. 2013; Sosinska et al. 2011; Yin et al. 2005).

III. Saccharomycetales

In this section, we will discuss several ascomycetous yeasts for which the cell walls have been studied intensively in recent years. Even though *C. albicans* is not strictly a yeast, as it is able to develop true hyphae depending on the environmental conditions, it is included in this section as it phylogenetically belongs to the Saccharomycetales and its cell wall architecture including the proteomic composition is very similar to *S. cerevisiae* and other yeast species.

A. *Saccharomyces cerevisiae*

As stated above, the composition and architecture of the cell wall in the baker's yeast *S. cerevisiae* have been described in great detail in the past (Klis et al. 2006; Lesage and Bussey

2006; Orlean 2012). It consists of an elastic and continuous network of moderately branched β-1,3-glucan molecules to which β-1,6-glucan chains and chitin (linear chains of N-acetylglucosamine) are covalently attached (Fig. 5.1). The outer part of the cell wall is a fibrillar layer mainly comprised of mannoproteins that are covalently bound to the glucan matrix and account for 30–40 % of the cell wall mass. β-1,6-Glucan chains account for about 10 % of the cell wall mass and play a central role in the cell wall architecture as they are covalently connected to β-1,3-glucan but at the same time are the acceptor molecules to which most cell wall mannoproteins are connected. Chitin is a minor component of *S. cerevisiae* walls. It is mainly present as a chitin ring in the neck of the mother cell, in the primary septum, and in bud scars. Lateral walls of daughter cells are devoid of chitin, but some chitin is incorporated after cytokinesis, indicating that in lateral walls of growing buds, chitin is not required for mechanical strength. Also, under circumstances where the synthesis of the glucan network is compromised, chitin synthesis becomes upregulated to compensate for the loss of the skeletal strength.

Cell wall proteomics, by direct trypsin digestion of purified walls followed by batch LC/MS/MS, identified a total of 19 cell wall proteins including twelve predicted GPI proteins and seven proteins that are connected to the cell wall glycan network via an alkali-sensitive linkage (Yin et al. 2005, 2007). Additional evidence for the linkages was provided by extracting the proteins from the wall by either HF-pyridine or mild alkali. Seven of the identified proteins have been classified in three different glycoside hydrolase (GH) families of the Carbohydrate-Active enZYmes Database (www.cazy.org). These hydrolases use the cell's own cell wall polymers as their substrates and therefore presumably play a role in maturation, ramnification, and remodeling of the cell wall structure during growth. Gas1, Gas3, and Gas5 belong to GH family 72. Studies with recombinant proteins by Mouyna and coworkers demonstrated that Gas1 (Mouyna et al. 2000), as well as homologous proteins in *C. albicans* and *Aspergillus fumigatus* (Mouyna et al. 2005; Gastebois et al. 2010a), has β-1,3-glucan transglucosylase activity in vitro. When incubated with β-1,3-glucan oligosaccharides, they first act as endoglucanases and then form β-1,3 linkages between the new reducing ends of the released short laminarioligosaccharides and other β-1,3-glucan molecules, which probably serve to elongate and/or otherwise modify nascent β-1,3-glucan chains during cell growth. In a similar fashion, elegant biochemical work with recombinant Crh1 and Utr2, two GPI proteins of the GH16 family, indicated that they are chitin transglycosylases that can cleave β-(1,4) links of chitin molecules and couple the new reducing ends of the formed oligosaccharides to β-1,3-glucan, β-1,6-glucan, and chitin acceptors (Mazáň et al. 2013). In addition, studies with a double mutant in which the *CRH1* and *UTR2* genes are lacking showed that no chitin was linked to glucan leading to the conclusion that these proteins are responsible for the cross-links between chitin and β-1,6- and β-1,3-glucan molecules (Cabib 2009). Scw4 and Scw10 are GH17 proteins with homology to the secreted protein Bgl2 that has been reported to have endoglucanase activity (Mrša et al. 1993). Studies on homologues in *A. fumigatus* (Gastebois et al. 2010b) suggested that these proteins may also have transglucosylase activity and create β-1,6-linked side chains on β-1,3-glucans. The type of linkage(s) of Scw4 and Scw10 to the cell wall is rather unclear. The proteins lack GPI anchors and are found in extracts of reducing agents (Cappellaro et al. 1998). They are also found, together with Pir proteins and Cwp1, in mild-alkali extracts that were pretreated with a hot solution containing the reducing agent β-mercaptoethanol (Yin et al. 2005).

The proteome that was identified by the cell wall shaving method included the four members of the Pir protein family (Yin et al. 2005, 2007). These proteins lack GPI anchors but contain a variable number of internal repeats with the core consensus sequence Q[I/V]XDGQ[I/V]Q. Pir4 has been shown to be linked to the cell wall by the formation of an ester linkage between the second glutamine residue in the repeat sequence and cell wall β-1,3-glucan (Ecker et al. 2006). This has given rise to the

suggestion that Pir proteins may strengthen the β-1,3-glucan network by simultaneously being linked to multiple β-1,3-glucan chains and is consistent with the notion that Pir protein encoding genes are upregulated under cell wall stress or in cell wall mutants in response to activation of the cell wall integrity pathway (Boorsma et al. 2004; Lagorce et al. 2003). Single copies of Pir repeats are also present in the GPI proteins Cwp1, Cwp2, Tir1, Tir2, and Ans1. Such hybrid Pir-GPI-CWPs may therefore simultaneously be linked to both β-1,6-glucan and β-1,3-glucan. Evidence for the existence of this hybrid linkage was shown for the abundant protein Cwp1 because of its presence in HF-pyridine, mild-alkali, and β-1,6-glucanase-resistant mild-alkali extracts (Yin et al. 2005; Kapteyn et al. 2001) as well as for two abundant Cwp1 homologues in *C. glabrata* (De Groot et al. 2008; Weig et al. 2004).

The functions of the remaining CWPs identified by cell wall shaving in the wall proteome of *S. cerevisiae*, Ccw14/Ssr1, Ecm33, Plb2, Pst1, Pry3, Tir1, Tip1, and Tos1, are less clear. Except for the phospholipase Plb2, they do not have similarity to described enzymes, suggesting that they may have structural or cementing roles. However, we cannot exclude that, for instance, Ecm33, Pst1, or Tos1 may have (unknown) enzymatic activity. In fact, using a combination of fold recognition and distant homology detection methods, Tos1 has been predicted to have endo-β-1,3-glucanase activity (Steczkiewicz et al. 2010). Deletion of the gene encoding Ecm33 caused severe cell wall defects and led to hypersecretion of CWPs containing β-1,6-glucan moieties into the growth medium (Pardo et al. 2004). Furthermore, as Ecm33 family members are widely present as part of the core wall proteome in many fungal species (see sections below), they seem to have a crucial role in fungal cell wall biosynthesis. Consistent with this, another member of this protein family, Pst1, was first identified in the culture medium of cell wall regenerating protoplasts (Pardo et al. 2000). Using isobaric tags for relative and absolute quantitation (iTRAQ), Crh1, Utr2, Ecm33, Gas5, Pst1, and Pir3 were shown to be three- to fivefold upregulated in *gas1Δ* deletion mutants, indicating increased incorporation of these proteins in response to cell wall weakening. By comparing to synthetic reference peptides, absolute quantitation was achieved for Cwp1, Crh1, Scw4, Gas1, and Ecm33 in wild-type log phase cells, showing that they had 67×10^3, 44×10^3, 38×10^3, 11×10^3, and 6.5×10^3 of wall-bound copies per cell, respectively. From this the total number of CWP molecules was deduced to be around 2×10^6 molecules per cell (Yin et al. 2007).

Finally, the snapshot of the wall proteome provided by the cell wall shaving technique did not identify all CWPs that are believed to be present in the cell wall of baker's yeast, for instance, Ccw12, Cwp2, Sed1, Flo family proteins, and Muc1 (Orlean 2012). It is plausible that some lack suitable tryptic peptides for mass spectrometric identification, while others simply may not be expressed to detectable levels under the given conditions.

B. *Candida albicans* and Related Pathogenic CTG-Clade *Candida* Species

Candida albicans is the most frequent cause of superficial and invasive fungal infections (Pfaller and Diekema 2007; Pfaller et al. 2012) and can grow in yeast and hyphal forms. It belongs to the CTG-clade of related *Candida* and *Lodderomyces* species in which the CUG codon encodes the amino acid serine instead of leucine. Because of its clinical importance, the cell wall of *C. albicans* has been intensively studied. Biochemical approaches using the architectural cell wall model for *S. cerevisiae* as starting point showed compositional and architectural resemblance with the wall in *C. albicans*. The same macromolecules, i.e., β-1,3-glucan, β-1,6-glucan, chitin, and mannoproteins, were identified as the major building blocks with apparently the same types of linkages between them. For reviews on cell wall organization in *C. albicans*, see Klis et al. (2001) and Ruiz-Herrera et al. (2006). Like in *S. cerevisiae*, the outer layer of the wall is electron-dense and seems to be populated with most of the cell wall proteins (Ene et al. 2012; Tokunaga et al. 1986). Because of their potential roles in primary host–pathogen interactions, several studies in recent years

have been focused on identification and subsequent functional characterization of CWPs. By making genome-wide predictions of GPI proteins in clinically relevant pathogens of the CTG-clade including *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, and comparing the generated GPI protein lists to species that are rarely associated with disease such as *Debaryomyces hansenii* and *Saccharomyces* clade species, three CWP families were found to be enriched in the more pathogenic species: Hyr/Iff proteins, Als adhesins, and Pga30-like proteins (Butler et al. 2009). All three families are highly enriched for gene duplications including tandem clusters of two to six genes and show high mutation rates. This variable repertoire of cell wall proteins is likely to be of profound importance to the niche adaptations and relative virulence of these organisms. Consistent with this, the Als family in *C. albicans* is associated with virulence (Hoyer et al. 2008) and in particular with adhesion to host surfaces, invasion of host cells, and iron acquisition (Phan et al. 2007; Almeida et al. 2008; Yeater et al. 2007). Proteins of the Hyr/Iff family also have properties resembling fungal adhesins (Boisramé et al. 2011; De Groot et al. 2013b), and Iff4 of this family has been implicated in adhesion to plastic and human epithelial cells (Fu et al. 2008). However, the precise biological role of this protein family remains unresolved, and this is also the case for the rather small and thus probably nonenzymatic Pga30-like proteins. Rhd3/Pga29 of this family is one of the most abundant CWPs in *C. albicans* yeast cells, while Pga31 is upregulated in regenerating protoplasts (Castillo et al. 2006) and has been implicated in cell wall stress response (Plaine et al. 2008; Sorgo et al. 2011). Other *Candida*-specific CWPs for which involvement in adhesion to host cell surface proteins and biofilm formation has been shown are the hyphae-specific Hwp1, Hwp2, Rbt1, and Eap1. These proteins are also considered to be part of a single family because they share a highly conserved 42-aa repeat unit (De Groot et al. 2013a) that is also present in seven other GPI proteins. The N-terminal part of Hwp1 is enriched in glutamine residues. Interestingly, these are used as substrates by human host transglutaminase enzymes resulting in covalent cross-linking of Hwp1 to extracellular matrix (ECM) proteins of epithelial host cells (Staab et al. 1999).

Because of its importance as the most prevalent human pathogenic fungus, cell wall proteome dynamics was studied in *C. albicans* under various in vitro culturing conditions with clinical relevance such as changes in pH, hypoxia, carbon source, stress imposed by the antifungal agent fluconazole or the cell wall perturbant Congo red, iron limitation, and conditions that induce filamentation (Ene et al. 2012; Heilmann et al. 2011; Sorgo et al. 2011, 2013; Sosinska et al. 2008, 2011). Using the cell wall shaving approach, a total of about 20 covalently bound CWPs are identified under most conditions analyzed. For instance, the ability of *C. albicans* to switch its morphology from yeast to hyphal growth is essential for its virulence. Using three different hyphal induction methods (serum, GlcNAc, and modified Dulbecco's medium), differences in the wall proteome between yeast cells and hyphae were analyzed using the cell wall shaving approach and metabolic ^{15}N-labeling. A total of 24 genuine CWPs were identified, and for 21, a quantitative analysis was provided (Heilmann et al. 2012). Three categories of proteins were considered: (1) proteins showing strongly increased incorporation levels during hyphal induction (Als3, Hwp2, Hyr1, Plb5, and Sod5), (2) strongly decreased levels in hyphae (Rhd3, Sod4, and Ywp1), and (3) a group of morphotype-independent proteins including members of the same carbohydrate-active enzyme families that in *S. cerevisiae* are also part of the core cell wall proteome (see Sect. III.A). The latter group included three members of the GH72 Gas family, Pga4, and the pH-responsive Phr1 and Phr2 proteins (Sosinska et al. 2011; Fonzi 1999). Also in this group are Cht2, Ecm33, Rbt1, Sap9, and Ssr1, while Als4 and Pir1 were associated to growth at low temperature (30 °C) rather than changes in growth morphology. Homologues of Sap9 and Cht2 are uncommon in walls of Saccharomycetaceae. Sap9 is one of two wall-bound GPI-modified aspartic proteases that can cleave covalently bound CWPs including the GH18 chitinase Cht2 (Schild et al. 2011) that is probably involved in chitin remodeling.

Pir1 is the only Pir-like protein in *C. albicans* containing multiple Pir repeats, thus having the potential to connect to multiple β-1,3-glucan chains, and seems to be vital for cell wall integrity (Martinez et al. 2004). Hyphally upregulated Sod5, together with Sod4 and Sod6, represents a group GPI-CWPs that degrade host-derived reactive oxygen species to escape innate immune surveillance (Frohner et al. 2009). Apart from the five proteins in group (1) mentioned earlier, wall protein Hwp1 is also well known to be hyphal-induced. It is detected in purified walls by immunoblotting (Sosinska et al. 2008); however, it appears difficult to identify Hwp1 using cell wall shaving and mass spectrometry, probably because it does not yield suitable unglycosylated tryptic peptides. In a different study (Sorgo et al. 2013), analysis of the *C. albicans* wall proteome upon iron restriction led to a strong increase in the levels of the multifunctional adhesin Als3, which also serves as a ferritin receptor, and of the CFEM (common in fungal extracellular membranes) domain-containing heme-binding proteins Csa1, Pga7, Pga10/Rbt51, and Rbt5, which have a role iron utilization from hemoglobin (Kuznets et al. 2014). Hypoxic conditions led to incorporation of the non-GPI protein Sim1/Sun42, a member of the Sun family (GH132). Together, Sun41 and Sun42 are essential for cell separation in *C. albicans* (Firon et al. 2007). Consistent with this, studies with homologous Sun proteins in *A. fumigatus* suggest that these Sun proteins are likely to have exo-β-1,3-glucanase activity (Gastebois et al. 2013). Importantly, these studies demonstrate the dynamic nature of the cell wall proteome in response to changes in environmental conditions or growth morphology.

Functions of several of the predicted GPI-CWPs in *C. albicans* (De Groot et al. 2003), including those that are uncommon or not detected by cell wall shaving, for instance, Pga13 (Gelis et al. 2012), Ywp1/Pga24 (Granger et al. 2005), Pga26 (Laforet et al. 2011), and Pga59 and Pga62 (Moreno-Ruiz et al. 2009), have been studied using reverse genetics, and in some cases, cell wall localization was demonstrated using tagged versions of the proteins. Two studies addressed functions of GPI proteins more systematically using deletion mutants (Plaine et al. 2008) or overexpression approaches (Cabral et al. 2014). Several GPI proteins were implicated in cell wall integrity, morphology, host cell adhesion, or virulence; however, in most cases, their real functions remain unresolved.

C. *Candida glabrata*

After *C. albicans*, *C. glabrata* is one of the most frequent causes of candidiasis (Pfaller et al. 2014), but it does not belong to the *Candida/Lodderomyces* CTG-clade group of species. *C. glabrata* belongs to the *Nakaseomyces/Candida* clade and is phylogenetically more related to *S. cerevisiae*. Biochemical studies performed to elucidate the cell wall structure in *C. glabrata* have been based mainly on the architectural wall model for *S. cerevisiae* and showed that the wall structures in the two organisms are very similar (De Groot et al. 2008; Weig et al. 2004). The main cell wall macromolecules are also the polysaccharides β-1,3-glucan, β-1,6-glucan, chitin, and mannoproteins. Like in *S. cerevisiae* and *C. albicans*, electron microscopy revealed a bilayered structure with an electron-dense outer layer and an inner layer that is more electron transparent, supporting the idea that the wall architecture in these organisms is largely comparable (De Groot et al. 2008).

The high similarity between *C. glabrata* and *S. cerevisiae* is also apparent when a genomic inventory is made of the genes involved in various aspects of cell wall biology. For instance, similar gene families involved in glucan and chitin synthesis and remodeling or cross-linking of cell wall polymers are present, and many of the cell wall proteins have clear orthologs. However, genome-wide predictions of GPI proteins in the *C. glabrata* reference strain CBS138 indicated, besides the presence of many of the same gene families, at least one large dissimilarity between both organisms; where *S. cerevisiae* has about 11 genes encoding GPI proteins with shown or presumed functions as flocculins or agglutinins, this class of proteins is hugely expanded in *C. glabrata* with 66 identified adhesin-like wall protein encoding

genes (De Groot et al. 2008, 2013a). Consistent with this, in addition to a core proteome comprised of orthologs of CWPs that are also present in the wall of *S. cerevisiae*, proteomic analysis of *C. glabrata* walls identified some adhesins whose incorporation seemed to be dependent on strain background, growth phase, and environmental conditions (De Groot et al. 2008; Kraneveld et al. 2011). For instance, in two clinical isolates showing hyper-adherence phenotypes, increased incorporation of specific uncharacterized non-Epa adhesins was observed in cell walls under in vitro biofilm-forming conditions (our unpublished observations). Rather than or in addition to being involved in self-adhesion (flocculation), it is conceivable that these putative adhesins are involved in binding to host cell surface molecules and that regulated expression and genomic variability of adhesin genes play an important role in the virulence of *C. glabrata*.

Based on phylogenetic analysis of their N-terminal presumed ligand-binding domains, the putative adhesins are divided in different clusters. Some members of one cluster, the Epa family of epithelial adhesins (counting 17 members in CBS138), have been studied in detail. Deletion of only *EPA1* diminished the binding capacity to human epithelial cells by 95 % (Cormack et al. 1999). One possible explanation for this striking result is that under standard laboratory conditions, most of the *EPA* genes are expressed at very low levels but may become induced under the specific conditions such as those imposed by the human host. For instance, *EPA6* and *EPA7* have been shown to be induced in the presence of urine and under biofilm-forming conditions (Domergue et al. 2005; Iraqui et al. 2005). Related to the apparent tight control of adhesin gene expression, two-third of the putative adhesins, including most *EPA* genes, are located in the telomeric regions of the chromosomes and may be subject to subtelomeric silencing (De Groot et al. 2008; Castaño et al. 2005).

Epa1, Epa6, and Epa7 were shown to have lectin activity and bound to galactosyl-containing oligosaccharides that are present as glycan additions onto human surface proteins such as O-type mucins (Maestre-Reyna et al. 2012; Zupancic et al. 2008). The binding was mediated through their N-terminal domains, which, like those in the flocculin family of *S. cerevisiae*, are categorized as PA14 (anthrax protective-antigen 14) domains. Functionality of most other putative adhesins is largely unexplored, and their binding ligands are unknown although one study suggested a role for Aed1 and Pwp7 in adhesion to endothelial cells (Desai et al. 2011). The C-terminal half of the putative adhesins usually is of low complexity and in many cases contains internal repeats (also termed megasatellites), which are present across different adhesin clusters (De Groot et al. 2013a; Thierry et al. 2010). The megasatellites may have a role as spacer molecules helping the fungus to make contact with the host surface (Verstrepen and Fink 2009); however, also a role as molecular zipper in amyloid formation has been proposed (Verstrepen and Fink 2009).

D. *Kluyveromyces lactis*

The milk budding yeast *Kluyveromyces lactis* is closely related to *S. cerevisiae*. However, rather than fruits, its natural habitat is milk with lactose being the major carbon source. Therefore, *K. lactis* is employed in the dairy industry as an enzyme source for lactose degradation (Breunig et al. 2000). A first systematic analysis of its cell wall structure was performed by Brackhaus and colleagues, including proteomic analysis (Backhaus et al. 2010). Electron microscopy of *K. lactis* walls showed a bilayered wall with an electron-dense layer surrounding a more transparent layer. The thickness of the wall varied from 64 nm, in cells growing on glucose as the carbon source to 105 nm when growing on 3 % ethanol. As in *S. cerevisiae*, glucose and mannose were the most abundant saccharides in sulfuric acid extracts, indicating that glucan and mannoproteins are the main cell wall constituents, with a low amount of chitin. Mass spectrometric wall analysis using cell wall shaving identified 19 predicted GPI proteins, two Pir proteins, and a Scw4 homologue (Backhaus et al. 2010). Most of these proteins are homologues of identified CWPs in *S. cerevisiae*

using the same approach, including Gas1, Crh1, Ecm33, Cwp1, Tos1, Plb2, and Ccw14 homologues, and thus appear to be part of the core cell wall proteome. In addition, four of the GPI proteins have homology with flocculins/adhesins in *S. cerevisiae* or *C. albicans*, but a systematic GPI protein prediction is lacking to see if adhesins/flocculins are overrepresented in the genome as is the case for *C. glabrata*. Interestingly, judged from the number of peptides found for an individual protein in a given condition, some growth phase- and carbon source-dependent incorporation of proteins was observed. In particular, two homologues of Muc1/Flo11 in *S. cerevisiae* were identified that appeared to be oppositely regulated both in terms of growth phase (exponential vs. stationary) and carbon source (glucose vs. lactose) applied, illustrating the dynamic nature of the cell wall of *K. lactis*.

IV. *Schizosaccharomyces pombe*

The fission yeast *Schizosaccharomyces pombe* is often used as a model system in molecular cell biology to study cell cycle regulation. In contrast to the budding yeasts described above, α-1,3-glucan is present as one of the major polysaccharides in the cell wall of *S. pombe*. It was shown to be essential for both secondary septum formation and for primary septum structural strength needed to withstand the physical forces of the cell turgor pressure during cell separation (Cortés et al. 2012). α-1,3-Glucan does not seem to be coupled to the other polysaccharides β-1,3-glucan and a very highly β-1,3-glucose-branched (therefore also named diglucan (Magnelli et al. 2005)) β-1,6-glucan or to cell wall proteins (Grün et al. 2005). Consistent with the absence of Crh family genes in the genome, cell walls of vegetative *S. pombe* cells lack chitin (De Groot et al. 2007). Ultrastructural studies showed that the *S. pombe* wall is also bilayered (Osumi 2012). The inner skeletal polysaccharide layer is surrounded by a fibrillar, layer probably representing galactomannoproteins (Osumi 2012; Bush et al. 1974). HPLC analysis of sulfuric acid-hydrolyzed walls indicated that 83–84 % of the cell wall mass in *S. pombe* is glucose originating from β-1,3-glucan, β-1,6-glucan, and α-1,3-glucan. Galactomannan accounts for 13–14 % and protein for about 3 % of the cell wall mass (De Groot et al. 2007). Pir proteins are not present in the translated genome of *S. pombe*. For more details concerning the cell wall architecture in *S. pombe*, we refer to Klis et al. (2007b).

Mass spectrometric analysis of *S. pombe* walls using cell wall shaving identified 6 proteins: the four predicted GPI proteins, Gas1, Gas5, Ecm33, and Pwp1, and two alkali-sensitive CWPs, Psu1 and Asl1 (De Groot et al. 2007). Compared to *S. cerevisiae*, the lower number of identified CWPs seems consistent with a lower number of predicted GPI proteins (66 vs. 33) (De Groot et al. 2003). The Gas1 and Ecm33 family proteins are homologues of core wall proteins in budding yeasts indicating that their cell wall-localized functions are conserved. Psu1 is a member of the Sun family and is likely to have exo-β-1,3-glucanase activity (Gastebois et al. 2013), consistent with its role in cell division (Omi et al. 1999). The functions of the very abundant Pwp1 and Asl1 remain uncharacterized.

V. *Aspergillus* spp.

Knowledge of the mechanisms underlying cell wall biosynthesis in *Aspergillus* spp. is of high relevance to medicine and food safety and for biotechnological applications. *Aspergillus niger* is a food contaminant. Hyphal walls of *A. niger* contain the polysaccharides β-1,3-glucan, α-1,3-glucan, and chitin (Klis et al. 2007b). Chitin accounts for about 10 % of the wall mass. This higher percentage as compared to ascomycetous budding yeasts is common in *Aspergillus* spp. and is reflected by largely expanded gene families involved in chitin synthesis and processing. Also present in the cell wall of *A. niger* is alkali-soluble galactosaminogalactan and galactomannan, possibly as protein-bound material (Klis et al. 2007b). Analysis of alkali-insoluble material from *A. fumigatus* walls further identified a linear

β-1,3/1,4-glucan (Fontaine et al. 2000). Less clear is if hyphal walls in *Aspergillus* species contain β-1,6-glucan to covalently link GPI-CWPs to the skeletal layer of the wall. The fact that HF-pyridine released multiple covalently bound wall proteins (Damveld et al. 2005) and that β-1,3-glucanase digestion released proteins carrying β-1,6-glucan epitopes (Brul et al. 1997) indicates that it is present. However, it was not found in the alkali-insoluble fraction of the wall of *A. fumigatus* (Fontaine et al. 2000). On the other hand, both species contain a homologue of Kre6 that in yeast plays an important role in β-1,6-glucan synthesis. Genomic inventories of wall-related genes in *A. niger* and *A. nidulans* further identified gene products possibly involved in the synthesis of β-1,3/1,4-glucan, as well as predicted GPI-modified mixed-linked β-glucanases and amylase-like α-glucanases with possible transglucosidic activities pertaining to the processing of β-1,3/1,4-glucan and α-1,3/1,4-glucan, respectively. Pir proteins, which are widely present in ascomycetous budding yeasts, are absent in *Aspergillus* (De Groot et al. 2009; Pel et al. 2007).

Wall proteome analysis of *A. nidulans* using wall shaving identified 12 proteins including 10 GPI proteins (De Groot et al. 2009), providing more support for the presence of β-1,6-glucan. The 12 proteins specify 7 putative carbohydrate-active enzymes (three Gas-, two Crh-, and two Bgl2-family proteins), an Ecm33 homologue, an aspartic protease, and three unknown proteins. Thus, *A. nidulans* walls contain many homologues of covalently bound core CWPs in yeast species. These results are also consistent with the identification of GPI-anchored Ecm33 and Bgl2 orthologs in cell walls of *Neurospora crassa* (Bowman et al. 2006). The two non-GPI proteins identified were another Bgl2 homologue and an unknown protein with tandem repeats in the C-terminal region, a feature that is often encountered in adhesin-like fungal wall proteins. By analogy to yeast, these proteins may be incorporated via a mild-alkali-sensitive linkage; however, a different type of linkage cannot be excluded.

VI. *Fusarium oxysporum*

Fusarium oxysporum is a soilborne fungus that causes vascular wilt disease on a wide range of crops. During initial stages of infection, fungal hyphae attach to roots, penetrate the cortex, and colonize xylem vessels. The wall composition of *Fusarium oxysporum* f.sp. *lycopersici*, a special race of this fungus that is able to cause wilt in tomato plants, was studied in detail (Schoffelmeer et al. 1999). Analysis of sulfuric acid-hydrolyzed wall carbohydrates showed the presence of glucose and N-acetylglucosamine, but also mannose, galactose, and uronic acids, presumably originating from cell wall glycoproteins. X-ray diffraction studies showed the presence of α-1,3-glucan in the alkali-soluble fraction and β-1,3-glucan and chitin in the alkali-insoluble fraction. Electron microscopy of walls and lectin-binding immunoblot studies of wall fractions upon glucanase and/or pronase digestion led to the conclusion that similar to *S. cerevisiae*, glycoproteins form an external layer covering an inner layer composed of chitin and glucan. Consistent with this idea, treatment of isolated hyphal walls with ice-cold anhydrous HF resulted in the release and subsequent identification of an abundant GPI-CWP, Fem1 (Schoffelmeer et al. 2001).

Proteomic analysis of purified hyphal walls of *F. oxysporum* using the wall shaving technique resulted in identification of a total of 174 proteins (Prados-Rosales et al. 2009). However, only 19 of those contain a predicted signal peptide and 10 are predicted to be GPI proteins. The very high number of intracellular proteins identified is surprising in view of the results obtained with other fungal species, including the filamentous fungus *A. nidulans*. However, there is one important difference between the cell wall isolation procedure in this study compared to other papers that have performed cell wall proteomics using wall shaving: in other wall proteome studies, extraction of walls was performed with a hot solution containing 0.1 M β-mercaptoethanol, whereas by Prados-Rosales and colleagues, this step was carried out with 10 mM DTT as the reducing agent. Although wall localization through alternative secretion

(see Sect. II.B.) cannot be excluded, we surmise that the DTT treatment may simply not have been stringent enough to clear the walls from intracellular contaminations. Fem1 was one the GPI proteins identified in hyphal walls. Two other identified GPI-CWPs contain a CFEM domain. Among the other identified GPI-CWPs were GH16 and GH17 enzymes (possible transglycosylases) and an Ecm33 homologue, all typical core wall proteins. Gas proteins of GH72, present on the list of predicted GPI proteins, were not identified. Possibly this is related to the high background of intracellular proteins in the samples.

VII. *Botrytis cinerea*

The filamentous ascomycete *Botrytis cinerea* is a ubiquitous plant pathogen affecting hundreds of different plant species and serves as a model for the study of necrotrophic fungal pathogens. In recent years, its genome has been published (Amselem et al. 2011; Staats and van Kan 2012) and several studies have focused on cell wall composition and related glycosylation mechanisms (Cantu et al. 2009; De Groot et al. 2013b; González et al. 2013, 2014; Plaza et al. 2015).

Cantu and colleagues studied the wall composition of *B. cinerea* hyphae from cultures in liquid malt extract medium after 3, 6, or 9 days of growth (Cantu et al. 2009). Mycelium mixed with 100 % ethanol was disrupted and boiled to yield crude alcohol-insoluble cell wall preparations. Sulfuric acid extractions yielded neutral sugars amounting, in terms of dry wall mass, to 57 % at day 3 and about 80 % at day 9. About 90 % of the neutral sugars at each time point was glucose, indicating that the wall matrix of *B. cinerea* consists for a large part of glucan(s). Consistent with this, the genome of *B. cinerea* contains many genes encoding glucan-modifying enzymes including exoglucanases, endoglucanases, and β-1,3-glucan transglycosylases. However, only a single-gene product of the Fks family encoding the catalytic subunit of β1,3-glucan synthase complex is present (De Groot et al. 2013b). A change was observed from predominantly alkali-soluble material at day 3 to more or less equal amounts of alkali-soluble and alkali-insoluble material at the later time points. This coincided with a decrease of protein from 37 % to 7 % of the wall dry mass and an increase in chitin from 1 % to 5 % (Cantu et al. 2009). As compared to *S. cerevisiae*, an expanded Crh family is present in the genome of *B. cinerea*. Together, these observations indicate that maturation of hyphal walls involves incorporation and coupling of chitin to the glucan matrix. The measured high protein content at the early time point is remarkable, especially considering the low levels of galactose (4 %), arabinose (2 %), and mannose (1 %). Possibly, rather than being genuine covalently bound wall proteins that transit through the secretory pathway, most of these proteins may have become associated with the wall upon hyphal breakage. In a recent paper by Plaza and colleagues, saccharides in a sulfuric acid extract of hyphal walls accounted for >80 % of the wall dry mass, the major saccharides being glucose (47 %) and mannose (34 %) (Plaza et al. 2015). Moreover, a *bcpmr1* null mutant lacking a putative Ca^{2+}/Mn^{2+}-ATPase that plays a role in protein glycosylation displayed a >80 % reduction in the relative amount of wall mannan, accompanied with increases in glucan and chitin (Plaza et al. 2015). Obviously, there are some clear discrepancies between the two papers described above. Nevertheless, taken together the data indicates that hyphal walls of *B. cinerea* consist of a glucan-chitin matrix to which glycosylated proteins are bound.

Genomic analysis of cell wall genes (De Groot et al. 2013b) indicated that *B. cinerea* contains two homologues of *S. cerevisiae* Kre6. Predictions of GPI proteins resulted in a list of 107 putative GPI proteins, and among them are members of the core CWP families Gas, Crh, Bgl, and Ecm33. As in other filamentous ascomycetes, the Dfg5 family is expanded in *B. cinerea* in comparison to yeast species. Dfg5 proteins are believed to be responsible for cleaving the sugar moiety of protein-bound GPI anchors at the plasma membrane and possibly are also involved in subsequently linking the protein to β-1,6-glucan. Altogether, these

genomic data suggest the presence β-1,6-glucan-linked GPI-CWPs in the hyphal wall of *B. cinerea*. However, wall proteome analysis using the wall shaving technique to identify covalently bound CWPs, for example, some of the classical core wall proteins mentioned above, has not been performed yet for *B. cinerea*.

Homologues of baker's yeast protein families involved in protein glycosylation, for instance, Pmt and Mnt, are also present in *B. cinerea*. The fungal Pmt family is phylogenetically classified into Pmt1, Pmt2, and Pmt4 subfamilies, which differ in protein substrate specificity (Girrbach and Strahl 2003). In *B. cinerea*, three *PMT* genes are present, one for each of the subgroups (De Groot et al. 2013b). This is similar to other filamentous ascomycetes such as, for instance, *A. nidulans* and *A. fumigatus* (Goto et al. 2009; Mouyna et al. 2010). In yeast species like *S. cerevisiae* (seven genes) and *C. albicans* (five genes), the Pmt families are more expanded (Prill et al. 2005; Girrbach and Strahl 2003). This probably reflects the fact that the *O*-glycans in these yeasts are mainly linear mannosyl chains, whereas in filamentous species, they can be branched and more variable in glycan composition (Goto 2007). In single-gene deletion studies, all three *bcpmt* mutants exhibited a reduction in infection of plant tissues, indicating functionality in *O*-glycosylation for all three *PMT* genes. The most pronounced was the effect of deleting *bcpmt2*. Deletion of this gene led to inability of infecting intact leaves, petals, or fruits of any plant tested. Furthermore, the *bcpmt2* null mutant most clearly recovered growth rate in the presence of osmotic stabilizers and showed the strongest increase in sensitivity to the cell wall perturbing compound Calcofluor white (González et al. 2013). Thus, of the three Pmt isoenzymes in *B. cinerea*, Pmt2 seems to be most critical for cell wall integrity and virulence.

VIII. Conclusions and Perspectives

Thanks to advances and development of DNA next-generation sequencing technologies, the number of completed fungal genome sequences that has come available in recent years is large and rapidly increasing. This also enabled new post-genomic approaches such as comparative genomics, transcript profiling using RNA sequencing, and proteomics. These techniques have also entered into the field fungal cell wall biology, greatly improving our knowledge about cell wall construction and its regulation in many different fungal species. For instance, the comparison of lists with predicted GPI proteins in pathogenic CTG-clade *Candida* species with nonpathogenic species pinpointed three families of GPI proteins that were enriched in pathogenic species. This suggests that these three families may play important roles in *Candida* virulence (Butler et al. 2009). Genomic analysis of *C. glabrata* uncovered a largely expanded and highly variable family of putative wall adhesins that may help the yeast to bind various different host tissues in different host niches.

During the last decade, many studies have focused on making inventories of cell wall proteomes in various ascomycetes. Emerging from these studies is that the walls of most species contain carbohydrate-active enzymes from families GH16, GH17, and GH72, which through biochemical studies were shown to be involved in chitin-β-glucan cross-linking, β-1,3-glucan branching, and β-1,3-glucan elongation, respectively. Ecm33 homologues are also part of the core proteome. It is very clear that Ecm33 proteins have an important function in cell wall synthesis; however, their function is still not uncovered. In *Candida albicans,* owing to its importance as major human pathogen, several studies have addressed dynamics of the cell wall proteome under infection-relevant conditions by quantitative mass spectrometry. This showed that the cell wall proteome of *C. albicans* is highly dynamic and that differential expression of members from CWP families enables *Candida* to adhere, invade, colonize, and evade host immune responses.

An important aspect that is still poorly understood in fungal cell wall biology is synthesis of β-1,6-glucan and its connection to GPI proteins. Although β-1,6-glucan is widely distributed in fungi, it is rare outside the fungal

kingdom. If β-1,6-glucan is formed directly from UDP-glucose donors, the β-1,6-glucosyltransferase would represent a new glycosyltransferase family. Another possibility could be that β-1,6-glucan is produced solely by transglycosylation (Orlean 2012). Despite all the genomic information that is nowadays available, thorough biochemical studies will be required to solve this issue.

Acknowledgments P.G. was supported by grants from the Spanish Ministry of Economy and Competitiveness (MINECO) [SAF2013-47570-P] and the Junta de Comunidades de Castilla-La Mancha [PPII11-2014-007-P]. E.V. was supported by a grant from MINECO (PI12/01797) that was co-financed by the Instituto de Salud Carlos III and the EU (FEDER).

References

Almeida RS, Brunke S, Albrecht A, Thewes S, Laue M, Edwards JE, Filler SG, Hube B (2008) The hyphal-associated adhesin and invasin Als3 of *Candida albicans* mediates iron acquisition from host ferritin. PLoS Pathog 4, e1000217

Amselem J, Cuomo CA, Van Kan JA, Viaud M, Benito EP, Couloux A, Coutinho PM, De Vries RP, Dyer PS, Fillinger S, Fournier E, Gout L, Hahn M, Kohn L, Lapalu N, Plummer KM, Pradier JM, Quévillon E, Sharon A, Simon A, Ten Have A, Tudzynski B, Tudzynski P, Wincker P, Andrew M, Anthouard V, Beever RE, Beffa R, Benoit I, Bouzid O, Brault B, Chen Z, Choquer M, Collémare J, Cotton P, Danchin EG, Da Silva C, Gautier A, Giraud C, Giraud T, Gonzalez C, Grossetete S, Güldener U, Henrissat B, Howlett BJ, Kodira C, Kretschmer M, Lappartient A, Leroch M, Levis C, Mauceli E, Neuvéglise C, Oeser B, Pearson M, Poulain J, Poussereau N, Quesneville H, Rascle C, Schumacher J, Segurens B, Sexton A, Silva E, Sirven C, Soanes DM, Talbot NJ, Templeton M, Yandava C, Yarden O, Zeng Q, Rollins JA, Lebrun MH, Dickman M (2011) Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genet 7, e1002230. doi:10.1371/journal.pgen.1002230

Backhaus K, Heilmann CJ, Sorgo AG, Purschke G, De Koster CG, Klis FM, Heinisch JJ (2010) A systematic study of the cell wall composition of *Kluyveromyces lactis*. Yeast 27:647–660. doi:10.1002/yea.1781

Boisramé A, Cornu A, Da Costa G, Richard ML (2011) Unexpected role for a serine/threonine-rich domain in the *Candida albicans* Iff protein family. Eukaryot Cell 10:1317–1330. doi:10.1128/EC.05044-11

Boorsma A, De Nobel H, Ter Riet B, Bargmann B, Brul S, Hellingwerf KJ, Klis FM (2004) Characterization of the transcriptional response to cell wall stress in *Saccharomyces cerevisiae*. Yeast 21:413–427

Bowman SM, Piwowar A, Al Dabbous M, Vierula J, Free SJ (2006) Mutational analysis of the glycosylphosphatidylinositol (GPI) anchor pathway demonstrates that GPI-anchored proteins are required for cell wall biogenesis and normal hyphal growth in *Neurospora crassa*. Eukaryot Cell 5:587–600

Breunig KD, Bolotin-Fukuhara M, Bianchi MM, Bourgarel D, Falcone C, Ferrero II, Frontali L, Goffrini P, Krijger JJ, Mazzoni C, Milkowski C, Steensma HY, Wesolowski-Louvel M, Zeeman AM (2000) Regulation of primary carbon metabolism in *Kluyveromyces lactis*. Enzyme Microb Technol 26:771–780, doi:S0141022900001708

Brul S, King A, Van der Vaart JM, Chapman J, Klis F, Verrips CT (1997) The incorporation of mannoproteins in the cell wall of *S. cerevisiae* and filamentous *Ascomycetes*. Antonie Van Leeuwenhoek 72:229–237

Bruneau JM, Magnin T, Tagat E, Legrand R, Bernard M, Diaquin M, Fudali C, Latgé JP (2001) Proteome analysis of *Aspergillus fumigatus* identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis. Electrophoresis 22:2812–2823

Buerth C, Heilmann CJ, Klis FM, de Koster CG, Ernst JF, Tielker D (2011) Growth-dependent secretome of *Candida utilis*. Microbiology 157:2493–2503. doi:10.1099/mic.0.049320-0

Bush DA, Horisberger M, Horman I, Wursch P (1974) The wall structure of *Schizosaccharomyces pombe*. J Gen Microbiol 81:199–206

Butler G, Rasmussen MD, Lin MF, Santos MAS, Sakthikumar S, Munro CA, Rheinbay E, Grabherr M, Forche A, Reedy JL, Agrafioti I, Arnaud MB, Bates S, Brown AJP, Brunke S, Costanzo MC, Fitzpatrick DA, De Groot PWJ, Harris D, Hoyer LL, Hube B, Klis FM, Kodira C, Lennard N, Logue ME, Martin R, Neiman AM, Nikolaou E, Quail MA, Quinn J, Santos MC, Schmitzberger FF, Sherlock G, Shah P, Silverstein KAT, Skrzypek MS, Soll D, Staggs R, Stansfield I, Stumpf MPH, Sudbery PE, Srikantha T, Zeng QD, Berman J, Berriman M, Heitman J, Gow NAR, Lorenz MC, Birren BW, Kellis M, Cuomo CA (2009) Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. Nature 459:657–662

Cabib E (2009) Two novel techniques for determination of polysaccharide cross-links show that Crh1p and Crh2p attach chitin to both b(1-6)- and b(1-3) glucan in the *Saccharomyces cerevisiae* cell wall. Eukaryot Cell 8:1626–1636

Cabral V, Znaidi S, Walker LA, Martin-Yken H, Dague E, Legrand M, Lee K, Chauvel M, Firon A, Rossignol T, Richard ML, Munro CA, Bachellier-Bassi S, d'Enfert C (2014) Targeted changes of the cell

wall proteome influence *Candida albicans* ability to form single- and multi-strain biofilms. PLoS Pathog 10, e1004542. doi:10.1371/journal.ppat.1004542

Cantu D, Greve LC, Labavitch JM, Powell AL (2009) Characterization of the cell wall of the ubiquitous plant pathogen *Botrytis cinerea*. Mycol Res 113:1396–1403, doi:S0953-7562(09)00184-1

Cappellaro C, Mrša V, Tanner W (1998) New potential cell wall glucanases of *Saccharomyces cerevisiae* and their involvement in mating. J Bacteriol 180:5030–5037

Castaño I, Pan SJ, Zupancic M, Hennequin C, Dujon B, Cormack BP (2005) Telomere length control and transcriptional regulation of subtelomeric adhesins in *Candida glabrata*. Mol Microbiol 55:1246–1258

Castillo L, Martínez AI, Garcerá A, García-Martínez J, Ruiz-Herrera J, Valentín E, Sentandreu R (2006) Genomic response programs of *Candida albicans* following protoplasting and regeneration. Fungal Genet Biol 43:124–134

Chaffin WL (2008) *Candida albicans* cell wall proteins. Microbiol Mol Biol Rev 72:495–544

Cormack BP, Ghori N, Falkow S (1999) An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. Science 285:578–582

Cortés JC, Sato M, Munoz J, Moreno MB, Clemente-Ramos JA, Ramos M, Okada H, Osumi M, Duran A, Ribas JC (2012) Fission yeast Ags1 confers the essential septum strength needed for safe gradual cell abscission. J Cell Biol 198:637–656. doi:10.1083/jcb.201202015

Cutler JE (2001) *N*-glycosylation of yeast, with emphasis on *Candida albicans*. Med Mycol 39(Suppl 1):75–86

Damveld RA, Arentshorst M, Vankuyk PA, Klis FM, Van den Hondel CAMJJ, Ram AFJ (2005) Characterisation of CwpA, a putative glycosylphosphatidylinositol anchored cell wall mannoprotein in the filamentous fungus *Aspergillus niger*. Fungal Genet Biol 42:873–885

De Groot PWJ, Schaap PJ, Sonnenberg AS, Visser J, Van Griensven LJ (1996) The *Agaricus bisporus hypA* gene encodes a hydrophobin and specifically accumulates in peel tissue of mushroom caps during fruit body development. J Mol Biol 257:1008–1018 doi:S0022-2836(96)90219-3

De Groot PWJ, Ruiz C, Vázquez de Aldana CR, Dueñas E, Cid VJ, Del Rey F, Rodríguez-Peña JM, Pérez P, Andel A, Caubín J, Arroyo J, García JC, Gil C, Molina M, García LJ, Nombela C, Klis FM (2001) A genomic approach for the identification and classification of genes involved in cell wall formation and its regulation in *Saccharomyces cerevisiae*. Comp Funct Genomics 2:124–142

De Groot PWJ, Hellingwerf KJ, Klis FM (2003) Genome-wide identification of fungal GPI proteins. Yeast 20:781–796

De Groot PWJ, De Boer AD, Cunningham J, Dekker HL, De Jong L, Hellingwerf KJ, De Koster C, Klis FM (2004) Proteomic analysis of *Candida albicans* cell walls reveals covalently bound carbohydrate-active enzymes and adhesins. Eukaryot Cell 3:955–965

De Groot PWJ, Ram AF, Klis FM (2005) Features and functions of covalently linked proteins in fungal cell walls. Fungal Genet Biol 42:657–675

De Groot PWJ, Yin QY, Weig M, Sosinska GJ, Klis FM, De Koster CG (2007) Mass spectrometric identification of covalently bound cell wall proteins from the fission yeast *Schizosaccharomyces pombe*. Yeast 24:267–278

De Groot PWJ, Kraneveld EA, Yin QY, Dekker HL, Groß U, Crielaard W, De Koster CG, Klis FM, Weig M (2008) The cell wall of the human pathogen *Candida glabrata*: differential incorporation of novel adhesin-like wall proteins. Eukaryot Cell 7:1951–1964

De Groot PWJ, Brandt BW, Horiuchi H, Ram AFJ, De Koster CG, Klis FM (2009) Comprehensive genomic analysis of cell wall genes in *Aspergillus nidulans*. Fungal Genet Biol 46:S72–S81

De Groot PWJ, Bader O, de Boer AD, Weig M, Chauhan N (2013a) Adhesins in human fungal pathogens: glue with plenty of stick. Eukaryot Cell 12:470–481. doi:10.1128/EC.00364-12

De Groot PWJ, Martinez AI, Castillo L (2013b) A genomic inventory of cell wall biosynthesis in the ubiquitous plant pathogen *Botrytis cinerea*. In: Mora Montes HM (ed) The fungal cell wall, 1st edn. Nova Science, Hauppauge

Desai C, Mavrianos J, Chauhan N (2011) *Candida glabrata* Pwp7p and Aed1p are required for adherence to human endothelial cells. FEMS Yeast Res 11:595–601. doi:10.1111/j.1567-1364.2011.00743.x

Domergue R, Castaño I, De las Peñas A, Zupancic M, Lockatell V, Hebel JR, Johnson D, Cormack BP (2005) Nicotinic acid limitation regulates silencing of *Candida* adhesins during UTI. Science 308:866–870

Ecker M, Deutzmann R, Lehle L, Mrša V, Tanner W (2006) PIR-proteins of *Saccharomyces cerevisiae* are attached to b-1,3-glucan by a new protein-carbohydrate linkage. J Biol Chem 281:11523–11529

Ene IV, Heilmann CJ, Sorgo AG, Walker LA, de Koster CG, Munro CA, Klis FM, Brown AJ (2012) Carbon source-induced reprogramming of the cell wall proteome and secretome modulates the adherence and drug resistance of the fungal pathogen *Candida albicans*. Proteomics 12:3164–3179. doi:10.1002/pmic.201200228

Ernst JF, Prill SK (2001) *O*-glycosylation. Med Mycol 39 (Suppl 1):67–74

Firon A, Aubert S, Iraqui I, Guadagnini S, Goyard S, Prevost MC, Janbon G, d'Enfert C (2007) The *SUN41* and *SUN42* genes are essential for cell sep-

aration in *Candida albicans*. Mol Microbiol 66:1256–1275

Fontaine T, Simenel C, Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M, Latgé JP (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. J Biol Chem 275:27594–27607

Fonzi WA (1999) *PHR1* and *PHR2* of *Candida albicans* encode putative glycosidases required for proper cross-linking of b-1,3-and b-1,6-glucans. J Bacteriol 181:7070–7079

Frieman MB, Cormack BP (2003) The omega-site sequence of glycosylphosphatidylinositol-anchored proteins in *Saccharomyces cerevisiae* can determine distribution between the membrane and the cell wall. Mol Microbiol 50:883–896

Frohner IE, Bourgeois C, Yatsyk K, Majer O, Kuchler K (2009) *Candida albicans* cell surface superoxide dismutases degrade host-derived reactive oxygen species to escape innate immune surveillance. Mol Microbiol 71:240–252

Fu Y, Luo G, Spellberg BJ, Edwards JE Jr, Ibrahim AS (2008) Gene overexpression/suppression analysis of candidate virulence factors of *Candida albicans*. Eukaryot Cell 7:483–492. doi:10.1128/EC.00445-07

Fujii T, Shimoi H, Iimura Y (1999) Structure of the glucan-binding sugar chain of Tip1p, a cell wall protein of *Saccharomyces cerevisiae*. Biochim Biophys Acta 1427:133–144

Gastebois A, Fontaine T, Latge JP, Mouyna I (2010a) b (1-3)Glucanosyltransferase Gel4p is essential for *Aspergillus fumigatus*. Eukaryot Cell 9:1294–1298. doi:10.1128/EC.00107-10

Gastebois A, Mouyna I, Simenel C, Clavaud C, Coddeville B, Delepierre M, Latge JP, Fontaine T (2010b) Characterization of a new b(1-3)-glucan branching activity of *Aspergillus fumigatus*. J Biol Chem 285:2386–2396. doi:10.1074/jbc.M109.077545

Gastebois A, Aimanianda V, Bachellier-Bassi S, Nesseir A, Firon A, Beauvais A, Schmitt C, England P, Beau R, Prevost MC, d'Enfert C, Latgé JP, Mouyna I (2013) SUN proteins belong to a novel family of b-(1,3)-glucan-modifying enzymes involved in fungal morphogenesis. J Biol Chem 288:13387–13396. doi:10.1074/jbc.M112.440172

Gelis S, De Groot PWJ, Castillo L, Moragues MD, Sentandreu R, Gómez MM, Valentín E (2012) Pga13 in *Candida albicans* is localized in the cell wall and influences cell surface properties, morphogenesis and virulence. Fungal Genet Biol 49:322–331, doi: S1087-1845(12)00027-8

Gil-Bona A, Llama-Palacios A, Parra CM, Vivanco F, Nombela C, Monteoliva L, Gil C (2015) Proteomics unravels extracellular vesicles as carriers of classical cytoplasmic proteins in *Candida albicans*. J Proteome Res 14:142–153. doi:10.1021/pr5007944

Gioti A, Nystedt B, Li W, Xu J, Andersson A, Averette AF, Münch K, Wang X, Kappauf C, Kingsbury JM, Kraak B, Walker LA, Johansson HJ, Holm T, Lehtiö J, Stajich JE, Mieczkowski P, Kahmann R, Kennell JC, Cardenas ME, Lundeberg J, Saunders CW, Boekhout T, Dawson TL, Munro CA, De Groot PWJ, Butler G, Heitman J, Scheynius A (2013) Genomic Insights into the atopic eczema-associated skin commensal yeast Malassezia sympodialis. MBio 4:e00572–00512. doi:10.1128/mBio.00572-12

Girrbach V, Strahl S (2003) Members of the evolutionarily conserved PMT family of protein *O*-mannosyltransferases form distinct protein complexes among themselves. J Biol Chem 278:12554–12562. doi:10.1074/jbc.M212582200

González M, Brito N, Frias M, González C (2013) *Botrytis cinerea* protein *O*-mannosyltransferases play critical roles in morphogenesis, growth, and virulence. PLoS ONE 8, e65924. doi:10.1371/journal.pone.0065924

González M, Brito N, González C (2014) Identification of glycoproteins secreted by wild-type *Botrytis cinerea* and by protein *O*-mannosyltransferase mutants. BMC Microbiol 14:254, doi:s12866-014-0254-y

Goto M (2007) Protein *O*-glycosylation in fungi: diverse structures and multiple functions. Biosci Biotechnol Biochem 71:1415–1427. doi:10.1271/bbb.70080

Goto M, Harada Y, Oka T, Matsumoto S, Takegawa K, Furukawa K (2009) Protein *O*-mannosyltransferases B and C support hyphal development and differentiation in *Aspergillus nidulans*. Eukaryot Cell 8:1465–1474. doi:10.1128/EC.00371-08

Granger BL, Flenniken ML, Davis DA, Mitchell AP, Cutler JE (2005) Yeast wall protein 1 of *Candida albicans*. Microbiology 151:1631–1644

Grün CH, Hochstenbach F, Humbel BM, Verkleij AJ, Sietsma JH, Klis FM, Kamerling JP, Vliegenthart JF (2005) The structure of cell wall a-glucan from fission yeast. Glycobiology 15:245–257

Hall RA, Gow NA (2013) Mannosylation in Candida albicans: role in cell wall function and immune recognition. Mol Microbiol 90:1147–1161. doi:10.1111/mmi.12426

Hamada K, Terashima H, Arisawa M, Yabuki N, Kitada K (1999) Amino acid residues in the omega-minus region participate in cellular localization of yeast glycosylphosphatidylinositol-attached proteins. J Bacteriol 181:3886–3889

Heilmann CJ, Sorgo AG, Siliakus AR, Dekker HL, Brul S, de Koster CG, de Koning LJ, Klis FM (2011) Hyphal induction in the human fungal pathogen *Candida albicans* reveals a characteristic wall protein profile. Microbiology 157:2297–2307. doi:10.1099/mic.0.049395-0

Heilmann CJ, Sorgo AG, Klis FM (2012) News from the fungal front: wall proteome dynamics and host-pathogen interplay. PLoS Pathog 8, e1003050. doi:10.1371/journal.ppat.1003050

Hochstenbach F, Klis FM, Van den Ende H, Van Donselaar E, Peters PJ, Klausner RD (1998)

Identification of a putative a-glucan synthase essential for cell wall construction and morphogenesis in fission yeast. Proc Natl Acad Sci USA 95:9161–9166

Hoyer LL, Green CB, Oh SH, Zhao X (2008) Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family - a sticky pursuit. Med Mycol 46:1–15

Iraqui I, Garcia-Sanchez S, Aubert S, Dromer F, Ghigo J-M, d'Enfert C, Janbon G (2005) The Yak1p kinase controls expression of adhesins and biofilm formation in *Candida glabrata* in a Sir4p-dependent pathway. Mol Microbiol 55:1259–1271

Jaafar L, Moukadiri I, Zueco J (2003) Characterization of a disulphide-bound Pir-cell wall protein (Pir-CWP) of *Yarrowia lipolytica*. Yeast 20:417–426

Jackson AP, Gamble JA, Yeomans T, Moran GP, Saunders D, Harris D, Aslett M, Barrell JF, Butler G, Citiulo F, Coleman DC, de Groot PWJ, Goodwin TJ, Quail MA, McQuillan J, Munro CA, Pain A, Poulter RT, Rajandream MA, Renauld H, Spiering MJ, Tivey A, Gow NAR, Barrell B, Sullivan DJ, Berriman M (2009) Comparative genomics of the fungal pathogens *Candida dubliniensis* and *Candida albicans*. Genome Res 19:2231–2244

Kapteyn JC, Ter Riet B, Vink E, Blad S, De Nobel H, Van den Ende H, Klis FM (2001) Low external pH induces *HOG1*-dependent changes in the organization of the *Saccharomyces cerevisiae* cell wall. Mol Microbiol 39:469–479

Katayama S, Hirata D, Arellano M, Pérez P, Toda T (1999) Fission yeast a-glucan synthase Mok1 requires the actin cytoskeleton to localize the sites of growth and plays an essential role in cell morphogenesis downstream of protein kinase C function. J Cell Biol 144:1173–1186

Kitagaki H, Wu H, Shimoi H, Ito K (2002) Two homologous genes, *DCW1* (*YKL046c*) and *DFG5*, are essential for cell growth and encode glycosylphosphatidylinositol (GPI)-anchored membrane proteins required for cell wall biogenesis in *Saccharomyces cerevisiae*. Mol Microbiol 46:1011–1022

Klis FM, De Groot P, Hellingwerf K (2001) Molecular organization of the cell wall of *Candida albicans*. Med Mycol 39(Suppl 1):1–8

Klis FM, Boorsma A, De Groot PWJ (2006) Cell wall construction in *Saccharomyces cerevisiae*. Yeast 23:185–202

Klis FM, de Jong M, Brul S, De Groot PWJ (2007a) Extraction of cell surface-associated proteins from living yeast cells. Yeast 24:253–258

Klis FM, Ram AFJ, De Groot PWJ (2007b) A molecular and genomic view of the fungal cell wall. In: Howard RJ, Gow NAR (eds) The mycota, vol 8. Springer, Berlin, pp 95–117

Klis FM, Sosinska GJ, de Groot PWJ, Brul S (2009) Covalently linked cell wall proteins of *Candida albicans* and their role in fitness and virulence. FEMS Yeast Res 9:1013–1028

Klis FM, de Koster CG, Brul S (2014) Cell wall-related bionumbers and bioestimates of *Saccharomyces cerevisiae* and *Candida albicans*. Eukaryot Cell 13:2–9. doi:10.1128/EC.00250-13

Kraneveld EA, De Soet JJ, Deng DM, Dekker HL, De Koster CG, Klis FM, Crielaard W, De Groot PWJ (2011) Identification and differential gene expression of adhesin-like wall proteins in *Candida glabrata* biofilms. Mycopathologia 172:415–427

Kurtz MB, Heath IB, Marrinan J, Dreikorn S, Onishi J, Douglas C (1994) Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-b-D-glucan synthase. Antimicrob Agents Chemother 38:1480–1489

Kuznets G, Vigonsky E, Weissman Z, Lalli D, Gildor T, Kauffman SJ, Turano P, Becker J, Lewinson O, Kornitzer D (2014) A relay network of extracellular heme-binding proteins drives *C. albicans* iron acquisition from hemoglobin. PLoS Pathog 10, e1004407. doi:10.1371/journal.ppat.1004407

Laforet L, Moreno I, Sánchez-Fresneda R, Martínez-Esparza M, Martínez JP, Argüelles J-C, De Groot PWJ, Valentín E (2011) Pga26 mediates filamentation and biofilm formation and is required for virulence in *Candida albicans*. FEMS Yeast Res 11:389–397. doi:10.1111/j.1567-1364.2011.00727.x

Lagorce A, Hauser NC, Labourdette D, Rodriguez C, Martin-Yken H, Arroyo J, Hoheisel JD, Francois J (2003) Genome-wide analysis of the response to cell wall mutations in the yeast *Saccharomyces cerevisiae*. J Biol Chem 278:20345–20357

Latgé JP (2007) The cell wall: a carbohydrate armour for the fungal cell. Mol Microbiol 66:279–290

Latgé JP, Beauvais A (2014) Functional duality of the cell wall. Curr Opin Microbiol 20:111–117. doi:10.1016/j.mib.2014.05.009

Lesage G, Bussey H (2006) Cell wall assembly in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 70:317–343

Lipke PN, Ovalle R (1998) Cell wall architecture in yeast: new structure and new challenges. J Bacteriol 180:3735–3740

Maddi A, Fu C, Free SJ (2012) The *Neurospora crassa dfg5* and *dcw1* genes encode a-1,6-mannanases that function in the incorporation of glycoproteins into the cell wall. PLoS ONE 7, e38872. doi:10.1371/journal.pone.0038872

Maestre-Reyna M, Diderrich R, Veelders MS, Eulenburg G, Kalugin V, Bruckner S, Keller P, Rupp S, Mösch HU, Essen LO (2012) Structural basis for promiscuity and specificity during *Candida glabrata* invasion of host epithelia. Proc Natl Acad Sci USA 109:16864–16869. doi:10.1073/pnas.1207653109

Magnelli PE, Cipollo JF, Robbins PW (2005) A glucanase-driven fractionation allows redefinition

of *Schizosaccharomyces pombe* cell wall composition and structure: assignment of diglucan. Anal Biochem 336:202–212

Martinez AI, Castillo L, Garcera A, Elorza MV, Valentin E, Sentandreu R (2004) Role of Pir1 in the construction of the *Candida albicans* cell wall. Microbiology 150:3151–3161

Mazáň M, Blanco N, Kováčová K, Firáková Z, Rehulka P, Farkaš V, Arroyo J (2013) A novel fluorescence assay and catalytic properties of Crh1 and Crh2 yeast cell wall transglycosylases. Biochem J 455:307–318. doi:10.1042/BJ20130354

Moreno-Ruiz E, Ortu G, De Groot PWJ, Cottier F, Loussert C, Prévost M-C, De Koster C, Klis FM, Goyard S, d'Enfert C (2009) The GPI-modified proteins Pga59 and Pga62 of *Candida albicans* are required for cell wall integrity. Microbiology 155:2004–2020

Morris VK, Sunde M (2013) Formation of amphipathic amyloid monolayers from fungal hydrophobin proteins. Methods Mol Biol 996:119–129. doi:10.1007/978-1-62703-354-1_7

Moukadiri I, Zueco J (2001) Evidence for the attachment of Hsp150/Pir2 to the cell wall of *Saccharomyces cerevisiae* through disulfide bridges. FEMS Yeast Res 1:241–245, doi:S1567135601000253

Moukadiri I, Jaafar L, Zueco J (1999) Identification of two mannoproteins released from cell walls of a *Saccharomyces cerevisiae mnn1 mnn9* double mutant by reducing agents. J Bacteriol 181:4741–4745

Mouyna I, Monod M, Fontaine T, Henrissat B, Lechenne B, Latgé JP (2000) Identification of the catalytic residues of the first family of b(1-3) glucanosyltransferases identified in fungi. Biochem J 347:741–747

Mouyna I, Morelle W, Vai M, Monod M, Lechenne B, Fontaine T, Beauvais A, Sarfati J, Prévost MC, Henry C, Latgé JP (2005) Deletion of *GEL2* encoding for a b(1-3)glucanosyltransferase affects morphogenesis and virulence in *Aspergillus fumigatus*. Mol Microbiol 56:1675–1688

Mouyna I, Kniemeyer O, Jank T, Loussert C, Mellado E, Aimanianda V, Beauvais A, Wartenberg D, Sarfati J, Bayry J, Prévost MC, Brakhage AA, Strahl S, Huerre M, Latgé JP (2010) Members of protein O-mannosyltransferase family in *Aspergillus fumigatus* differentially affect growth, morphogenesis and viability. Mol Microbiol 76:1205–1221. doi:10.1111/j.1365-2958.2010.07164.x

Mrša V, Klebl F, Tanner W (1993) Purification and characterization of the *Saccharomyces cerevisiae BGL2* gene product, a cell wall endo-b-1,3-glucanase. J Bacteriol 175:2102–2106

Nombela C, Gil C, Chaffin WL (2006) Non-conventional protein secretion in yeast. Trends Microbiol 14:15–21

Omi K, Sonoda H, Nagata K, Sugita K (1999) Cloning and characterization of *psu1*$^{+}$, a new essential fission yeast gene involved in cell wall synthesis. Biochem Biophys Res Commun 262:368–374

Orlean P (2012) Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. Genetics 192:775–818. doi:10.1534/genetics.112.144485

Osumi M (2012) Visualization of yeast cells by electron microscopy. J Electron Microsc (Tokyo) 61:343–365. doi:10.1093/jmicro/dfs082

Ouyang H, Chen X, Lu Y, Wilson IB, Tang G, Wang A, Jin C (2013) One single basic amino acid at the w-1 or w-2 site is a signal that retains glycosylphosphatidylinositol-anchored protein in the plasma membrane of *Aspergillus fumigatus*. Eukaryot Cell 12:889–899. doi:10.1128/EC.00351-12

Pardo M, Ward M, Bains S, Molina M, Blackstock W, Gil C, Nombela C (2000) A proteomic approach for the study of *Saccharomyces cerevisiae* cell wall biogenesis. Electrophoresis 21:3396–3410

Pardo M, Monteoliva L, Vazquez P, Martinez R, Molero G, Nombela C, Gil C (2004) *PST1* and *ECM33* encode two yeast cell surface GPI proteins important for cell wall integrity. Microbiology 150:4157–4170

Pel HJ, De Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ, Turner G, De Vries RP, Albang R, Albermann K, Andersen MR, Bendtsen JD, Benen JAE, Van den Berg M, Breestraat S, Caddick MX, Contreras R, Cornell M, Coutinho PM, Danchin EGJ, Debets AJM, Dekker P, Van Dijck PWM, Van Dijk A, Dijkhuizen L, Driessen AJM, d'Enfert C, Geysens S, Goosen C, Groot GSP, De Groot PWJ, Guillemette T, Henrissat B, Herweijer M, van den Hombergh JPTW, Van den Hondel CAMJJ, Van der Heijden RTJM, Van der Kaaij RM, Klis FM, Kools HJ, Kubicek CP, Van Kuyk PA, Lauber J, Lu X, Van der Maarel MJEC, Meulenberg R, Menke H, Mortimer MA, Nielsen J, Oliver SG, Olsthoorn M, Pal K, van Peij NNME, Ram AFJ, Rinas U, Roubos JA, Sagt CMJ, Schmoll M, Sun J, Ussery D, Varga J, Vervecken W, van de Vondervoort PJJ, Wedler H, Wosten HAB, Zeng AP, Van Ooyen AJ, Visser J, Stam H (2007) Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. Nat Biotechnol 25:221–231

Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20:133–163

Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D (2012) Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance(R)) registry, 2004-2008. Diagn Microbiol Infect Dis 74:323–331. doi:10.1016/j.diagmicrobio.2012.10.003

Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, Franks B, Azie NE (2014) Epidemiology and outcomes of invasive candidiasis due to non-albicans species of *Candida* in 2,496

patients: data from the Prospective Antifungal Therapy (PATH) registry 2004-2008. PLoS ONE 9, e101510. doi:10.1371/journal.pone.0101510

Phan QT, Myers CL, Fu Y, Sheppard DC, Yeaman MR, Welch WH, Ibrahim AS, Edwards JE Jr, Filler SG (2007) Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol 5, e64

Pitarch A, Jimenez A, Nombela C, Gil C (2006) Decoding serological response to *Candida* cell wall immunome into novel diagnostic, prognostic, and therapeutic candidates for systemic candidiasis by proteomic and bioinformatic analyses. Mol Cell Proteomics 5:79–96

Plaine A, Walker L, Da Costa G, Mora-Montes HM, McKinnon A, Gow NA, Gaillardin C, Munro CA, Richard ML (2008) Functional analysis of *Candida albicans* GPI-anchored proteins: roles in cell wall integrity and caspofungin sensitivity. Fungal Genet Biol 45:1404–1414. doi:10.1016/j.fgb.2008.08.003

Plaza V, Lagües Y, Carvajal M, Pérez-García LA, Mora-Montes HM, Canessa P, Larrondo LF, Castillo L (2015) *bcpmr1* encodes a P-type Ca(2+)/Mn(2+)-ATPase mediating cell-wall integrity and virulence in the phytopathogen *Botrytis cinerea*. Fungal Genet Biol 76:36–46. doi:10.1016/j.fgb.2015.01.012

Prados-Rosales R, Luque-Garcia JL, Martínez-López R, Gil C, Di Pietro A (2009) The *Fusarium oxysporum* cell wall proteome under adhesion-inducing conditions. Proteomics 9:4755–4769

Prill SKH, Klinkert B, Timpel C, Gale CA, Schroppel K, Ernst JF (2005) *PMT* family of *Candida albicans*: five protein mannosyltransferase isoforms affect growth, morphogenesis and antifungal resistance. Mol Microbiol 55:546–560

Rodrigues ML, Nimrichter L, Oliveira DL, Nosanchuk JD, Casadevall A (2008) Vesicular trans-cell wall transport in fungi: a mechanism for the delivery of virulence-associated macromolecules? Lipid Insights 2:27–40

Ruiz-Herrera J, Elorza MV, Valentin E, Sentandreu R (2006) Molecular organization of the cell wall of *Candida albicans* and its relation to pathogenicity. FEMS Yeast Res 6:14–29

Schild L, Heyken A, De Groot PWJ, Hiller E, Mock M, De Koster C, Horn U, Rupp S, Hube B (2011) Proteolytic cleavage of covalently linked cell wall proteins by *Candida albicans* Sap9 and Sap10. Eukaryot Cell 10:98–109

Schoffelmeer EAM, Klis FM, Sietsma JH, Cornelissen BJ (1999) The cell wall of *Fusarium oxysporum*. Fungal Genet Biol 27:275–282

Schoffelmeer EAM, Vossen JH, Van Doorn AA, Cornelissen BJ, Haring MA (2001) FEM1, a *Fusarium oxysporum* glycoprotein that is covalently linked to the cell wall matrix and is conserved in filamentous fungi. Mol Genet Genomics 265:143–152

Smits GJ, Kapteyn JC, van den Ende H, Klis FM (1999) Cell wall dynamics in yeast. Curr Opin Microbiol 2:348–352

Sorgo AG, Heilmann CJ, Dekker HL, Bekker M, Brul S, De Koster CG, De Koning LJ, Klis FM (2011) Effects of fluconazole on the secretome, the wall proteome, and wall integrity of the clinical fungus *Candida albicans*. Eukaryot Cell 10:1071–1081

Sorgo AG, Brul S, De Koster CG, De Koning LJ, Klis FM (2013) Iron restriction-induced adaptations in the wall proteome of *Candida albicans*. Microbiology 159:1673–1682. doi:10.1099/mic.0.065599-0

Sosinska GJ, De Groot PWJ, Teixeira de Mattos MJ, Dekker HL, De Koster CG, Hellingwerf KJ, Klis FM (2008) Hypoxic conditions and iron restriction affect the cell wall proteome of *Candida albicans* grown under vagina-simulative conditions. Microbiology 154:510–520

Sosinska GJ, de Koning LJ, De Groot PWJ, Manders EM, Dekker HL, Hellingwerf KJ, de Koster CG, Klis FM (2011) Mass spectrometric quantitation of the adaptations in the wall proteome of *Candida albicans* in response to ambient pH. Microbiology 157:136–146

Spreghini E, Davis DA, Subaran R, Kim M, Mitchell AP (2003) Roles of *Candida albicans* Dfg5p and Dcw1p cell surface proteins in growth and hypha formation. Eukaryot Cell 2:746–755

Staab JF, Bradway SD, Fidel PL, Sundstrom P (1999) Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. Science 283:1535–1538

Staats M, van Kan JA (2012) Genome update of *Botrytis cinerea* strains B05.10 and T4. Eukaryot Cell 11:1413–1414. doi:10.1128/EC.00164-12

Steczkiewicz K, Knizewski L, Rychlewski L, Ginalski K (2010) TOS1 is circularly permuted 1,3-b-glucanase. Cell Cycle 9:201–204

Tanner W, Lehle L (1987) Protein glycosylation in yeast. Bba-Gen Subjects 906:81–99

Terashima H, Hamada K, Kitada K (2003) The localization change of Ybr078w/Ecm33, a yeast GPI-associated protein, from the plasma membrane to the cell wall, affecting the cellular function. FEMS Microbiol Lett 218:175–180

Thierry A, Dujon B, Richard GF (2010) Megasatellites: a new class of large tandem repeats discovered in the pathogenic yeast *Candida glabrata*. Cell Mol Life Sci 67:671–676. doi:10.1007/s00018-009-0216-y

Tokunaga M, Kusamichi M, Koike H (1986) Ultrastructure of outermost layer of cell wall in *Candida albicans* observed by rapid-freezing technique. J Electron Microsc (Tokyo) 35:237–246

Vargas G, Rocha JD, Oliveira DL, Albuquerque PC, Frases S, Santos SS, Nosanchuk JD, Gomes AM, Medeiros LC, Miranda K, Sobreira TJ, Nakayasu ES, Arigi EA, Casadevall A, Guimaraes AJ, Rodrigues ML, Freire-de-Lima CG, Almeida IC,

Nimrichter L (2015) Compositional and immunobiological analyses of extracellular vesicles released by *Candida albicans*. Cell Microbiol 17:389–407. doi:10.1111/cmi.12374

Verstrepen KJ, Fink GR (2009) Genetic and epigenetic mechanisms underlying cell-surface variability in protozoa and fungi. Annu Rev Genet 43:1–24, PMID: 19640229

Weig M, Jansch L, Gross U, De Koster CG, Klis FM, De Groot PWJ (2004) Systematic identification in silico of covalently bound cell wall proteins and analysis of protein-polysaccharide linkages of the human pathogen *Candida glabrata*. Microbiology 150:3129–3144

Wolf JM, Espadas-Moreno J, Luque-Garcia JL, Casadevall A (2014) Interaction of *Cryptococcus neoformans* extracellular vesicles with the cell wall. Eukaryot Cell 13:1484–1493. doi:10.1128/EC.00111-14

Wösten HA (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55:625–646

Yeater KM, Chandra J, Cheng G, Mukherjee PK, Zhao X, Rodriguez-Zas SL, Kwast KE, Ghannoum MA, Hoyer LL (2007) Temporal analysis of *Candida albicans* gene expression during biofilm development. Microbiology 153:2373–2385, doi:153/8/2373

Yin QY, De Groot PWJ, Dekker HL, De Jong L, Klis FM, De Koster CG (2005) Comprehensive proteomic analysis of *Saccharomyces cerevisiae* cell walls: identification of proteins covalently attached via glycosylphosphatidylinositol remnants or mild alkali-sensitive linkages. J Biol Chem 280:20894–20901

Yin QY, de Groot PWJ, De Jong L, Klis FM, De Koster CG (2007) Mass spectrometric quantitation of covalently bound cell wall proteins in *Saccharomyces cerevisiae*. FEMS Yeast Res 7:887–896

Zupancic ML, Frieman M, Smith D, Alvarez RA, Cummings RD, Cormack BP (2008) Glycan microarray analysis of *Candida glabrata* adhesin ligand specificity. Mol Microbiol 68:547–559

6 Heterogenic Incompatibility in Fungi

K. Esser[1]

CONTENTS

I. Introduction

In biology, incompatibility is usually defined as restriction of mating competence controlled by genes other than those determining sexual differentiation. It has been long recognised that incompatibility concerns not only the sexual phase but also the vegetative phase. The latter becomes apparent especially in fungi and was first termed heterokaryon incompatibility. In both sexual and vegetative incompatibility, the action of the genetic traits involved precludes the exchange of genetic material. Thus, inhibition of recombination results by a lack of karyogamy (sexual incompatibility) as well as by an inability of the nuclei to coexist in a common cytoplasm and to undergo somatic recombination (vegetative incompatibility). Since recombination is of paramount importance in evolution, the biological significance of incompatibility as a factor controlling recombination is immediately apparent.

Nature has evolved two principal systems to control incompatibility. According to their mode of genetic determination, these have been called homogenic and heterogenic incompatibility (Esser 1962). The genetic basis of homogenic incompatibility consists in a sexual incompatibility of nuclei carrying identical incompatibility factors. **Heterogenic incompatibility consists in a genetic difference of at least one single gene which inhibits the coexistence of the nuclei concerned in a common cytoplasm.**

From these definitions, it follows that homogenic incompatibility enhances outbreeding and favours recombination and evolution of the species. Heterogenic incompatibility, however, restricts outbreeding and thereby favours the evolution of isolated groups within a single species. Both systems, despite controlling recombination in an antagonistic way, are integrated constituents of evolution.

Homogenic incompatibility has been known since Darwin's time and its various mechanisms have been analysed in great detail, in both higher plants and fungi. Its actions and its distribution are the subject of many books and reviews. This type of incompatibility is described in Freihorst et al. (2016) and Dyer et al. (2016).

The genetics of heterogenic incompatibility was first revealed 60 years ago, in studies involving the ascomycete *Podospora anserina*

[1]Lehrstuhl für Allgemeine Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany; e-mail: karl.esser@rub.de

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

(Rizet and Esser 1953; Esser 1954). Meanwhile, this subject has attracted much attention, and many cases of heterogenic incompatibility, dealing with the vegetative and/or the sexual phases of fungi, have been described and analysed (see Table 6.1).

It is understandable that heterogenic incompatibility was less intensively studied than homogenic incompatibility, since the former rarely occurs within true breeding laboratory strains, but rather between geographical races differing in their genetic constituency. In addition, heterogenic incompatibility has often been overlooked and sometimes misinterpreted as "sterility", merely because of a failure to mate.

This review is a revised and updated version of the chapter on heterogenic incompatibility in the second edition of this volume (Esser 2006).

Other pertinent reviews on heterogenic incompatibility can be found in Esser and Kuenen (1967), Esser (1971), Lemke (1973), Carlile and Gooday (1978), Lane (1981), Esser and Meinhardt (1984), Jennings and Rayner (1984), Perkins and Turner (1988), Glass and Kuldau (1992), Leslie (1993), Bégueret et al. (1994), Leslie and Zeller (1996), Worall (1997), Glass et al. (2000), Saupe (2000) and Glass and Kaneko (2003).

II. Barrage Formation

More than 100 years ago, Reinhardt (1892) first observed that when certain fungal mycelia approached one another, sometimes an interaction phenotypically recognisable as repulsion occurred. This phenomenon was subsequently described by others (Cayley 1923, 1931; Nakata 1925), and it was probably Vandendries (1932) who introduced the term barrage to describe it. Certainly, older descriptions of barrages, based on quite different phenomena, were assigned different names. Since **barrage is a phenotypic expression of heterogenic incompatibility**, it is at first necessary to define the concept of barrage.

If fungal hyphae from different mycelia grow towards each other, in general four main types of interaction occur, and these can be easily demonstrated on agar media.

1. Mutual Intermingling = Normal Contact
   After approach, the hyphae intermingle in the zone of contact and show (with a few exceptions, as in Oomycota) numerous hyphal fusions via anastomosis. After a time, the border zone between the two mycelia becomes unrecognisable (Fig. 6.1a–d).

2. Inhibition
   When opposing hyphae approach each other, an inhibition zone free of hyphae is formed between the two mycelia. This phenomenon may be caused by unilateral or mutual interaction, due to the secretion and diffusion of inhibitory substances.

3. Mutual Intermingling and Inhibition = Barrage Formation
   When two mycelia grow into each other and intermingle, an antagonistic reaction ensues. In contrast to inhibition by diffusible substances, the barrage reaction requires cytoplasmic contact via hyphal fusions. The phenotype of the barrage varies depending on the species and mode of genetic control (Fig. 6.1). However, in all barrages known so far, nuclear exchange is not inhibited, but in most cases the two types of mycelia form abnormal and even lethal fusions. The hyphal tips may branch profusely. A clear line of contact appears with increasing age of the culture. Barrages are mainly found in intraspecific (interracial) matings. Depending on the species, the barrage may be colourless or pigmented (Fig. 6.1a, c). A recent study in *Neurospora* shows that different types of barrages may occur also within one and the same species (Micali and Smith 2003).

4. Mutual Repulsion = Border Line, Demarcation Line
   Especially noted in matings of wood-rotting basidiomycetes, a mutual repulsion and antagonistic reaction is evident which leads to the formation of a more or less strongly pigmented zone of intermingled hyphae. This demarcation line (Adams and Roth 1967) or border line (Esser and Hoffmann 1977) occurs mainly in interspecific matings. It is visible in nature on cuttings of logs (Rayner and Todd 1977; Esser and Meinhardt 1984; Fig. 6.1d) as well as in axenic cultures (Fig. 6.1c). Border lines are often used as criteria for species delineations. However, this commonly creates some problems in interpretation because, in most cases investigated to date, there are no analyses of the microscopic structure of these

Table 6.1 Examples of occurrence of heterogenic incompatibility in fungi, as interpreted from symptoms described by the investigators

Species	Symptoms	References
Oomycota		
* *Phytophthora* spp.	Restriction of mating competence	Savage et al. (1968), Boccas (1981)
P. infestans	Influence of nutrient media on incompatibility	Cherepennikova-Anikina et al. (2002)
Eumycota Glomeromycota		
Glomus mosseae	v-c groups	Giovanetti et al. (2003)
Ascomycetes, saprophytes		
**Ascobolus immersus*	Bipolar; v-c groups; barrage; sexual incompatibility	Meinhardt et al. (1984)
**Aspergillus* spp.	Self-compatible; v-c groups; up to eight *het*-genes	Grindle (1963a, b), Jinks et al. (1966), Butcher (1968, 1969), Butcher et al. (1972), Caten et al. (1971), Dales and Croft (1977, 1990), Croft and Dales (1984)
Aspergillus flavus *A. parasiticus*	i-c groups; morphological and physiological diversities	Horn et al. (1996)
A. tamarii **Aspergillus niger*	i-c groups; virus transfer inhibited Mating-type idiomorphs; up to 11 *het*-genes	Van Diepeningen et al. (1997), Pal (2005), Garnjobst and Wilson (1956), Pittenger and Brawner (1961), Wilson (1961, 1963), Wilson et al. (1961), Pittenger (1964), Wilson and Garnjobst (1966), Moreau and Moruzi (1933), Newmeyer and Taylor (1967), Newmeyer (1968, 1970), Williams and Wilson (1968), Turner et al. (1969), Mylyk (1975, 1976), Leslie (1987), Perkins and Turner (1988), Shiu and Glass (2015)
**Neurospora* spp.		
* *Podospora anserina*	Bipolar; v-c groups; barrage; up to nine *het*-genes; reciprocal and nonreciprocal sexual incompatibility	See Sect. II, this chapter
Ascomycetes, parasites		
* *Botrytis cinerea*	v-c groups; gene *Bc-hch* homolog to *het*-genes of *N. crassa* and *P. anserina*	Fournier et al. (2003)
Cochliobolus heterostrophus	Bipolar; v-c groups; *het*-genes	Leach and Yoder (1983), Nelson (1963, 1965a, b 1966, 1970)
**Cochliobolus* spp. (imperf. *Helminthosporium*)	Heterogenic incompatibility in the sexual phase	Nelson and Kline (1964), Webster and Nelson (1968)
Crumenolopsis soriora	Bipolar; restriction of mating competence between subpopulations	Ennos and Swales (1987)
Diaporthe phaseolorum **Endothia (Cryphonectria) parasitica*	Self-compatible; barrage Bipolar; barrage; v-c groups; six or seven *het*-genes identified, allelic and non-allelic mechanism	Ploetz and Shokes (1986), Anagnostakis (1977,1982a, b, 1983), Nuss and Koltin (1990), Cortesi and Milgroom (1998), Smith et al. (2006)
Erysiphe cichoracearum	Bipolar; sexual incompatibility	Morrison (1960)
Ophiostoma (Ceratocystis ulmi)	Bipolar; barrage	Brasier (1984)
Gaeumannomyces graminis	v-c groups	Jamil et al. (1984)
* *Gibberella fujikuroi* (*Fusarium moniliforme*)	Bipolar; v-c groups; *het*-genes	Kuhlmann (1982), Puhalla and Spieth (1983, 1985), Sidhu (1986), Leslie et al. (2004)

(continued)

Table 6.1 (continued)

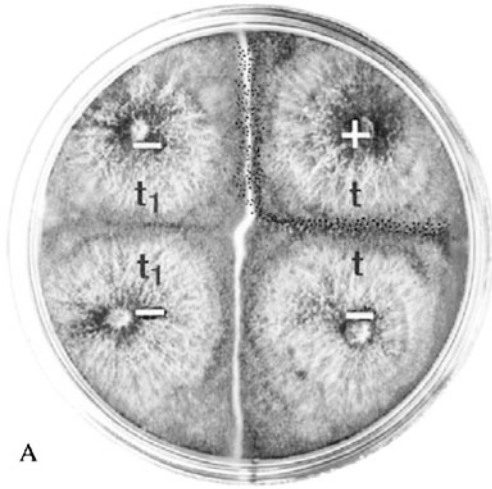
Species	Symptoms	References
Fusarium oxysporum	Imperfect; v-c groups	Correl et al. (1986), Jacobson and Gordon (1988), Katan and Katan (1988)
Fusarium spp.	Sexual and vegetative incompatibility	Hornok (2007)
Leucocytospora kunzei	v-c groups; six *het*-loci	Proffer and Hart (1988)
Monascus purpureus	Self-fertile; six v-c groups	Chaisrisook (2002)
Nannizzia spp. (imperf. *Microsporum)*	Bipolar; heterogenic incompatibility in the sexual phase?	Padhaye and Carmichel (1971)
Sclerotinia sclerotiorum	v-c groups	Kohn et al. (1991), Ford et al. (1995)
Sclerotinium rolfsii (teleomorph: *Athelia rolfsii)*	71 v-c groups	Punja and Sun (2002)
Sclerotinium delphini	Five v-c groups	
Stagonospora nodorum (teleomorph: *Leptosphaeria nodorum*)	v-c groups	Newton et al. (1998)
Venturia inaequalis	Bipolar; nonreciprocal incompatibility between different mating types of geographical races	Kiebacher and Hoffmann (1981)
Verticillium dahliae	Imperfect; v-c groups partially without barrage formation	Puhalla and Hummel (1983), Papaioannou and Typas (2015)
Basidiomycetes (in considering the wood-destroying basidiomycetes in this group, there is no distinction between saprophytes and parasites)		
Auricularia spp.	Tetrapolar; intraspecific i-s groups; barrage formation	Wong (1993)
Agaricus spp.	Border lines between species	Anderson et al. (1984)
Armillaria mellea	Bipolar; i-s groups; partially compatible	Anderson and Ullrich (1979), Anderson et al. (1980), Anderson (1986)
Athelia (*Sclerotium*) *rolfsii*	Bipolar? Barrage; i-s groups	Punja and Grogan (1983a, b)
Bjerkandera fumosa	Bipolar; i-s groups	Lombard et al. (1992)
Ceratobasidium bicorne	Self-fertile; demarcation line; i-s groups	Hietala et al. (2003)
**Collybia dryophila*	Bipolar; i-s groups; reduced sexual compatibility	Vilgalys and Miller (1987), Vilgalys and Johnson (1987)
Collybia subnuda	Bipolar; i-s groups; barrage	Murphy and Miller (1993)
Coprinus bisporus (Coprinellus bisporus)	Bipolar; heterogenic incompatibility sexual phase	Kemp (1989)
* *Coprinus cinereus* (*Coprinopsis cinerea*)	Tetrapolar; barrage, due to heterogenic mitochondrial DNA	May (1988)
Coriolus versicolor (*Trametes versicolor*)	Tetrapolar; i-s groups; barrage	Rayner and Todd (1977)
Cyathus spp.	Tetrapolar; unilateral dikaryotization within two species	Brodie (1970)
Fomes cajanderi	Barrage between geographical races	Adams and Roth (1967)
Ganoderma boninense	Tetrapolar incompatibility; i-s groups; border line	Pilotti et al. (2002), Goh et al. (2014)
Helicobasidium mompa	i-s groups; border line	Ikeda et al. (2003)
**Heterobasidion annosum*	Bipolar; i-s groups; 4–5 *het*-genes; epistatic; multiple alleles	Chase and Ullrich (1990a, b), Hansen et al. (1993a, b)
Heterobasidion insulare	Bipolar; three i-s groups	Dai et al. (2002)

(continued)

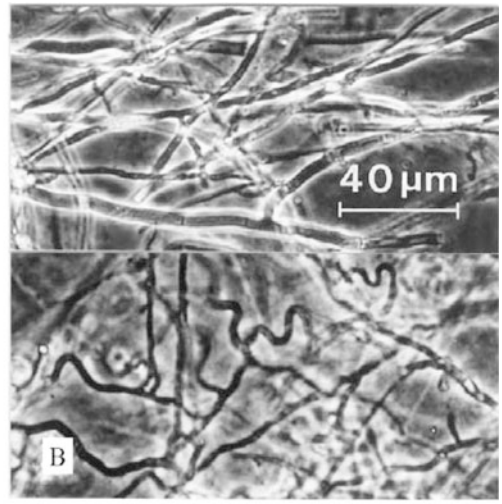
Table 6.1 (continued)

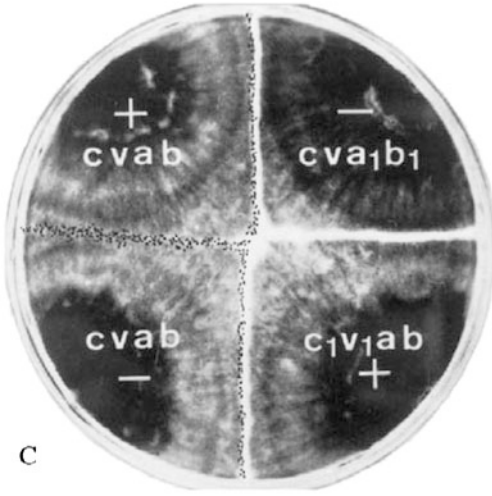
Species	Symptoms	References
Inonotus arizonicus	Self-compatible; i-s groups; barrage	Goldstein and Gilbertson (1981)
Laccaria spp.	Bipolar; i-s groups within one and between species	Fries and Mueller (1984)
Lentinula edodes	Tetrapolar; i-s groups; barrage	Yindeeyoungyeon and Triratana (1992)
Marasmiellus parasiticus	Tetrapolar; i-s groups; barrage	Murphy and Miller (1993)
Marasmius spp.	Tetrapolar; i-s groups	Gordon and Petersen (1992)
Paxillus involutus	Bipolar; i-s groups	Fries (1985)
Peniophora spp.	Bipolar; host-dependent intra- and interspecific incompatibility	McKeen (1952)
Phellinus gilvus	Tetrapolar; i-s groups; one or several *het*-genes	Rizzo et al. (1996)
Phellinus pini	Bipolar; i-s groups	Fischer (1994)
Phellinus weirii	Bipolar; barrage; i-s groups	Hansen (1979)
**Phellinus torulosus*	Bipolar; barrage; i-s groups	Fischer and Bresinsky (1992)
Phlebia spp.	Bipolar; intraspecific barrage; interspecific border line; i-s groups	Boddy and Rayner (1983)
Pisolithus arhizus (syn. *P. tinctorius)*	Tetrapolar; unilateral dikaryotization	Kope (1992)
**Pleurotus ostreatus*	Tetrapolar; barrage; i-s groups	Kay and Vilgalys (1992)
**Polyporus* spp.	Interspecific crosses sterile (border line); intraspecific crosses, tetrapolar incompatibility superimposed by heterogenic incompatibility; barrage formation caused by three genes	Macrae (1967), Barrett and Uscuplic (1971), Hoffmann and Esser (1978)
**Sistotrema brinkmannii*	Different breeding systems superimposed by heterogenic incompatibility	Lemke (1969)
**Stereum hirsutum*	Bipolar; compatibility superimposed by multiallelic *het*-genes; barrage	Coates and Rayner (1985a, b, c), Coates et al. (1981, 1985)
Stereum rugosum	Bipolar; i-s groups	Rayner and Turton (1982)
Stereum gausapatum	Bipolar; i-s groups	Boddy and Rayner (1982)
Stereum sanguinolentum	Self-compatible; i-s groups	Rayner and Turton (1982)
Stereum rameale	Self-compatible; i-s groups	Rayner and Turton (1982)
**Thanatephorus cucumeris*	Self-compatible; i-s groups; host-dependent	Stretton and Flentje (1972a, b)
Thanatephorus practicola (Rhizoctonia solani)	Self-compatible; i-s groups; barrage	Cubeta et al. (1993)
Trametes versicolor	Self-compatible i-s groups, barrage	Guler and Bicer (2014)
Typhula spp.	Tetrapolar; interspecific border line	Bruehl et al. (1975)
**Ustilago maydis*	Killer phenomenon	Puhalla (1968), Hankin and Puhalla (1971)

Because of the diversity involved in examples cited, there is risk of misinterpretations. I do not claim to present a complete list of all examples published. There are many papers, especially in basidiomycetes, which only vaguely indicate the existence of heterogenic incompatibility, and this needs to be further investigated. Abbreviations: v-c groups, fungal isolates which show vegetative compatibility, used mostly for ascomycetes; for the same concept in basidiomycetes, the terms intersterility (i-s) groups, biological races and biological species are used; *het*-genes, genes responsible for heterokaryon incompatibility; idiomorphs, mating-type genes showing slight differences of their genetic code; bipolar and tetrapolar, homogenic incompatibility controlled by a mono- and bifactorial mechanism, respectively. All these terms are also defined at appropriate places in the text. Asterisks indicate fungi treated in detail in the text

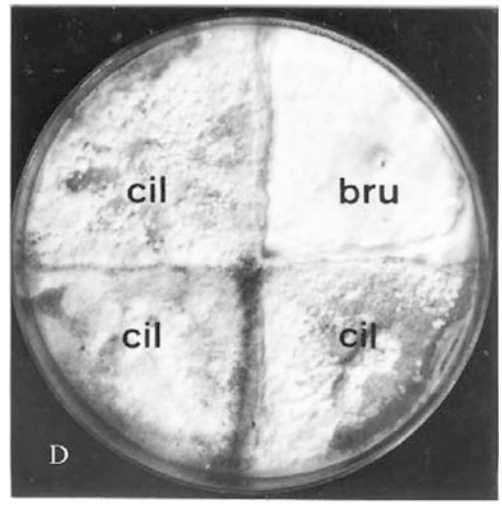


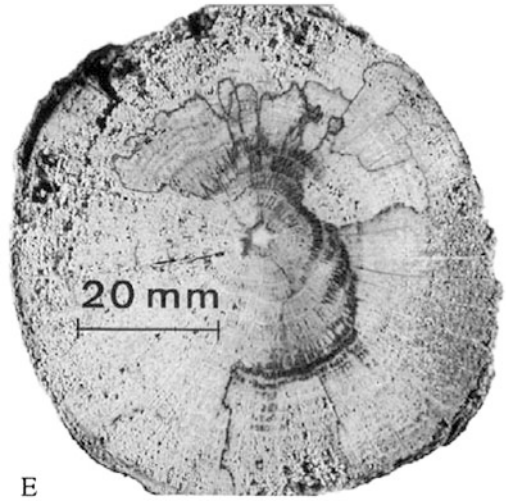
A





C





E

lines, nor of whether there is hyphal fusion allowing cytoplasmic contact and nuclear exchange. Therefore, it is often rather difficult to evaluate experimental reports with respect to barrage or border line formation and, consequently, to distinguish between intraspecific and interspecific matings.

Accordingly, I shall henceforth use the term barrage only if the microscopic observations show that a zone of aversion occurring between two mycelia is associated with hyphal fusions. I am well aware that this distinction is not always possible on the basis of exact data.

III. Heterogenic Incompatibility in the Ascomycete *Podospora anserina*

A. The Phenomenon

Although barrage formation is found in mycelial interactions with various higher fungi, the best analysed case concerns the ascomycete *Podospora* anserina. As in many ascomycetes, the mating competence of *P. anserina* is controlled by the bipolar mechanism of homogenic incompatibility (heterothallism) due to an interaction of the two idiomorphs + and − of the mating-type locus (see Dyer et al. 2016). In analogy to the *Neurospora* terminology, these alleles were later renamed and termed *mat*+ and *mat*−, respectively.

In studying various races of *P. anserina* with different geographical origins, the mycelia of which showed no recognisable macroscopic differences, Rizet (1952, 1953) found that in interrace combinations a barrage was formed irrespective of mating type. As may be seen from Fig. 6.1a, this barrage is macroscopically characterised by a sharp white zone between two darkly pigmented (melanotic) paired mycelia. A microscopic examination revealed that in this zone the hyphae forming anastomoses become curled, swollen and degenerative (Fig. 6.1b). This barrage zone eventually consists of dead hyphae.

In some cases the barrage formation does not affect fruiting between different mating types, because perithecia are formed on both sides of the barrage (Fig. 6.1a).

This peculiar phenomenon is explained by the fact that the trichogynes of the + female sex organs (protoperithecia) fuse only with the − male gametes (spermatia), and vice versa. Spermatia, however, are never formed in the barrage zone. From this it follows that the trichogynes pass the barrage unimpaired, since no anastomoses involving trichogynes take place. Obviously, the fusion of the tip of the trichogyne with a spermatium does not necessarily bring about the incompatibility reaction occurring between two hyphae, although the reason for this remains obscure.

Nevertheless, as summarised in Fig. 6.2, a comprehensive study of the mating interactions between 19 geographical races revealed that in addition to the barrage formation, the fruit body formation (perithecia) also was quantitatively and/or qualitatively disturbed. In the first instance, the number of perithecia was drastically reduced on one or both sides of the barrage. In the second case, one or both of the reciprocal crosses between the two mates were incompatible. In the 13 (7.6 %) interracial combinations which showed no barrage, there was also no effect on fruiting—in other words, **sexual incompatibility in interrace crosses is always linked with barrage formation.**

In this context, it should be noted that at least one incompatibility mechanism may also

Fig. 6.1 (**a–e**) Compilation of mycelial interactions in fungi. (**a**) *Podospora anserina*. Barrage formation between different geographical races. Sexual reproduction is not affected. Perithecia are produced in any combination between different mating types. (**b**) *Podospora* anserina. Hyphal morphology. *Above hyphae* in the contact zone of an intra-race combination, *below hyphae* within the barrage zone. (**c**) *Podospora anserina*. Barrage formation linked with sexual incompatibility (for details, see Fig. 3 and text). (**d**) Intra- and interspecific interactions between monokaryons of *Polyporus ciliatus (cil)* and *Polyporus brumalis (bru)* showing normal contact *(left)*, and barrage *(bottom)* and border line *(top* and *right)*. All monokaryons are compatible in mating type. (**e**) Cross section of a log colonised by wood-destroying basidiomycetes. *Dark zones* indicate the aversion lines of mycelia. Further details are given at various sites in the text (adapted from Esser 1956, and Esser and Meinhardt 1984)

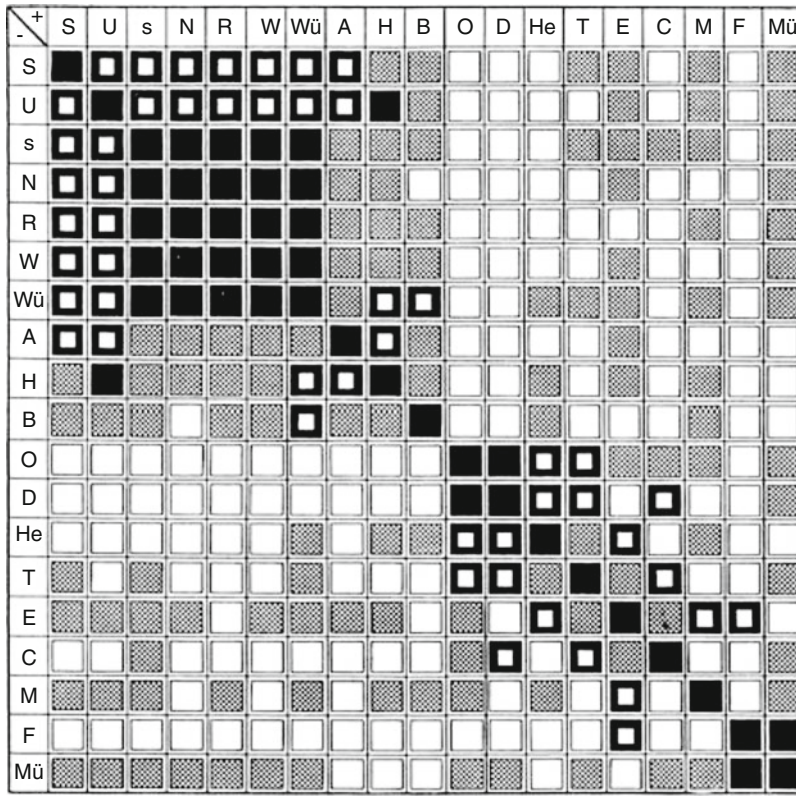


Fig. 6.2 Scheme of the mating reactions between various races of *Podospora anserina* isolated from different localities in France and Germany. +/− Mating type, *uppercase letters* designation of races, *closed squares* compatible in both vegetative and sexual phase, *closed squares with inserted open squares* sexual compatibility and vegetative incompatibility (barrage formation only), *hatched squares* vegetative incompatibility and reduced fruit body formation, *open squares* incompatibility in both vegetative and sexual phase (Adapted from Esser 1971)

lead to distortions in meiotic segregation due to spore killer effects (van der Gaag et al. 2003; Hamann and Osiewacz 2004).

B. Genetic Control

The genetic background of heterogenic incompatibility was revealed by the analysis of the two races *s* and *M* (Rizet and Esser 1953; Esser 1954, 1956). Six loci were identified as instrumental in two different mechanisms, as summarised in Fig. 6.3 and explained below:

1. The **allelic mechanism** caused by alleles of the *t* and *u* loci does not interfere with sexual compatibility. If strains differ at either one (Fig. 6.1a) or both loci, a vegetative incompatibility is provoked, showing up as barrage formation.
2. The **non-allelic mechanism** depends on the interaction of two specific alleles at two different loci. In the interracial cross of *s* and *M*, four loci *a*, *b*, *c*, *v* were identified showing an incompatibility of the alleles a_1/b and c_1/v, respectively, leading to barrage formation as well as to unilateral incompatibility (middle of Fig. 6.1c). If both mechanisms overlap in recombinants from the cross $s \times M$ in the combination a, b, c_1, $v_1 \times a_1$, b_1, c, v, then a complete sexual incompatibility is brought about (Fig. 6.1c, right side).

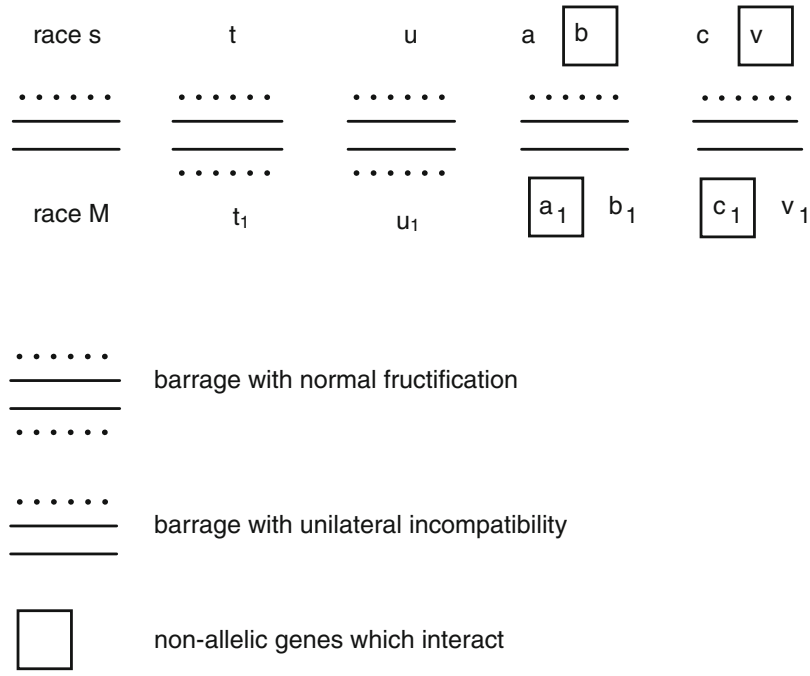


Fig. 6.3 *Podospora anserina*. Scheme of the action of the two mechanisms of heterogenic incompatibility operating in a cross of the races *s* and *M*. The different alleles are symbolised by *lowercase letters*. For further information, see text (Adapted from Blaich and Esser 1970)

C. Physiological Expression

As mentioned above, a prerequisite for the expression of heterogenic incompatibility is that two nuclei showing a specific allelic and/or non-allelic difference are brought together in a common cytoplasm. This occurs very frequently because in *Podospora* as in many other ascomycetes, hyphae fuse by anastomosis when they come into contact. This is followed by a mutual nuclear migration leading to heterokaryosis.

Incompatibility of heterogenic nuclei can be brought about by a unilateral or by a bilateral action. It was found that in those heterokaryons in which the allelic mechanism was effective, a destabilisation took place, leading to a formation of homokaryotic sectors of either nuclear type, separated by barrage formation. In heterokaryons in which the non-allelic mechanism was instrumental, however, no sectoring occurred, because one nuclear species was eliminated. For example, in the combination $ab+a_1b_1$, only the *ab* nuclei survived. Thus, it follows that the allelic mechanism brings about a mutual interaction, whereas the non-allelic mechanism is realised through unilateral gene action (Esser 1956, 1959a, b).

This explains as well the unilateral sexual incompatibility in the non-allelic mechanism present, for instance, in the crossing of strains $ab \times a_1b_1$ (Fig. 6.3). It may be deduced from these heterokaryon experiments that *b* is the "aggressive" allele and that a_1 is its target. Since during the mating procedure in *Podospora* in the differentiated trichogynes the nuclei degenerate in the combination $ab \times a_1b_1$, the active *b*-nuclei are no longer present and there is no inhibition for the sensitive a_1-nucleus, which may thus migrate into the ascogonial cell of the protoperithecium. In the alternate case, the active *b*-nucleus of the spermatium is able to initiate destruction when entering the trichogyne. As in the case of the allelic mechanism, there is also no explanation why the non-allelic mechanism does not become effective during the steps of sexual differentiation and is expressed only when the ascospores germinate, as demonstrated by the assay of heterokaryons.

If as a result of sexual propagation, two *het*-genes come together in one nucleus and in comparable heterokaryons, respectively, they are not viable (a phenomenon found also in other fungi). This has resulted to use in recent publications programmed cell death or apoptosis for heterokaryon incompatibility (Glass and Demnthon 2006; Goncalves and Videira 2014).

These findings were later confirmed by Bernet (1965), who studied the same races *s* and *M*. By analysis of other races, six more incompatibility loci were identified (Bernet 1967; Bourges et al. 1998), one being involved in the allelic and five in the non-allelic mechanism. Unfortunately, Bernet did not use our gene designations. All these genes were later summarised under the heading *het*-genes, e.g. *het-c*. In the following, I shall give the original names of these genes in parentheses.

D. Function of the *het*-Genes

During the last years, many efforts were undertaken to understand the function of the *het*-genes on the molecular and biochemical level.

1. The Non-allelic Mechanism

a) Application of Biochemical Techniques
In heterokaryons, specific proteins (Esser 1959b; Blaich and Esser 1970), catabolic enzymes (Blaich and Esser 1971) and new enzyme activities, such as for several proteases, phenoloxidases, malate and NADH dehydrogenase and amino acid oxidase (Boucherie and Bernet 1978; Boucherie et al. 1981; Paoletti et al. 1998), were found, but their involvement with the function of the *het*-genes was not evident.

b) Mutations Which Interfere with het-Genes
A series of modifier mutants (mod-genes) were found, most of which suppress in specific combinations the barrage formation caused by the allelic or by the non-allelic mechanism. In active combinations, these mutants also suppress the formation of female sex organs (protoperithecia). One of these mod-genes, *mod-E* (*a*), codes for a member of the Hsp90 family of heat-shock proteins acting in various types of stress responses (Loubradou et al. 1997). A second cloned gene, the *mod-D* gene *(v)*, encodes a Gα protein. The gene displays functional interactions with *mod-E* and *Pa AC*, a gene for an adenylate cyclase. Although addition of cyclic AMP can partially suppress growth defects caused by *mod-D* mutations, a molecular connection of cAMP with the effect of *mod-D* mutations on the *het*-genes was not found (Loubradou et al. 1999).

c) Analyses of mRNA
During the incompatibility reaction, a strong decrease of mRNA synthesis and the appearance of a new set of proteins occur. Therefore, the vegetative incompatibility is regulated, at least in part, by variation of the mRNA content of specific genes. Genes induced during the incompatibility reactions have been termed *idi*.

- idi-1 is a cell wall protein and resides in the septum during normal growth (Dementhon et al. 2003).
- idi-4 is abZIP transcription factor regulating autophagy and cell fate and also expression of other *idi*-genes (Dementhon et al. 2004).
- *idi-6/pspA* encodes a vacuolar protease involved in autophagy (Pinan-Lucarré et al. 2003).
- *idi-7* acts in the formation of autophagosomes, vesicles which target cytoplasmic material to the vacuole (Pinan-Lucarré et al. 2003).

Rapamycin treatment of *Podospora anserina* causes *idi*-gene expression and cellular effects typical for heterogenic incompatibility (Dementhon et al. 2003).

d) Molecular Analysis of the *het-c*, *het-d* and *het-e* Genes
Alleles of *het-c* and *het-e* genes correspond to the genes b/b_1 and a/a_1 in Esser's studies. Alleles of the *het-c* locus have similar ORFs but lead to protein products with some amino acid differences (Saupe et al. 1995). The *het-c* gene products are members of a family of ancestral sphingolipid transfer proteins (Mattjus et al. 2003). By inactivation of the *het-c* gene, abnormal ascospores are formed. *het-c* alleles

interact in different ways with the *het-e* and *het-d* alleles (Saupe et al. 1995). Bastiaans et al. (2014) identified 11 *het-c* alleles, which define 7 distinct incompatibility specifities.

Both the *het-d* and the *het-e* genes code for proteins which display a GTP-binding site and a WD40 repeat domain, typical for a β-subunit of a G-protein. Sequence comparison of different *het-e* alleles showed that *het-e* specificity is determined by the sequence of the WD40 domain, which may confer the incompatibility interactions (for references, see Espagne et al. 2002). Physical interactions with the *het-c* proteins need to be demonstrated to clarify this.

2. The Allelic Mechanism

The first *het*-genes of all described are the alleles *het-s* and *het-S* (Rizet 1952). They cause barrage formation by the allelic mechanism but do not interfere with fruit body production. Their co-expression in a common cytoplasm causes cell death. Both alleles encode 30-kd proteins consisting of 289 amino acids. The alleles differ in 43 amino acid positions (Turcq et al. 1990, 1991).

A disruption of either gene resulted in a lack of the 30-kd protein. When mated, these strains no longer formed a barrage. Sexual compatibility was not affected. It was further shown by detailed analyses of the *het-s/S* locus of 13 wild strains that the specificity of the *s* and *S* proteins to provoke heterogenic incompatibility depends on a single amino acid difference only (Deleu et al. 1993).

Strains with the genotype *het-s* exist in two phenotypic states: the neutral phenotype *het-s** and the active phenotype *het-s*. The neutral phenotype is characterised by the fact that it does not show the barrage reaction, when crossed with *het-S* strains. The *het-s* phenotype is infective and is able to transform, via hyphal fusions, a neutral *het-s** strain.

The *het-s* protein which provokes the transformation is a prion which adopts an amyloid structure and propagates in vivo as a self-perpetuating amyloid aggregate (Nazabal et al. 2003; Balguerie et al. 2004). Amyloid structures formed in vitro were shown to be infectious, in contrast to soluble *het-s* protein and amorphous aggregates, supporting the prion nature of the amyloid fibres (Maddelein et al. 2002).

The analysis of deletion constructs and site-directed mutants showed that a short C-terminal peptide (112 amino acids) allows the propagation of the prion analogue (Cousteau et al. 1997). This part of the protein contains the amyloid core regions of the *het-s* prion protein (Balguerie et al. 2003, 2004).

In conclusion: The many studies of heterogenic incompatibility performed with *Podospora anserina* have given deep insights into the genetic mechanism of the *het*-genes and shown many aspects of their action. However, a complete understanding of their function in causing their mutual antagonism still needs further research.

IV. Further Examples of Heterogenic Incompatibility

As one may suppose, the discovery of heterogenic incompatibility was not through focussed research. By contrast, in the ascomycetes it was observed as a "by-product" of genetic research on breeding competence. In the basidiomycetes, as discussed below, data on heterogenic incompatibility are very often a result of studies in population genetics dealing with intergeneric and interspecific delineation and evolution. In the literature, there is a diversity of names, definitions and gene symbols for effects which can be interpreted as manifestations of heterogenic incompatibility. Thus, it is understandable that in describing the antagonistic mycelial interactions and the various groups of natural isolates showing compatibility or incompatibility, different terms and expressions are used (as stated in the legend in Table 6.1), which I consider also as a source of information for a reader who is not familiar with this area of research and who wishes to gain more detailed information. Therefore, I shall discuss only some other cases of heterogenic incompatibility which have been studied in more detail.

Although the **myxozoa** are no longer grouped with the fungi, they have at least to be briefly mentioned, because these organisms have been the subject of intensive studies of heterogenic incompatibility. Vegetative incompatibility is widespread between geographical races of the genera and species analysed to date. In analogy with *Podospora*, plasmodial matings may lead to a visible zone of aversion, which does not allow nuclear migration, because there is either unilateral or mutual disintegration of the nuclei. Collectively from these studies, various genes acting according to the allelic mechanism have been identified. There are no data concerning the physiological actions of these genes.

For pertinent information, the reader is referred to literature on *Didymium iridis* and related species (Betterley and Collins 1984; Clark 1984, 2003), *Physarum polycephalum* (Knowles and Carlile 1978a,b; Lane and Carlile 1979; Schrauwen 1979; Lane 1981), and *Dictyostelium discoideum* (Robson and Williams 1979, 1980).

A. Oomycota

Oomycota have scarcely been used for genetic studies. This may be partially due to the fact that in contrast to ascomycetes and basidiomycetes, they are vegetative diploids, thus complicating genetic analysis of progeny. It is not surprising that our knowledge of genetic control of their breeding systems, and especially evidence for heterogenic incompatibility, is very limited. Furthermore, anastomoses between the coenocytic hyphae having cellulose walls do not occur. The only indication for the existence of heterogenic incompatibility in Oomycota concerns the genus *Phytophthora* (for details, this is referenced in Table 6.1).

B. Glomeromycota

In the recently defined Glomeromycota, heterogenic incompatibility has been reported between isolates of the arbuscular mycorrhizal fungus *Glomus mosseae* (Giovanetti et al. 2003).

C. Dikaryomycota

1. Ascomycotina

This class includes some of the most thoroughly studied saprophytic genera, for example, *Neurospora*, *Aspergillus* and *Podospora*. There are also numerous data proving the existence of heterogenic incompatibility in a great number of parasitic ascomycetes, but detailed genetic data are lacking in many cases.

a) Saprophytic Ascomycetes

Neurospora: Apart from *Podospora*, the genus *Neurospora* is the best analysed taxonomic entity for heterogenic incompatibility in fungi (Table 6.1).

Over 3900 isolates have been collected from nature from over 500 sampling sites. Perkins and his collaborators have classified and assessed this impressive dataset in terms of inter- and intraspecies mating relations (Perkins et al. 1976; Perkins and Turner 1988). The species most studied is *Neurospora crassa*, but the closely related species *Neurospora sitophila* is also used for investigating heterogenic incompatibility.

Heterogenic incompatibility in *Neurospora* has, according to my knowledge, been reported only as **heterokaryon incompatibility** concerning the vegetative phase. It is under polygenic control and involves **several allelic mechanisms**. According to Debets et al. (1994), mitochondrial plasmids might be involved in heterogenic incompatibility.

1. The Mating-Type Locus

   As early as 1933, Moreau and Moruzi reported "cross sterility" between opposite mating types in interracial crosses of *N. sitophila*. A comparable phenomenon of "cross sterility" was also described by Lindegren (1934) for *N. crassa*. In both cases, no genetic analyses were performed. A more substantial indication for the occurrence of heterogenic incompatibility originates from the classical "heterokaryon paper" of Beadle and Coonradt (1944), showing that strains of *N. crassa* must be of the same mating type in order to form a vigorous and stable heterokaryon.

Newmeyer and her collaborators (cf. Table 6.1) proved that the two mating types MAT A and MAT ***a*** are not able to coexist in a common cytoplasm. They found that the gene ***tol*** (linkage group IV), which is not linked with the mating-type locus, suppresses this vegetative incompatibility. ***tol*** does not interfere with the sexual compatibility initiated in an ***A/a*** cross. These authors proposed that the mating-type locus is a complex genetic trait

controlling both heterokaryon formation and sexual compatibility.

The *tol*-gene encodes a putative reading frame for a 1011-amino-acid polypeptide with a coiled-coil domain and a leucine-rich repeat. It is suggested that the TOL-locus may interact with the mating-type proteins MAT *A*-1 and/or MAT *a*-1 to form a death-triggering complex (Shiu and Glass 1999).

The concept of complex mating-type loci was verified by molecular analyses. The MAT *A* gene consists of 5301 bp (Glass et al. 1990), whereas the MAT *a* gene is much smaller and comprises 3225 bp only (Staben and Yanofsky 1990). However, sexual compatibility and heterokaryon incompatibility were found to be inseparable. Thus, a single gene product in both mating types MAT *A* and MAT *a* is responsible for the completion of the sexual cycle and for heterogenic incompatibility in the vegetative phase (Shiu and GlassFdeets 2015). In order to emphasise the rather strong structural dissimilarity of the mating-type alleles, Metzenberg (1990) has proposed to use the term **idiomorphs**, rather than alleles.

The structure of the mating-type alleles in *Podospora anserina* is very similar to those of *Neurospora crassa* (Debuchy and Coppin 1992, Dyer et al. 2016). However, as mentioned above, the mating idiomorphs in *P. anserina* control only homogenic incompatibility.

The involvement of mating loci in heterogenic incompatibility is rather unusual in fungi. It seems to be restricted to some of the self-incompatible *Neurospora* species. Here again, the same question as in *Podospora* is raised: why do the genes responsible for heterokaryon incompatibility not interfere with the overall sexual process? It is not possible at present to answer this question. Maybe there are additional genes which, like the above-mentioned *tol*-gene, are able to act as switches to stop the interaction as soon the nuclei enter the sexual phase.

In this context, the spore killer genes of *Neurospora* should be mentioned (Raju 1979, 2002; Turner and Perkins 1979). These *sk*-genes are widely distributed in wild-type collections. They cause (albeit only in crosses with sensitive strains), after meiotic segregation, lethality of the four ascospores carrying the genes. This phenomenon does not occur in *sk/sk* matings. This observation also strengthens the idea that there are two different, genetically controlled phases in the sexual cycle of fungi: the bringing together of genetic material via cytoplasmic contact and the true sexual cycle leading eventually to recombination.

2. The *het*-Genes
   There are some other genes, apart from the mating idiomorphs, which control heterokaryon formation in *Neurospora*. This was earlier postulated by Gross (1952) and Holloway (1955). A detailed analysis of these so-called *het*-genes was performed by Garnjobst, Wilson and collaborators (cf. Table 6.1). At present, 11 *het*-genes are known.

Two unlinked allelic pairs *(C/c* and *D/d)* were identified which control heterokaryon formation according to the allelic mechanism. Heterokaryons are formed only if the two partners have identical alleles at both loci. If one or both factors are heterogenic, then there will be an incompatibility reaction, as in the barrage zone of *Podospora*, leading to a destruction of the hyphae which have anastomosed. The *C* and *D* genes are neither linked with the mating-type locus nor suppressed by the tol-gene, nor do they interfere with sexual compatibility of different mating types. A third locus *(E/e)* was identified which resembles in its effect the *C* and *D* genes. Subsequently, these genes were given the prefix *het.*

In analysing different geographical races, Perkins (1968) has detected multiple alleles at the *het-C* locus. The similarity of the action of the *het*-genes to that of the *Podospora* barrage genes is also supported by the observation that a clear barrage zone between the two lines of perithecia may be seen when strains of opposite mating types, but heteroallelic for the *het*-genes, meet (Griffith and Rieck 1981; Perkins 1988). Degradation of nuclear DNA indicating a form of programmed cell death has also been visualised (Marek et al. 2003).

Another *het*-gene, but not leading to cell death, was found by Pittenger (cf. Table 1). The allelic pair *I/i* controls the capacity of nuclei to divide. Allele *I* is weakly dominant over allele *i*. When the proportion of *I* nuclei in an (*I*+*i*) heterokaryon is more than 30 %, the *i* nuclei are lost and the heterokaryon becomes an *I* homokaryon. By use of different marker genes, it became evident that this incompatibility is independent of the genetic background and, hence, from the action of the *C*, *D*, *E het*-genes mentioned above.

In recent years, more detailed knowledge about the structure and function of the *het*-genes has accumulated. By deletions within *het*-genes, their action is suppressed and compatibility achieved (Smith et al. 1996). Two *het*-loci were studied in more detail.

The ***het-c* gene** encodes a 966-amino-acid polypeptide with a putative signal peptide, a coiled-coil motif and a C-terminal glycine-rich domain, found also in cell wall proteins. Deletions showed that this region is responsible for the activity of the *het-c* gene (Saupe et al. 1996). *het-c* specificities reside in a 38–48 aa domain at the N-terminal end (Saupe and Glass 1997; Wu and Glass 2001). *Het-c* alleles of *N. crassa* function also in *Podospora anserina* in cell death reaction related to heterokaryon incompatibility, whilst the *P. abserina* homolog *Pahch* causes no heterokaryon incompatibility in its host (Saupe 2000).

Three deletion mutants were identified within an open reading frame (named vib = vegetative incompatibility blocked). These mutants relieved growth inhibition and repression of conidiation caused by the *het-c* gene. Thus, it was suggested that the vib region is a regulator for conidiation (Xiang and Glass 2002). Rather often, these suppressor mutants exhibited chromosome rearrangements (Xiang and Glass 2004).

The ***het-6* gene** maps to a region of 250 kbp. Within this region, two genes were identified which show incompatibility activity. One of these shows sequence similarity to the *het-e* product of *Podospora anserina* and the *tol*-gene product. The other encodes the large subunit of the ribonucleotide reductase. Both genes are inherited as a block. Thus, it was suggested that these genes act through a non-allelic mechanism to cause heterogenic incompatibility (Smith et al. 2000; Mir-Rashed et al. 2000).

Regarding the comprehensive new data obtained for *Neurospora*, like in *Podospora*, a breakthrough in understanding the molecular mechanism of the mutual interaction of the *het*-genes requires still further research.

Aspergillus: Some *Aspergillus* species were also studied for heterogenic incompatibility. The failure of heterokaryon formation between various natural isolates was already described by Gossop et al. (1940) for *Aspergillus niger* and by Raper and Fennell (1953) for *Aspergillus fonsecaeus* (both imperfect). Comprehensive studies with the perfect (teleomorphic) species *Aspergillus nidulans* were performed by Jinks and his coworkers (cf. Table 6.1). A synopsis of these observations and experiments, including numerous related and unrelated isolates from all over the world, allows the following conclusions:

1. Heterokaryon incompatibility is not due to geographical isolation, since the various vegetative compatibility (v-c) groups comprise isolates from adjacent as well as from distant areas. Nor is it linked with minor morphological differences of the isolates.
2. Vegetative incompatibility does not prevent heterokaryon formation. Heterokaryons seem to have selective disadvantage and are supposed to be overgrown by the homokaryons.
3. Fruit body formation for teleomorphic species is not inhibited. However, the number of fruit bodies is reduced, as observed also in *Podospora* (Fig. 6.2).
4. Eight *het*-loci were identified, two of which are multiallelic; *het-B* has four and *het-C* has three alleles. The interaction of the *het*-genes follows the allelic mechanism, as described above for *Podospora* and *Neurospora.*
5. The physiological action of these genes is not yet understood. A "killing reaction" like the one in *Podospora* and *Neurospora* seems not to take place.

Comparable results were obtained with two other teleomorphic species, *A. glaucus* (Jones 1965) and *A. heterothallicus* (Kwon and Raper 1967).

Studies with some anamorphic species (*A. versicolor*, *A. terreus*, *A. amstelodami)* performed by Caten (cf. Table 6.1) in general confirmed the observations made on the teleomorphic species, although without having the opportunity to identify *het*-genes.

A comprehensive study of the species *Aspergillus flavus*, *A. parasiticus* and *A. tamarii* revealed an association of morphology and mycotoxin production with the various i-c groups within each species (Horn et al. 1996).

Ascobolus: In *Ascobolus immersus* (cf. Table 6.1) the mating pattern of 38 strains collected at various places in Europe and southern India has been determined. There were at least three compatibility groups: A (23 strains) and B

(nine strains) comprise the European isolates, and C, the Indian isolates. Within each group sexual reproduction is, as expected, controlled by a bipolar mechanism of homogenic incompatibility. No fertile offspring are obtained in any intergroup crossing, showing that there is genetic separation by heterogenic incompatibility. However, the European group B seems to be more closely related to the Indian group (C) in that sterile fruit bodies are produced between + and − mating types. An indication for further subdivision is the occurrence of barrages between representatives of all three groups. These data thus indicate how speciation may be initiated in *Ascobolus immersus* by means of both spatial and genetic isolation, the latter mediated by heterogenic incompatibility.

b) Parasitic Ascomycetes

For a number of plant pathogenic fungi, heterokaryon tests between different isolates led to barrage or to border line formation and to the classification of the so-called vegetative compatibility groups (**v-c**). Partially due to the difficulty of breeding pathogens under laboratory conditions, genetic data are often not available (see Table 6.1). However, in some of the recently published papers, biochemical techniques such as RAPD analysis were used to characterise the v-c groups, e.g. Punja and Sun (2002).

More comprehensive data are available for *Botrytis cinerea*. It was found that heterokaryon incompatibility in this fungus is caused by the gene Bc-*hch* which is homolog to Nc-*het-c* and the Pa-*hch* loci of *Neurospora crassa* and *Podospora anserina*, respectively. A PCR-RFLP analysis on a 1171-bp section was used to screen for polymorphism for this locus among 117 wild isolates and revealed two allelic types, thus allowing scientists to structure the natural populations into two groups.

For some other parasites, more detailed studies are available. In the case of the chestnut blight, *Cryphonectria (Endothia) parasitica*, different v-c groups characterised by barrage formation of varied intensity were detected. Weak barrages did not inhibit heterokaryon formation. Over 75 v-c groups were identified, controlled by at least seven incompatibility loci, some with multiple alleles. The heterogenic incompatibility followed mostly an allelic mechanism, but a non-allelic mechanism was also observed. Sexual compatibility was not affected by these genes (Choi et al. 2012).

According to Nuss and Koltin (1990), hypovirulence is related to the presence of a virus-like double-stranded RNA which can be transmitted via heterokaryosis. The efficiency of the transfer is highly reduced between incompatible strains and leads therefore to a lack of horizontal transfer of this parasite and contributes to its biocontrol (Milgroom and Cortesi 2004; Smith et al. 2006).

In the *Gibberella fujikuroi* species complex, in addition to the mating-type genes (+/−), mating groups termed A, B, C and D are recognised. They are considered varieties according to their host specificity. Within each group, heterogenic incompatibility was found. This allelic mechanism is controlled by at least 10 loci in group A, five loci in group B and three loci each in both groups C and D.

Within parasitic ascomycetes, there are only two indications for **heterogenic incompatibility which affect the sexual phase**. Both are not linked with heterokaryon incompatibility. The genus *Cochliobolus* includes plant parasites causing leaf and inflorescence diseases in Gramineae (anamorphic: *Helminthosporium)*. Nelson and collaborators have studied extensively the mating system within this genus. A detailed analysis of the mating reactions of nearly 10,000 isolates from North and South America, comprising more than 40,000 matings, has led to the following results:

1. The bipolar mechanism of homogenic incompatibility is responsible for the basic control of mating, insofar as only the combination of the alleles *A* and *a* leads to fructification.
2. Fertility in crosses between opposite mating types originating from different hosts or origin is about 29 %. Most of the infertile crosses produce perithecia with immature or sterile ascospores. The others show no fruit body formation.
3. Several sterility genes blocking the normal ontogenesis at different stages were identified and were predominantly responsible for the formation of sterile perithecia.

4. The fact that some strains exhibiting incompatibility in certain combinations were compatible in all others can be explained only by the action of heterogenic incompatibility, despite the fact that the appropriate genes have not yet been identified.
5. The objection that the incompatibility might be provoked by gross genetic diversities or species differences could be excluded. Furthermore, Nelson was able to assign 92.2 % of his strains to five distinct morphological types which might correspond to a single species.

2. Basidiomycotina

The first phenomena which may be attributed to heterogenic incompatibility came from this group of fungi.

Apart from the description of barrage phenomena between geographical races of *Fomes* species (Mounce 1929; Mounce and Macrae 1938), Bauch (1927) found in the smut fungus, *Microbotryum violaceum (Ustilago violacea)*, that in interracial crosses additional genes interfere with the bipolar mating system and cause unilateral or reciprocal incompatibility. Similar findings were later reported by Grasso (1955) who studied interracial crosses in two other species, *U. avenae* and *U. levis*, originating from Italy and the United States, respectively.

Many phenomena resulting in heterokaryon incompatibility or inhibition of fruit body formation were poorly understood in older publications. They were mostly referred to as demarcation lines, barrages and/or crossing barriers. Thus, it is understandable that in a very comprehensive review (Burnett 1965), the mating restrictions in 17 species of basidiomycetes were treated only under the general heading "restrictions of outbreeding".

In this review, I distinguish between those cases in which the existence of heterogenic incompatibility is proved and supported by genetic data and cases in which antagonistic mycelial interactions may only be interpreted as the expression of heterogenic incompatibility. Only the better-analysed cases will be presented in detail here (for others, see Table 6.1).

In this context, it needs to be stressed that in contrast to ascomycetes where heterogenic incompatibility may concern either the vegetative and/or the sexual phase, in basidiomycetes it is instrumental only in the vegetative phase, because basidiomycetes do not form sex organs. From this it follows that if a heterokaryon incompatibility occurs, then the sexual propagation is automatically inhibited.

An impetus to study heterogenic incompatibility stems from the interest in the population structure of saprophytic basidiomycetes, involving matings between natural isolates in order to obtain information for classification of genera and species (cf. Boidin 1986). These studies have not only revealed basic mating systems but also indicate a variety of antagonistic mycelial interactions correlated with heterokaryon and/or sexual incompatibility.

However, during the last years the interest in heterogenic incompatibility of basidiomycetes seems to have decreased, because there have been fewer publications describing this phenomenon. Instead, many comprehensive studies were published in establishing intra- or interspecific relationships by using molecular techniques. Thus, there is not much progress in understanding the genetic and physiological control of heterogenic incompatibility within this group of fungi.

In **wood-rotting fungi** the antagonistic interaction is easily recognised in cross sections from logs, as a narrow zone of interwoven hyphae in a region of relatively undecayed wood. These **border lines**, also called interaction zones or demarcation lines, are usually darkly pigmented, in contrast to the adjacent decay zones (Fig. 6.1e). They also show up on agar-grown cultures, depending on the composition of the medium. Without microscopic examination, it is not possible to say whether hyphal interactions inhibiting heterokaryon formation take place, as is evident in the barrage zone of *Podospora*, or simply antagonistic repulsions occur. Thus, a distinction between delimitation of species or races is a priori not possible. In the literature, the terms **biological**

races and **intersterility groups** (**i-c**) are often used to characterise the interacting mycelia.

In analogy to the evaluation of data concerning the ascomycetes, I shall start the discussion of the basidiomycetes with a subject for which information on both heterogenic incompatibility and genetic control of speciation has been obtained. This is the wood-rotting fungus *Polyporus*.

Macrae (1967) studied the mating reactions of 31 single spore isolates of the tetrapolar *Polyporus abietinus* (syn. *Hirschioporus abietinus)* collected in different places in North America and Europe. According to differences in the morphology of their hymenial surfaces, the isolates were assigned to three morphological groups. The North American strains could be subdivided into the two classes A and B which are incompatible with each other, but which are both compatible with a third class C comprising the European strains. Since geographical isolation could be excluded, Macrae concluded that genes additional to the mating-type factors were involved. Similar data were also reported for *P. schweinitzii* (Barrett and Uscuplic 1971). Unfortunately, in both cases no genetic data are available.

Comparable phenomena were found and could be interpreted after comprehensive studies of other species of the genus (Hoffmann and Esser 1978). We had chosen the wood-rotting genus *Polyporus* in order to investigate, by genetic parameters, the validity of the classical species concept based on typological characters. In performing these studies, we "accidentally" detected evidence for heterogenic incompatibility.

As a result of matings of single spore-derived mycelia from 26 races of different origin, all races could unequivocally be grouped into three separate entities corresponding with the typological species *P. arcularius*, *P. brumalis* and *P. ciliatus*, on the basis of the following results (Fig. 6.4):

1. As expected, the basic breeding system in *Polyporus* is the tetrapolar mechanism of homogenic incompatibility controlled by multiple alleles of the mating-type factors *A* and *B*.

2. All intraspecific combinations were fertile. A conspicuous barrage formed in those crosses where dikaryotisation and fruiting were impaired. This barrage is characterised by a clear zone, about 1–2 mm wide, free of aerial hyphae, and of reduced hyphal density in the medium (Fig. 6.1d).

3. Using two races of *P. ciliatus* as an example, it was revealed that barrage formation is induced by the specific interaction of three unlinked genes (b^+/b^- = barrage initiation, bfI_1/bfI_2 and $bfII_1/bfII_2$ = barrage formation) in a way characteristic for systems of heterogenic incompatibility. Barrage formation requires the presence of the allele bi^+ in at least one mating partner, in addition to heterogeneity of both *bf*-genes.

4. Interspecific combinations were sterile. There is no hyphal fusion between mating partners, and because of the mutual repulsion, a sharp border line is formed in the area of contact. Its formation is independent of both mating type and the nuclear status (monokaryons or dikaryons) of the confronted mycelia (see also Silveira et al. 2002).

From the experimental data, the following conclusions may be drawn:

1. The analysis of intraspecific matings has shown that within each species, the so-called biological races (intersterility groups, i-s) exist. They are delineated by barrage formation, which, as deduced from the genetic data, is an unequivocal example of heterogenic incompatibility. The unilateral inhibition of fruiting, not caused by the mating-type factors, can also be considered as an expression of heterogenic incompatibility, although genetic data for this are not yet available.
2. The analysis of interspecific matings, all characterised by a strong macroscopic border line (Fig. 6.1d), is in good agreement with the species limits derived from morphological data. This indicates the validity of both the typological and the biological species concept. The latter, however, proved superior in compensating the variability of morphological characters, at least in higher fungi.

The **biological species concept** can thus be modified as follows: **populations (races) belong to different species if the failure to interbreed and to produce viable offspring is caused by**

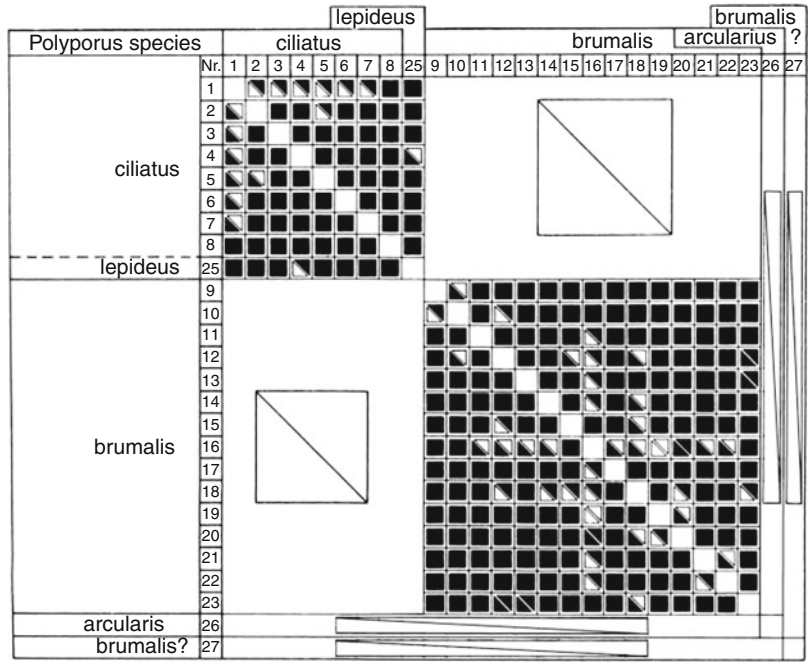


Fig. 6.4 Mating relations in intra- and interspecies combinations of monokaryons from 26 races of different species of *Polyporus*. ■ Normal contact, clamp connections and fruit bodies formed when $A \neq B \neq$; ◥ barrage formation, fruit body production delayed; ◥ barrage formation with unilateral nuclear migration (only in *dark part)*; ◹ *border line*, neither clamp connections nor dikaryotic fruit bodies formed in any combination (Adapted from Esser and Hoffmann 1977)

genetic mechanisms other than those operating upon completion of the sexual cycle.

There are some more examples where genetic control of heterogenic incompatibility is available.

In *Sistotrema brinkmannii* (syn. *Corticium coronilla)*, strains obtained from different geographical locations exhibit three types of basic sexual control:

1. Homokaryotic fruiting, i.e. homokaryons produce dikaryons and fruit bodies with viable spores.
2. Homogenic incompatibility determined by the bipolar mechanism, i.e. one mating-type locus with multiple alleles.
3. Homogenic incompatibility determined by the tetrapolar mechanism, i.e. two incompatibility factors, each with multiple alleles (Biggs 1937).

Lemke (1969) has confirmed and extended the work of Biggs by analysing the interstrain relations of 11 isolates from different parts of the world. In using the technique of forced heterokaryons between auxotrophs, he found that there are fertility barriers within each of the three above-mentioned fruiting classes as well as in interclass crosses. This phenomenon was interpreted by Lemke as heterogenic incompatibility for two reasons:

1. In incompatible interracial matings, the two auxotrophic partners form unbalanced mycelia with poor vegetative vigour and no clamp connections. This points to an antagonistic reaction similar to that in *Podospora* heterokaryons.
2. In one compatible interracial mating, recombinant types were obtained with an altered incompatibility pattern. This excluded the presence of sterility genes, which were sometimes found in other combinations.

In the oyster mushroom, *Pleurotus ostreatus*, 60 heterokaryons of a natural population were examined by pair-

wise matings for mycelial antagonisms (Kay and Vilgalys 1992). Most pairings (93–100 %) between sib-composed heterokaryons gave somatic incompatibility responses, showing that most isolates represent discrete individuals, with as many as 15 individuals occupying a single log. A total of 53 somatically distinct individuals were identified from the population, distributed among 21 logs. Test with homokaryons showed that genetic elements not identical with the incompatibility factors of the tetrapolar system are responsible for the formation of intersterility groups.

In *Stereum hirsutum*, mating is controlled by a bipolar mechanism of homogenic incompatibility (C-factor with multiple alleles). In analysing mating homokaryons from different geographical isolates, a mycelial aversion (bow-tie reaction) was observed. This involved the formation of a migrating or stationary band of suppressed mycelium. This was followed by partial or complete replacement of one homokaryon by another. This reaction is brought about by a heterozygosity at a single locus (B-factor), which is not linked with the mating-type locus. The fact that the heterogenic nuclei reject each other, reminiscent of the barrage formation of *Podospora*, is a further example for heterogenic incompatibility as an isolation mechanism within a single species. In four other species of *Stereum*, as in *Polyporus*, all interspecific matings were sterile and delineated by strong border lines.

In the litter-decomposing bipolar *Collybia dryophila*, collected from different continents, several intersterility groups (i-s) were identified, three of which are distributed over two or more continents. In some matings within one i-c, reduced sexual compatibility was found. Genetic diversity of some strains was proved by DNA-DNA hybridisation.

In the bipolar *Heterobasidion annosum* are at least three intersterility groups, P and S (from pine and spruce) and F (from firs), the representatives of which in general show no compatibility of different mating types. However, there are exceptions, because there is a significant degree of fertility in i-s matings. Five loci were identified controlling this system, superimposed upon the mating-type alleles. In contrast to the results obtained with *Podospora*, for instance, the heterogenic incompatibility between the i-s strains requires a heterogeneity of all five loci. A homogeneity at only one locus acts epistatically and suppresses the mating barrier. This does not exclude that under "fully" heterogenic conditions, interracial incompatibility is present. Comparable data for the *Heterobasidion insulare* complex were reported by Dai et al. (2002).

Hansen et al. (1993a, b) published data which lead to a contradictory interpretation. They suggested that mating between the incompatibility groups is controlled at 3–4 multiallelic loci. Each genotype acts independently in causing vegetative incompatibility. Thus, in accordance with the *Podospora* system, a single genetic difference would be sufficient to cause heterogenic incompatibility.

Perhaps the **control of heterogenic incompatibility is not restricted to nuclear genes.** In the tetrapolar *Coprinus cinereus (Coprinopsis cinerea)*, barrage formation was observed in matings between heterokaryons from different geographical locations having different mitochondrial genomes but common nuclear genomes (May 1988). Unfortunately, no further details of this novel interaction were given.

Yet another example should be mentioned, which is caused by an interaction of nuclear genes and cytoplasmic genetic elements. In the tetrapolar *Ustilago maydis*, an antagonism between genetically different strains, which does not depend on the mating-type genes, was observed which is similar to the killer phenomenon of yeast (cf. Stark et al. 1990). There are three genotypes:

1. Antagonistic strains, producing a heat-labile protein which inhibits the growth of sensitive strains but does not interfere with growth of the producing strain. Genetic configurations: a nuclear gene with the alleles *s* or s^+, and cytoplasmic elements *I* and *S*. The s^+ allele confers insensitivity, the *s* allele sensitivity which is suppressed by the cytoplasmic element *S*; the element *I* is responsible for the production of the killer substance.
2. Sensitive strains, which do not produce inhibitor protein but are sensitive to it. Genetic configuration: gene *s*, but no cytoplasmic element *O*.

3. Neutral strains, which do not produce inhibitor protein and are insensitive to it. Genetic configuration: s^+ *O*, *s S* or s^+ *S*.

There are phenotypic differences to the killer system in yeasts, since only growth inhibition of the sensitive cells occurs with no cell death, and there is no interference with the fusion of different mating types; hence, sexual propagation is not prevented. Thus, this phenomenon reveals similarity to heterogenic vegetative incompatibility in *Neurospora* and *Podospora*. Moreover, in the yeast killer system the genetic determinate for killer protein is a viral-related double-stranded RNA or DNA (cf. Tipper and Bostian 1984 and Stark et al. 1990, respectively).

Conclusion: The evaluation of the experimental data obtained with fungi with respect to the occurrence, distribution and mechanisms of heterogenic incompatibility allows one to make the following statements:

1. The existence of heterogenic incompatibility is unequivocally proved among Dikaryomycota. Although *het*-genes were identified for a number of species, its genetic control certainly needs more experimental investigation.
2. This holds even more true for an understanding of the expression and functions of the *het*-genes.
3. The existence of many v-c and i-s groups in ascomycetes and basidiomycetes, respectively, shows the necessity to support taxonomic classification for speciation by means of comprehensive genetic data, and not just morphological criteria.

V. Correlations with Heterogenic Incompatibility in Plants and Animals, with DNA Restriction in Bacteria and with Histoincompatibility

As reviewed earlier (Esser and Blaich 1973), **in plants** there are also many examples for the existence of heterogenic incompatibility, manifesting as either unilateral or bilateral failures of matings between individuals of different isolates or races. Sometimes the genes responsible for homogenic incompatibility are involved, but mostly the action of other genes is superimposed. In addition, extrachromosomal genetic elements such as plastid-derived DNA have been found as determinative agents (de Nettancourt 1977; Barrett 1992). In plants, according to my knowledge, vegetative incompatibility has not been described.

In comparison to the predominantly hermaphroditic plants, sexual incompatibility of the homogenic type does not play a role in **animal breeding systems**. Increasing in outbreeding is in general achieved in animals by dioecism. There are some examples known in which karyogamy between female and male nuclei is prevented by genetic differences not identical with sex factors (cf. Esser and Blaich 1973).

The spectrum of heterogenic incompatibility comprises not only eukaryotes but also **prokaryotes**. The destruction of bacterial DNA by endonucleases, when brought into a genetically different host and as a defence mechanism to escape phage infection, is also a manifestation of this phenomenon.

Heterogenic incompatibility is not restricted to cell fusion and subsequent nuclear migration, because there is also a close correlation between heterogenic incompatibility and **histoincompatibility**, occurring after tissue transplantation. In the latter case, however, a complicated immune-response mechanism is involved. It seems justifiable to conclude that both heterogenic incompatibility and histoincompatibility, which seem to have convergently developed during evolution, exhibit one and the same effect, i.e. inability of genetically different material to coexist or tolerate a common physiological machinery, and simply represent different mechanisms of a fundamental biological process.

VI. Conclusions

As shown by this survey of the literature, most cases of heterogenic incompatibility can be

supported by genetic data. A number of important special cases are known under different names. In other cases, however, effects indicative of heterogenic incompatibility have been attributed to other causes or relegated by investigators as inexplicable secondary effects. In any case, **heterogenic incompatibility** must be regarded as a **basic biological phenomenon** controlling the coexistence of different genetic determinants, whose impact may be summarised as follows.

1. Occurrence
   Heterogenic incompatibility is widespread in both prokaryotes and eukaryotes. Special cases such as DNA restriction and histoincompatibility may be considered different expressions of one basic biological phenomenon.
2. Nature of Genetic Determinants
   The widespread occurrence is also indicative of the general importance of heterogenic incompatibility, with a varied genetic basis ranging from single to multiple nuclear genes and even to extranuclear genetic elements.
3. Biochemical Basis
   There are not yet sufficient biochemical data regarding the action of the nuclear genes which bring about heterogenic incompatibility in fungi. By contrast, the molecular mechanisms of heterogenic incompatibility provoked by extranuclear genetic traits, such as bacterial DNA restriction, are well known. Evidently, many of the genetic mechanisms leading to heterogenic incompatibility have developed independently, and this may be reflected by a variety of mechanisms at the molecular level.
4. Biological Impact
   The effect of heterogenic incompatibility is threefold:

   (a) As stated in the Introduction, heterogenic incompatibility has to be considered a **breeding system** which, in contrast to homogenic incompatibility, **favours inbreeding** by restricting the exchange of genetic material. Since there is no fundamental difference between recombinational events in the sexual and the parasexual cycle, it is not surprising that heterogenic incompatibility influences both. This also applies to the initiation of plasmogamy, which may occur either by sexual processes or simply through heterokaryosis. This mode of isolating strains or races leads to further speciation. Thus, heterogenic incompatibility must have been and still is one of the **basic genetic events acting in evolution**.

   (b) Genetic isolation has a second effect which should not be overlooked. The suppression of cell fusion **stops the transfer of harmful cytoplasmic components**, such as mutated mitochondria, viruses or plasmids, between individuals and thereby inhibits the spread of cell diseases and favours the survival of uninfected cells or tissues. This is particularly important for organisms without strict cellular compartmentation, such as the majority of fungi.

   (c) **Consequences for taxonomy**: In many studies of natural isolates of fungi, the formation of antagonistic zones of mycelial aversion, such as barrages and/or border lines, is used by taxonomists as criteria for speciation. Although being a valuable tool, this parameter as a taxonomic criterion should be judged with great care to avoid creating taxonomic distinctions which are not valid and which could depend on only a single gene difference. This especially concerns the term "biological species", often used without any genetic basis. It is better to use the terms "race" or "geographical isolate", without a detailed evaluation of breeding patterns.

5. Practical Implications
   During the last decades, concerted breeding for biotechnologically relevant fungi has gained more and more importance (Esser 1985; Esser and Mohr 1990). Breed-

ing techniques employing new isolates from nature in order to exploit varied genetic backgrounds require a profound knowledge of the breeding systems involved. The existence of heterogenic incompatibility could be a serious handicap for any genetic exchange via either sexual or parasexual matings. Detailed experimental work would allow one in most cases to reach the desired goal, if based on alternative genetic manipulations such as DNA-mediated transformation.

6. Relation with Histoincompatibility and DNA Restriction

   Both of these phenomena have the same effect: hostile interaction of different genetic material originating from closely related organisms. This brings up the question: will it be possible in the future, based on further experimental work, to interrelate these events and the many manifestations of heterogenic incompatibility in considering the diversity of the genetic mechanisms promulgating the **failure of coexistence of genetically different material?**

References

Adams DH, Roth LF (1967) Demarcation lines in paired cultures of *Fomes cajanderi* as a basis for detecting genetically distinct mycelia. Can J Bot 45:1583–1589

Anagnostakis SL (1977) Vegetative incompatibility in *Endothia parasitica*. Exp Mycol 1:306–316

Anagnostakis SL (1982a) The origin of ascogenous nuclei in *Endothia parasitica*. Genetics 100:413–416

Anagnostakis SL (1982b) Genetic analyses of *Endothia parasitica:* linkage data for four single genes and three vegetative compatibility types. Genetics 102:25–28

Anagnostakis SL (1983) Conversion to curative morphology in *Endothia parasitica* and its restriction by vegetative compatibility. Mycologia 75:777–780

Anderson JB (1986) Biological species of *Armillaria* in North America: redesignation of groups IV and VIII and enumeration of voucher strains for other groups. Mycologia 78:837–839

Anderson JB, Ullrich RC (1979) Biological species of *Armillaria mellea* in North America. Mycologia 71:402–414

Anderson JB, Korhonen K, Ullrich RC (1980) Relationship between European and North American biological species of *Armillaria mellea*. Exp Mycol 4:87–95

Anderson JB, Petsche DM, Herr FB, Horgien PA (1984) Breeding relationship among several species of *Agaricus*. Can J Bot 62:1884–1889

Balguerie A, Reis SD, Ritter C, Chaignepain S, Coulary-Salin B, Forge V, Bathany K, Lascu I, Schmitter JM, Riek R et al (2003) Domain organization and structure-function relationship of the *HET-s* prion of *Podospora anserina*. EMBO J 22:2071–2081

Balguerie A, Reis SD, Coulary-Salin B, Chaignepain S, Sabourain M, Schmitter JM, Saupe SJ (2004) The sequences appended to the amyloid core region of the *HET-s* prion protein determine higher-order aggregate organization in vivo. J Cell Sci 117:2599–2610

Barrett SCH (ed) (1992) Evolution and function of heterostyly, vol 15, Monographs on theoretical and applied genetics. Springer, Berlin

Barrett DK, Uscuplic M (1971) The field distribution of interacting strains of *Polyporus schweinitzii* and their origin. New Phytol 70:581–598

Bastiaans E, Debets AM, Aanen DK, Von Depeningen AD, Saupe SJ, Paoletti M (2014) Natural variation of heterokaryon incompatibility gene *het-c* in *Podospora anserine* reveals diversifying selection. Mol Biol Evol 3:962–974

Bauch R (1927) Rassenunterschiede und sekundäre Geschlechtsmerkmale beim Antherenbrand. Biol Zentralbl 47:370–383

Beadle GW, Coonradt VL (1944) Heterocaryosis in *Neurospora crassa*. Genetics 29:291–308

Bégueret J, Turc B, Clavé C (1994) Vegetative incompatibility in filamentous fungi – *het* genes begin to talk. Trends Genet 10:441–446

Bernet J (1965) Mode d'action des gènes de barrage et la relation entre l'incompatibilité cellulaire et l'incompatibilité sexuelle chez *Podospora anserina*. Ann Sci Nat Bot Biol Veg 6:611–768

Bernet J (1967) Les systèmes d'incompatibilité chez le *Podospora anserina*. C R Acad Sci Paris Ser D 256:1330–1333

Betterley DA, Collins OR (1984) Vegetative incompatibility and myxomycete biology. Mycologia 76:785–792

Biggs R (1937) The species concept in *Corticium coronilla*. Mycologia 29:686–706

Blaich R, Esser K (1970) The incompatibility relationships between geographical races of *Podospora anserina*. IV. Biochemical aspects of the heterogenic incompatibility. Mol Gen Genet 109:186–192

Blaich R, Esser K (1971) The incompatibility relationships between geographical races of *Podospora anserina*. V. Biochemical characterization of heterogenic incompatibility on cellular level. Mol Gen Genet 111:265–272

Boccas BR (1981) Interspecific crosses between closely related heterothallic *Phytophthora* species. Phytopathology 71:60–65

Boddy L, Rayner ADM (1982) Population structure, intermycelial interactions and infection biology of *Stereum gausapatum*. Trans Br Mycol Soc 78:337–351

Boddy L, Rayner ADM (1983) Mycelial interactions, morphogenesis and ecology of *Phlebia radiata* and *P. rufa* from oak. Trans Br Mycol Soc 80:437–448

Boidin J (1986) Intercompatibility and the species concept in the saprobic basidiomycotina. Mycotaxon 76:319–336

Boucherie H, Bernet J (1978) Protoplasmic incompatibility and self-lysis in *Podospora anserina*: enzyme activities associated with cell destruction. Can J Bot 56:2171–2176

Boucherie H, Dupont CH, Bernet J (1981) Polypeptide synthesis during protoplasmic incompatibility in the fungus *Podospora anserina*. Biochim Biophys Acta 653:18–26

Bourges N, Groppi A, Barreau C, Clavé C, Bégueret J (1998) Regulation of gene the expression during the vegetative incompatibility reaction in *Podospora anserina*: characterization of three induced genes. Genetics 150:633–641

Brasier CM (1984) Inter-mycelial recognition systems in *Ceratocystis ulmi:* their physiological properties and ecological importance. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 451–498

Brodie HJ (1970) Sexuality patterns in some new and little-known species of *Cyathus*. Sver Bot Tidskr 64:44–50

Bruehl GW, Machtmes R, Kiyomoto R (1975) Taxonomic relationships among *Typhula* species as revealed by mating experiments. Phytopathology 65:1108–1114

Burnett JH (1965) The natural history of recombination systems. In: Esser K, Raper JR (eds) Incompatibility in fungi. Springer, Berlin/New York, pp 98–113

Butcher AC (1968) The relationship between sexual outcrossing and heterokaryon incompatibility in *Aspergillus nidulans*. Heredity 23:443–452

Butcher AC (1969) Non-allelic interactions and genetic isolation in wild populations of *Aspergillus nidulans*. Heredity 24:621–631

Butcher AC, Croft J, Grindle M (1972) Use of genotype-environmental interaction analysis in the study of natural populations of *Aspergillus nidulans*. Heredity 29:263–283

Carlile MJ, Gooday GW (1978) Cell fusion in myxomycetes and fungi. In: Poste G, Nicholson GL (eds) Membrane fusion. Elsevier, Amsterdam, pp 219–265

Caten CE, Butcher AC, Croft JH (1971) Genetic control of heterokaryon formation in *Aspergillus*. Aspergillus Newslett 12:1–2

Cayley DM (1923) The phenomenon of mutual aversion between mono-spore mycelia of the same fungus (*Diaporthe perniciosa* Marchal) with a discussion of sex heterothallism in fungi. J Genet 13:353–370

Cayley DM (1931) The inheritance of the capacity for showing mutual aversion between monospore mycelia of *Diaporthe perniciosa*. J Genet 24:1–63

Chaisrisook C (2002) Mycelial reactions and mycelial compatibility groups of red rice mould (*Monascus purpureus*). Mycol Res 106:298–304

Chase TE, Ullrich RC (1990a) Genetic basis of biological species in *Heterobasidion annosum*: Mendelian determinants. Mycologia 82:67–72

Chase TE, Ullrich RC (1990b) Five genes determining intersterility in *Heterobasidion annosum*. Mycologia 82:73–81

Cherepennikova-Anikina MI, Savenkova LV, Dolgova AV, Shaw DS, Dyakov Yu T (2002) Vegetative incompatibility in *Phytophthora infestans*. J Russ Phytopathol Soc 3:19–28

Choi GH, Dawe AL, Churbanow A, Smith ML, Milgroom MG, Nuss D (2012) Molecular characterization of vegetative incompatibility genes in the chestnut blight *Cryphonectria parasitica*. Genetics 190:113–127

Clark J (1984) Three-clone *Didymium iridis* crosses and plasmodial incompatibility phenotype. Mycologia 76:810–815

Clark J (2003) Plasmodial incompatibility in the myxomycete *Didymium squamulosum*. Mycologia 95:24–26

Coates D, Rayner ADM (1985a) Genetic control and variation in expression of the "bow-tie" reaction between homokaryons of *Stereum hirsutum*. Trans Br Mycol Soc 84:191–205

Coates D, Rayner ADM (1985b) Heterokaryon-homokaryon interactions in *Stereum hirsutum*. Trans Br Mycol Soc 84:637–645

Coates D, Rayner ADM (1985c) Evidence for a cytoplasmically transmissible factor affecting recognition and somato-sexual differentiation in the basidiomycete *Stereum hirsutum*. J Gen Microbiol 131:207–219

Coates D, Rayner ADM, Todd NK (1981) Mating behaviour, mycelial antagonism and the establishment of individuals in *Stereum hirsutum*. Trans Br Mycol Soc 76:41–51

Coates D, Rayner ADM, Boddy L (1985) Interactions between mating and somatic incompatibility in the basidiomycete *Stereum hirsutum*. New Phytol 99:473–483

Correl JC, Puhalla JE, Schneider RW (1986) Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396–400

Cortesi P, Milgroom MG (1998) Genetics of vegetative incompatibility in *Cryphonectria parasitica*. Appl Environ Microbiol 64:2988–2994

Cousteau V, Delau C, Saupe S, Bégueret J (1997) The protein product of the het-s heterokaryon incompatibility gene of the fungus *Podospora anserine* behaves as a prion analog. Proc Natl Acad Sci USA 94:9773–9778

Croft JH, Dales RBG (1984) Mycelial interactions and mitochondrial inheritance in *Aspergillus*. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 433–450

Cubeta MA, Briones-Ortega R, Vigalys R (1993) Reassessment of heterokaryon formation in *Rhizoctonia solani* anastomosis group 4. Mycologia 85:777–787

Dai YC, Vainio EJ, Hantula J, Niemelä T, Korhonen K (2002) Sexuality and intersterility within the *Heterobasidion insulare* complex. Mycol Res 106:1435–1448

Dales RBG, Croft JH (1977) Protoplast fusion and the isolation of heterokaryons and diploids from vegetatively incompatible strains of *Aspergillus nidulans*. FEMS Microbiol Lett 1:201–203

Dales RBG, Croft JH (1990) Investigation of the *het* genes that control heterokaryon incompatibility between members of heterokaryon compatibility (*h-c*) groups *A* and *G1* of *Aspergillus nidulans*. J Gen Microbiol 136:1717–1724

De Nettancourt D (1977) Incompatibility in Angiosperms, vol 3, Monographs on theoretical and applied genetics. Springer, Berlin/New York

Debets F, Yang X, Griffiths AJF (1994) Vegetative incompatibility in *Neurospora:* its effect on horizontal transfer of mitochondrial plasmids and senescence in natural population. Curr Genet 26:113–119

Debuchy R, Coppin E (1992) The mating types of *Podospora anserina:* functional analysis and sequence of the fertilization domains. Mol Gen Genet 233:113–121

Deleu C, Clavé C, Bégueret J (1993) A single amino acid difference is sufficient to elicit vegetative incompatibility in the fungus *Podospora anserina*. Genetics 135:45–52

Dementhon K, Paoletti M, Pinan-Lucarré B, Loubradou-Bourges N, Sabourin M, Saupe SJ, Clavé C (2003) Rapamycin mimics the incompatibility reaction in the fungus *Podospora anserina*. Eukaryot Cell 2:238–246

Dementhon K, Saupe SJ, Clavé C (2004) Characterization of *idi-4,* a bZIP transcription factor inducing autophagy and cell death in the fungus *Podospora anserina*. Mol Microbiol 53:1625–1640

Dyer PS, Inderbitzin P, Debuchy R (2016) Mating-type structure, function, regulation and evolution in the pezizomycotina. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 351–385

Ennos RA, Swales KW (1987) Estimation of the mating system in a fungal pathogen *Crumenulopsis sororia* (Karst.) groves using isozyme markers. Hereditas 59:423–430

Espagne E, Balhadère P, Penin ML, Barreau C, Turcq B (2002) HET-E and HET-D belong to a new subfamily of WD40 proteins involved in vegetative incompatibility specificity in the fungus *Podospora anserina*. Genetics 161:71.81

Esser K (1954) Sur le déterminisme génétique d'un nouveau type d'incompatibilité chez *Podospora*. C R Acad Sci Paris 238:1731–1733

Esser K (1956) Die Incompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (CES) REHM I. Genetische Analyse der Semi-Incompatibilität. Z Indukt Abstammungs Vererbungslehre 87:595–624

Esser K (1959a) Die Incompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (CES) REHM II. Die Wirkungsweise der Semi-Incompatibilitäts-Gene. Z Vererbungslehre 90:29–52

Esser K (1959b) Die Incompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (CES) REHM III. Untersuchungen zur Genphysiologie der Barragebildung und der Semi-Incompatibilität. Z Vererbungslehre 90:445–456

Esser K (1962) Die Genetik der sexuellen Fortpflanzung bei Pilzen. Biol Zbl 81:161–172

Esser K (1971) Breeding systems in fungi and their significance for genetic recombination. Mol Gen Genet 110:86–100

Esser K (1985) Potentials and prospects of genetics in biotechnology. In: Proceedings 3rd European congress biotechnology, München, 1984, vol IV. DECHEMA, Frankfurt/Main, pp 241–256

Esser K (2006) Heterogenic incompatibility in fungi. In: Kües U and Fischer R (eds) Growth, diferentiation and sexuality, 2nd edn. The mycota, vol I, Springer, Heidelberg, pp 141–165

Esser K, Blaich R (1973) Heterogenic incompatibility in plants and animals. Adv Genet 17:107–152

Esser K, Hoffmann P (1977) Genetic basis for speciation in higher basidiomycetes with special reference to the genus *Polyporus*. In: Clemencon H (ed) The species concept in hymenomycetes. Cramer, Vaduz, pp 189–214

Esser K, Kuenen R (1967) Genetics of fungi. Translated by Erich Steiner. Springer, Berlin/New York

Esser K, Meinhardt F (1984) Barrage formation in fungi, vol 17, Encyclopedia of plant physiology, new series. Springer, Berlin/New York, pp 350–361

Esser K, Mohr G (1990) Stammverbesserung von Hyphenpilzen durch die Gentechnik: Fakten und Perspektiven. Bioengineering 6:44–55

Fischer M (1994) Pairing tests in the *Phellinus pini* group. Mycologia 86:524–539

Fischer M, Bresinsky A (1992) *Phellinus torulosus:* sexuality and evidence of intersterility groups. Mycologia 84:823–833

Ford EJ, Miller RV, Gray, Sherwood JE (1995) Heterokaryon formation and vegetative incompatibility in *Sclerotina sclerotium*. Mycol Res 99:241–247

Fournier E, Levis C, Fortini D, Leroux P, Giraud T, Brygoo Y (2003) Characterization of *Bc-hch,* the *Botrytis cinerea* homolog of the *Neurospora crassa het-c* vegetative incompatibility locus, and its use as, a population marker. Mycologia 95:251–261

Freihorst D, Fowler TJ, Bartholomew K, Raudaskoski M, Horton JS, Kothe E (2016) The mating type genes of the basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 351–385

Fries N (1985) Intersterility groups in *Paxillus involutus*. Mycotaxon 14:403–409

Fries N, Mueller GM (1984) Incompatibility systems, cultural features and species circumscriptions in the ectomycorrhizal genus *Laccaria* (agaricales). Mycologia 76:633–642

Garnjobst L, Wilson JF (1956) Heterokaryosis and protoplasmic incompatibility in *Neurospora crassa*. Proc Natl Acad Sci USA 42:613–618

Giovanetti M, Sbrana C, Strani P, Agnolucci M, Rinaudo V, Avio L (2003) Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical molecular analysis. Appl Environ Microbiol 69:616–624

Glass NL, Demnthon K (2006) Non self recognition and programmed cell death in filamentous fungi. Curr Opin Microbiol 9:553–558

Glass NL, Kaneko I (2003) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 2:1–8

Glass NL, Kuldau GA (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. Annu Rev Phytopathol 30:201–224

Glass NL, Grotelueschen J, Metzenberg RL (1990) *Neurospora crassa* a mating-type region. Proc Natl Acad Sci USA 87:4912–4916

Glass NL, Jacobson DJ, Shiu PKT (2000) The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu Rev Genet 34:165–186

Goh KM, Ganeson M, Subramaniam N (2014) Infection potential of vegetative incompatible *Ganoderma boninense* isolates with known ligninolytic enzyme production. Afr J Biotechnol 13:1056–1066

Goldstein D, Gilbertson RL (1981) Cultural morphology and sexuality of *Inonotus arizonicus*. Mycologia 73:167–180

Goncalves AP, Videira A (2014) Programmed cell death in *Neurospora crassa*. New J Sci 2014:7

Gordon SA, Petersen RH (1992) Interbreeding populations of some *Marasmius* species. Mycologia 84:204–208

Gossop GH, Yuill E, Yuill JL (1940) Heterogeneous fructifications in species of *Aspergillus*. Trans Br Mycol Soc 24:337–344

Grasso V (1955) Studio sulla genetica dei carboni dell'avena: *Ustilago avenae* e *U. levis*. Boll Staz Patol Veg Roma 12:115–126

Griffith AJF, Rieck A (1981) Perithecial distribution in standard and variant strains of *Neurospora crassa*. Can J Bot 59:2610–2617

Grindle M (1963a) Heterokaryon compatibility of unrelated strains in the *Aspergillus nidulans* group. Heredity 18:191–204

Grindle M (1963b) Heterokaryon compatibility of closely related wild isolates of *Aspergillus nidulans*. Heredity 18:397–405

Gross SR (1952) Heterokaryosis between opposite mating types in *Neurospora crassa*. Am J Bot 39:574–577

Guler P, Bicer H (2014) The somatic incompatibility in *Trametes versicolor*. J Appl Biol Sci 8:22–26

Hamann A, Osiewacz HD (2004) Genetic analysis of spore killing in the filamentous ascomycete *Podospora anserina*. Fungal Genet Biol 41:1088–1098

Hankin L, Puhalla JE (1971) Nature of a factor causing interstrain lethality in *Ustilago maydis*. Phytopathology 61:50–53

Hansen EM (1979) Sexual and vegetative incompatibility reactions in *Phellinus weirii*. Can J Bot 57:1573–1578

Hansen EM, Stenlid J, Johansson M (1993a) Somatic incompatibility and nuclear reassortment in *Heterobasidion annosum*. Mycol Res 97:1223–1228

Hansen EM, Stenlid J, Johansson M (1993b) Genetic control of somatic incompatibility in the root-rotting basidiomycete *Heterobasidion annosum*. Mycol Res 97:1229–1233

Hietala AM, Korhonen K, Sen R (2003) An unknown mechanism promotes somatic incompatibility in *Ceratobasidium bicorne*. Mycologia 95:239–250

Hoffmann P, Esser K (1978) Genetics of speciation in the basidiomycetous genus *Polyporus*. Theor Appl Genet 53:273–282

Holloway BW (1955) Genetic control of heterocaryosis in *Neurospora crassa*. Genetics 40:117–129

Horn BW, Greene RL, Sobolov VS, Dorner JW, Powell JH, Layton RC (1996) Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus, A. parasiticus* and *A. tamarii*. Mycologia 88:574–587

Hornok L (2007) Sexual and vegetative compatibility/incompatibility in *Fusarium* species. Acta Phytopathologica and Entomologica Hungarica 42:2–11

Ikeda K, Nakamura H, Matsumato N (2003) Mycelial incompatibility operative in pairings between single basidiospore isolates of *Helicobasidium mompa*. Mycol Res 107:847–853

Jacobson DJ, Gordon TR (1988) Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. Phytopathology 78:668–672

Jamil N, Buck KW, Carlile MJ (1984) Sequence relationships between virus double-stranded RNA from isolates of *Gaeumannomyces graminis* in different vegetative compatibility groups. J Gen Virol 65:1741–1747

Jennings DH, Rayner ADM (eds) (1984) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge

Jinks JL, Caten CE, Simchen G, Croft JH (1966) Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans*. Heredity 21:227–239

Jones DA (1965) Heterokaryon compatibility in the *Aspergillus glaucus* link group. Heredity 20:49–56

Katan T, Katan J (1988) Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfec-*

tum from tissue and the rhizosphere of cotton plants. Phytopathology 78:852–855
Kay E, Vilgalys R (1992) Spatial distribution and genetic relationships among individuals in a natural population of the oyster mushroom *Pleurotus ostreatus*. Mycologia 84:173–182
Kemp RFO (1989) Incompatibility in basidiomycetes – the heterogenic pentax. FORK Publ 1:1–19
Kiebacher J, Hoffmann GM (1981) Zur Entwicklung des Perfektstadiums *Venturia inaequalis* in vitro (Development of the perfect stage of *Venturia inaequalis* in vitro). J Plant Dis Protect 88:1–8
Knowles DJC, Carlile MJ (1978a) Growth and migration of plasmodia of the myxomycete *Physarum polycephalum*: the effect of carbohydrates, including agar. J Gen Microbiol 108:9–15
Knowles DJC, Carlile MJ (1978b) The chemotactic response of plasmodia of the myxomycete *Physarum polycephalum* to sugars and related compounds. J Gen Microbiol 108:17–25
Kohn E, Stasovcki F, Carbone I, Royer J, Anderson JB (1991) Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. Phytopathology 81:480–485
Kope HH (1992) Interactions of heterokaryotic and homokaryotic mycelium of sibling isolates of the ectomycorrhizal fungus *Pisolithus arhizus*. Mycologia 84:659–667
Kuhlmann EG (1982) Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola*. Mycologia 74:759–768
Kwon KJ, Raper KB (1967) Heterokaryon formation and genetic analyses of color mutants in *Aspergillus heterothallicus*. Am J Bot 54:49–60
Lane EB (1981) Somatic incompatibility in fungi and myxomycetes. In: Gull K, Oliver SG (eds) The fungal nucleus. Cambridge University Press, Cambridge
Lane EB, Carlile MJ (1979) Post-fusion somatic incompatibility in plasmodia of *Physarum polycephalum*. J Cell Sci 35:339–354
Leach J, Yoder OC (1983) Heterokaryon incompatibility in the plant-pathogenic fungus, *Cochliobolus heterostrophus*. J Heredity 74:149–152
Lemke PA (1969) A reevaluation of homothallism, heterothallism and the species concept in *Sistotrema brinkmanni*. Mycologia 61:57–76
Lemke PA (1973) Isolating mechanisms in fungi – prezygotic, postzygotic, and azygotic. Persoonia 7:249–260
Leslie JF (1987) Heterokaryon incompatibility haplotypes on linkage group VII of *Neurospora crassa* (abstract). Mycol Soc Am Newslett 38:35
Leslie JF (1993) Fungal vegetative compatibility. Annu Rev Phytopathol 31:127–150
Leslie JF, Zeller KA (1996) Heterokaryon incompatibility in fungi: more than just a way to die. J Genet 75:416–424
Leslie JF, Zeller KA, Wohler M, Summerell BA (2004) Interfertility in two mating populations of *Gibberella fujikuroi*. Eur J Plant Pathol 90:1–8
Lindegren CC (1934) The genetics of *Neurospora*. VI. Bisexual and akaryotic ascospores from N. crassa. Genetica 16:315–320
Lombard FF, Larsen MJ, Dorworth EB (1992) Reassessment of the sexual incompatibility system and cultural characteristics of *Bjerkandera fumosa*. Mycologia 83:406–410
Loubradou G, Béguret J, Turcq B (1997) A mutation in aHSP90 gene affects the sexual cycle and suppresses vegetative incompatibility in the fungus *Podospora anseria*. Genetics 147:581–588
Loubradou G, Bégueret J, Turcq B (1999) Mod-D, a G α subunit of the fungus *Podospora anserina,* is involved in both regulation of development and vegetative incompatibility. Genetics 152:519–528
Macrae R (1967) Pairing incompatibility and other distinctions among *Hirschioporus* (*Polyporus*) *abietinus, H. fusco-violaceus* and *H. laricinus*. Can J Bot 45:1371–1398
Maddelein ML, Reis SD, Duvezin-Caubet S, Coulary-Salin B, Saupe SJ (2002) Amyloid aggregates of the *HET-s* prion protein are infectious. PNAS 99:7402–7407
Marek SM, Wu J, Glass NL, Gilchrist DG, Bostock RM (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 40:126–137
Mattjus P, Turcq B, Pike HM, Molotkovsky JG, Brown RE (2003) Glycolipid intermembrane transfer is accelerated by HET-C2, a filamentous fungus gene product involved in the cell-cell incompatibility response. Biochemistry 42:535–542
May G (1988) Somatic incompatibility and individualism in the coprophilous basidiomycete *Coprinus cinereus*. Trans Br Mycol Soc 91:443–451
McKeen CG (1952) Studies of Canadian theleophoraceae. IX. A cultural and taxonomic study of three species of *Peniophora*. Can J Bot 30:764–787
Meinhardt F, Koch H, Esser K (1984) Compatibility groups in *Ascobolus immersus*, an indication of speciation. Theor Appl Genet 68:365–367
Metzenberg RL (1990) Anecdotal, historical and critical commentaries on genetics. In: Crow JF, Dove WF (eds). Genetics 125:457–462
Micali CO, Smith ML (2003) On the independence of barrage formation and heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 38:209–219
Milgroom MG, Cortesi P (2004) The biological control of chestnut blight with hypervirulence: a critical analysis. Annu Rev Phytopathol 42:311–338
Mir-Rashed N, Jacobson DJ, Deghgany MR, Micali OC, Smith ML (2000) Molecular and functional analyses of incompatibility genes at *het-6* in a population of *Neurospora crassa*. Fungal Genet Biol 30:197–205

Moreau F, Moruzi C (1933) Sur de nouvelles irrégularités de la bipolarité sexuelle chez les Ascomycetes du genre *Neurospora*. Bull Soc Bot Fr 80:574–576

Morrison RM (1960) Compatibility of several clonal lines of *Erysiphe cichoracearum*. Mycologia 52:786–794

Mounce I (1929) Studies in forest pathology. II. The biology of *Fomes pinicola* (Sw) Cooke. Can Dept Agric Bull 111:1–77

Mounce I, Macrae R (1938) Interfertility phenomena in *Fomes pinicola*. Can J Bot 16:354–376

Murphy JF, Miller OK Jr (1993) The population biology of two litter decomposing agarics on a southern Appalachian mountain. Mycologia 85:769–776

Mylyk OM (1975) Heterokaryon incompatibility genes in *Neurospora crassa* detected using duplication-producing chromosome rearrangements. Genetics 80:107–124

Mylyk OM (1976) Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora crassa*. Genetics 83:275–284

Nakata K (1925) Studies on *Sclerotium rolfsii*. I. The phenomenon of aversion and its relation to the biologic forms of the fungus. II. The possible cause of the phenomenon of aversion in the fungus and morphological features of the phenomenon. Bull Sci Fak Terkulturen Kjusa Imp Univ 1:4

Nazabal A, Reis SD, Bonneu M, Saupe SJ, Schmitter JM (2003) Conformational transition occurring upon amyloid aggregation of the *HET-s* prion protein of *Podospora anserina* analyzed by hydrogen/deuterium exchange and mass spectrometry. Biochemistry 42:8852–8861

Nelson RR (1963) Interspecific hybridization in the fungi. Annu Rev Microbiol 17:31–48

Nelson RR (1965a) Bridging interspecific incompatibility in the ascomycetous genus *Cochliobolus*. Evolution 18:700–704

Nelson RR (1965b) Assessing biological relationship in the fungi. Phytopathology 55:823–826

Nelson RR (1966) The genetic control of conidial morphology and arrangement in *Cochliobolus carbonum*. Mycologia 58:208–214

Nelson RR (1970) Variation in mating capacities among isolates of *Cochliobolus carbonum*. Can J Bot 48:261–263

Nelson RR, Kline DM (1964) Evolution of sexuality and pathogenicity. III. Effects of geographic origin and host association on cross-fertility between isolates of Helminthosporium with similar conidial morphology. Phytopathology 54:963–967

Newmeyer D (1968) A suppressor of mating type incompatibility in *Neurospora*. Genetics 60:207

Newmeyer D (1970) A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. Can J Genet Cytol 12:914–926

Newmeyer D, Taylor CW (1967) A pericentric inversion in *Neurospora,* with unstable duplication progeny. Genetics 56:771–791

Newton AC, Osbourn AE, Caten CE (1998) Heterokaryosis and vegetative incompatibility in *Stagonospora nodorum*. Mycologia 90:215–225

Nuss DI, Koltin Y (1990) Significance of dsRNA genetic elements in plant pathogenic fungi. Annu Rev Phytopathol 28:37–58

Padhaye AA, Carmichel JW (1971) Incompatibility in *Microsporum cookei*. Sabouraudia 9:27–29

Pal K (2005) Vegetative incompatibility in *Aspergillus niger*. Acta Biologica Szegediensis 49:57

Paoletti M, Clave C, Begueret J (1998) Characterization of a gene from the filamentous fungus *Podospora anserina* encoding an aspartyl protease induced upon carbon starvation. Gene 210:45–52

Papaioannou I, Typas M (2015) Barrage formation is independent from heterokaryon incompatibility. Eur J Plant Pathol 141:71–82

Perkins DD (1968) Multiple alleles at a heterokaryon-compatibility locus in *Neurospora*. Genetics 61:47

Perkins DD (1988) Main features of vegetative incompatibility in *Neurospora*. Fungal Genet Newslett 35:44–46

Perkins DD, Turner BC (1988) *Neurospora* from natural populations: toward the population biology of a haploid eukaryote. Exp Mycol 12:91–131

Perkins DD, Turner BC, Barry EG (1976) Strains of *Neurospora* collected from nature. Evolution 30:281–313

Pilotti CA, Sanderson FR, Aitken AB (2002) Sexuality and interactions of monokaryotic and dikaryotic mycelia of *Ganoderma boninense*. Mycol Res 106:1315–1322

Pinan-Lucarré B, Paoletti M, Dementhon K, Coulary-Salin B, Clavé C (2003) Autophagy is induced during cell death by incompatibility and is essential for differentiation in the filamentous fungus *Podospora anserina*. Mol Microbiol 47:321–333

Pittenger TH (1964) Spontaneous alterations of heterokaryon compatibility factors in *Neurospora*. Genetics 50:471–484

Pittenger TH, Brawner TG (1961) Genetic control of nuclear selection in *Neurospora* heterokaryons. Genetics 46:1645–1663

Ploetz RC, Shokes FM (1986) Evidence for homothallism and vegetative compatibility in southern *Diaporthe phaseolorum*. Can J Bot 64:2197–2200

Proffer TJ, Hart JH (1988) Vegetative compatibility groups in *Leucocytospora kunzei*. Phytopathology 78:256–260

Puhalla EJ (1968) Compatibility reactions on solid medium and interstrain inhibition in *Ustilago maydis*. Genetics 60:461–474

Puhalla EJ, Hummel M (1983) Vegetative compatibility groups within *Verticillium dahliae*. Phytopathology 73:1305–1308

Puhalla JE, Spieth PT (1983) Heterokaryosis in *Fusarium moniliforme*. Exp Mycol 7:328–335

Puhalla JE, Spieth PT (1985) A comparison of heterokaryosis and vegetative incompatibility among

varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp Mycol 9:39–47
Punja ZK, Grogan RG (1983a) Basidiocarp induction, nuclear condition, variability, and heterokaryon incompatibility in *Athelia* (*Sclerotium*) *rolfsii*. Phytopathology 73:1273–1278
Punja ZK, Grogan RG (1983b) Hyphal interactions and antagonism among field isolates and single-basidiospore strains of *Athelia* (*Sclerotium*) *rolfsii*. Phytopathology 73:1279–1284
Punja ZK, Sun LJ (2002) Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (teleomorph *Athelia rolfsii*) and *S. delphinii*. Mycol Res 105:537–546
Raju NB (1979) Cytogenetic behavior of spore killer genes in *Neurospora*. Genetics 93:607–623
Raju NB (2002) Spore killers: meiotic drive elements that distort genetic ratios. In: Osiewacz HD (ed) Molecular biology of the fungal development. Dekker, New York, pp 275–296
Raper KB, Fennell DL (1953) Heterokaryosis in *Aspergillus*. J Elisha Mitchell Sci Soc 69:1–29
Rayner ADM, Todd NK (1977) Intraspecific antagonism in natural populations of wood-decaying basidiomycetes. J Gen Microbiol 103:85–90
Rayner ADM, Turton MN (1982) Mycelial interactions and population structure in the genus *Stereum: S. rugosum, S. sanguinolentum* and *S. rameale*. Trans Br Mycol Soc 78:483–493
Reinhardt OM (1892) Das Wachstum der Pilzhyphen. Jahrb Wiss Bot 23:479
Rizet G (1952) Les phénomènes de barrage chez *Podospora anserina*. I. Analyse génétique des barrages entre souches S et s. Rev Cytol Biol Vég 13:51–92
Rizet G (1953) Sur la multiplicité des mécanismes génétiques conduisant à des barrages chez *Podospora anserina*. C R Acad Sci Paris 237:666–668
Rizet G, Esser K (1953) Sur des phénomènes d'incompatibilité entre souches d'origines différentes chez *Podospora anserina*. C R Acad Sci Paris 237:760–761
Rizzo DM, Rentmeester RM, Bursdall J (1996) Sexuality and somatic incompatibility in *Phellinus gilvus*. Mycologia 87:805–820
Robson GE, Williams KL (1979) Vegetative incompatibility and the mating-type locus in the cellular slime mold *Dictyostelium discoideum*. Genetics 93:861–875
Robson GE, Williams KL (1980) The mating system of the cellular slime mold *Dictyostelium discoideum*. Curr Genet 1:229–232
Saupe SJ (2000) Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. Microbiol Mol Biol Rev 64:489–502
Saupe S, Glass NL (1997) Allelic specificity at the *het-c* heterokaryon incompatibility locus of *Neurospora crassa* is determined by a highly variable domain. Genetics 146:1299–1309
Saupe S, Turcq B, Bégueret J (1995) Sequence diversity and unusual variability at the *het-c* locus involved in the vegetative incompatibility in the fungus *Podospora anserina*. Curr Genet 27:466–471
Saupe SJ, Kuldau GA, Smith ML, Glass NL (1996) The product of the *het-C* heterokaryon incompatibility gene of *Neurospora crassa* has characteristics of glycine cell rich wall protein. Genetics 143:1589–1600
Savage EJ, Clayton CW, Hunter JH, Brenneman JA, Laviola C, Gallegly ME (1968) Homothallism, heterothallism and interspecific hybridization in the genus *Phytophthora*. Phytopathology 58:1004–1021
Schrauwen JAM (1979) Post-fusion incompatibility in *Physarum polycephalum*. Arch Microbiol 122:1–7
Shiu PKT, Glass NL (1999) Molecular characterization of *tol*, a mediator of mating-type-associated vegetative incompatibility in *Neurospora crassa*. Genetics 154:545–555
Shiu PKT, Glass NL (2015) Sequence important for heterokaryon incompatibility function in *MAT A-1* of *Neurospora crassa*. Fungal Genet Newslett 53:15–19
Sidhu GS (1986) Genetics of *Gibberella fujikuroi*. VIII. Vegetative compatibility groups. Can J Bot 64:117–121
Silveira RM, Borges DA, Wright JE (2002) *Polyporus* s. strictu in southern South America: mating tests. Mycol Res 106:1323–1330
Smith ML, Yang CJ, Metzenberg R, Glass NL (1996) Escape from *het-6* incompatibility in *Neurospora crassa*. Partial diploids involves preferential deletion within ectopic segment. Genetics 144:523–531
Smith ML, Micali OC, Hubbard SP, Mir-Rashed N, Jacobson DJ, Glass NL (2000) Vegetative incompatibility in the *het-6* region of *Neurospora crassa* is mediated by two linked genes. Genetics 155:1095–1104
Smith ML, Gibbs CC, Milgroom MC (2006) Heterokaryon incompatibility function of barrage-associated vegetative incompatibility genes (*vic*) in *Cryphonectria parasitica*. Mycologia 98:43–50
Staben C, Yanofsky C (1990) *Neurospora crassa* a mating-type region. Proc Natl Acad Sci USA 87:4917–4921
Stark JR, Boyd A, Mileham AJ, Romanos MA (1990) The encoded killer system of *Kluyveromyces lactis*: a review. Yeast 6:1–29
Stretton HM, Flentje NT (1972a) Inter-isolate heterokaryosis in *Thanatephorus cucumeris*. I. Between isolates of similar pathogenicity. Aust J Biol Sci 25:293–303
Stretton HM, Flentje NT (1972b) Inter-isolate heterokaryosis in *Thanatephorus cucumeris*. II. Between isolates of different pathogenicity. Aust J Biol Sci 25:305–318
Tipper DJ, Bostian KA (1984) Double stranded ribonucleic acid killer systems in yeasts. Microbiol Rev 48:125–156

Turcq B, Denayrolles M, Bégueret J (1990) Isolation of two allelic incompatibility genes *s* and *S* of the fungus *Podospora anserina*. Curr Genet 17:297–303

Turcq B, Deleu C, Denayrolles M, Bégueret J (1991) Two allelic genes responsible for vegetative incompatibility in the fungus *Podospora anserina* are not essential for cell viability. Mol Gen Genet 228:265–269

Turner BC, Perkins DD (1979) Spore killer, a chromosomal factor in *Neurospora* that kills meiotic products not containing it. Genetics 93:587–606

Turner BL, Taylor CW, Perkins DD, Newmeyer D (1969) New duplication-generating inversions in *Neurospora*. Can J Genet Cytol 11:622–638

Van der Gaag M, Debets AJM, Hoekstra RF (2003) Spore killing in the fungus *Podospora anserina*: a connection between meiotic drive and vegetative incompatibility? Genetics 117:59–65

Van Diepeningen AD, Debets JM, Hockstra RF (1997) Heterokaryon incompatibility blocks virus transfer among natural isolates of black *Aspergilli*. Curr Genet 32:209–217

Vandendries R (1932) La tétrapolarité sexuelle de *Pleurotus columbinus*. Démonstrations photographiques d'un tableau de croisement. Cellule 41:267–279

Vilgalys RJ, Johnson JL (1987) Extensive genetic divergence associated with speciation in filamentous fungi. Proc Natl Acad Sci USA 84:2355–2358

Vilgalys RJ, Miller OK (1987) Mating relationships within the *Collybia dryophila* group in Europe. Trans Br Mycol Soc 89:295–300

Webster RK, Nelson RR (1968) The genetics of *Cochliobolus spiciferus*. I. Genetic inhibition of perithecial and ascus formation. Can J Bot 46:197–202

Williams CA, Wilson JF (1968) Factors of cytoplasmic incompatibility. Neurospora Newslett 13:12

Wilson JF (1961) Microsurgical techniques for *Neurospora*. Am J Bot 48:46–51

Wilson JF (1963) Transplantation of nuclei in *Neurospora crassa*. Am J Bot 50:780

Wilson JF, Garnjobst L (1966) A new incompatibility locus in *Neurospora crassa*. Genetics 53:621–631

Wilson JF, Garnjobst L, Tatum EL (1961) Heterokaryon incompatibility in *Neurospora crassa* – microinjection studies. Am J Bot 48:299–305

Wong GJ (1993) Mating and fruiting studies of *Auricularia delicata* and *A. fuscosuccinea*. Mycologia 85:187–194

Worall JJ (1997) Somatic incompatibility in basidiomycetes. Mycologia 89:24–36

Wu J, Glass NL (2001) Identification of specificity determinants and generation of alleles with novel specificity at the *het-c* heterokaryon incompatibility locus of *Neurospora crassa*. Mol Cell Biol 21:1045–1057

Xiang Q, Glass NL (2002) Identification of *vib-1,* a locus involved in vegetative incompatibility mediated by *hetc* in *Neurospora crassa*. Genetics 162:89–101

Xiang Q, Glass NL (2004) Chromosome rearrangement in isolates that escape from *het-c* heterokaryon incompatibility. Curr Genet 44:329–338

Yindeeyoungyeon W, Triratana S (1992) Hyphal interaction among dikaryotic pairings of *Lentinula edodes*. Micol Neotrop Apl 5:17–27

7 The Art of Networking: Vegetative Hyphal Fusion in Filamentous Ascomycete Fungi

André Fleißner[1], Antonio Serrano[1]

CONTENTS

I. Introduction

The central and eponymous feature of filamentous fungi is their growth as mycelial colonies. These multicellular structures comprise a network of syncytial filaments, the fungal hyphae. Hyphae are highly polarized cells, which grow by tip extension and branching, thereby forming highly interconnected networks (Riquelme et al. 2011). In ascomycete species, hyphae are compartmentalized by cross walls; however, these septa contain central open pores, which allow fast and efficient translocation of cytoplasm, organelles, and other cellular constituents, including nutrients and signaling factors. Cytoplasmic continuity within these hyphal networks is however dynamic and appears to be regulated during hyphal differentiation and in response to environmental stimuli (Bleichrodt et al. 2012). For example, the formation of functionally specialized structures, such as vegetative spores or sexual fruiting bodies, often involves compartmentalization by sealing of septal pores (Markham 1994; Simon et al. 2005; Jedd 2007; Maruyama et al. 2010). Similarly, hyphal aging or starvation often results in controlled plugging of the cross walls (Glass et al. 2000; Fleißner and Glass 2007; Maruyama et al. 2010). The opposite process, the expansion of hyphal connectivity, is accomplished by the formation of secondary hyphae-to-hyphae connections, so-called **hyphal anastomoses**, via fusion of hyphal branches (Ward 1888; Hickey et al. 2002; Read et al. 2010). Similarly, early colony establishment often involves fusion between germinated or ungerminated spores, a process in which originally independent individuals merge into one functional unit, which further develops into the mycelial colony (Roca et al. 2003, 2005a). While these different types of fungal cellular fusion already caught the attention of the early mycologists, their physiological roles and molecular mechanisms are still not fully understood. In recent years, interest in the role and function of hyphal fusion has seen a great revival, and the numbers of scientific reports on this subject appear to be on the rise. While the majority of these studies employ the red bread mold

[1]Institut für Genetik, Technische Universität Braunschweig, Spielmannstraße 7, 38106 Braunschweig, Germany;
e-mail: a.fleissner@tu-bs.de

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

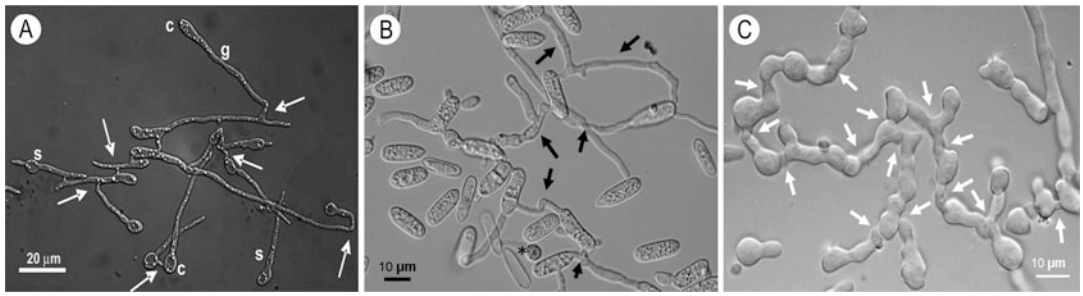


Fig. 7.1 Germinating spores form networks via germling fusion. (**a**) *Fusarium oxysporum* spores grown for 15 h in PDB. *Arrows* point to conidial anastomosis tubes (CATs). *c* conidium, *g* germ tube, *s* septum (Adapted from Ruiz-Roldan et al. 2010). (**b**) Spore germlings of *Colletotrichum lindemuthianum* after incubation in water for 72 h. *Arrows point* to CATs; the *asterisk* indicates an appressorium (Adapted from Ishikawa et al. 2010). (**c**) *Neurospora crassa* germlings growing on solid synthetic medium. *Arrows* point to fusion via CATs or germ tube tips (Adapted from Weichert and Fleißner 2015)

Neurospora crassa as a model organism, important contributions are also made by various saprophytic, pathogenic, and epiphytic species, including *Sordaria macrospora*, *Fusarium oxysporum*, *Alternaria brassicicola*, and *Epichloe festucae* (Engh et al. 2007; Craven et al. 2008; Prados Rosales and Di Pietro 2008; Ruiz-Roldan et al. 2010; Bernhards and Poggeler 2011; Charlton et al. 2012; Becker et al. 2015). Interest in fungal anastomosis extends beyond the scope of fungal biology and includes the question of general eukaryotic cell merger. Cell-cell fusion is essential for the development of most eukaryotic organisms and is involved in such diverse processes as fertilization, organ and muscle development and repair, or bone homeostasis (Aguilar et al. 2013). Despite these important functions, the molecular mechanisms underlying eukaryotic cell fusion remain mostly elusive. In recent years, *N. crassa* has advanced as one of various eukaryotic model organisms for studying these questions. Its many positive traits as a model organism include its fast growth, relatively large cell size, and simple handling procedures but first and foremost its ease of genetic manipulation. In addition, hyphal fusion readily occurs in this fungus in high numbers in axenic culture and can easily be analyzed by light microscopy. The identification of numerous mutants of *N. crassa* and other fungi affected in the process of hyphal fusion has revealed an intricate molecular network regulating anastomosis formation. It comprises many well-conserved eukaryotic signaling pathways but partially adapts them in very unusual and surprising ways. Studying hyphal fusion therefore promises not only novel insight into fungal development and cell merger but also holds much potential for basic cell biological research.

In the following sections, we will review the different types of vegetative fungal cell fusion, their physiological roles, and their mechanistic basis. Since the vast majority of the respective studies were conducted on the red bread mold *N. crassa*, we mostly focused on this fungus but also strived to include the most relevant reports on other species.

II. Types of Vegetative Hyphal Fusion

A. Germling Fusion

As sessile organisms, filamentous fungi typically disperse and translocate via spores, specialized cellular structures, which are adapted to transmission through air or water or are disseminated by biological vectors, such as insects (Dahlberg and Van Etten 1982). As part of their vegetative life cycle, many ascomycete species form conidia at specialized spore-bearing structures, as the main form of transmission. Novel colony establishment based on conidia often involves fusion of these spores briefly after or in some species even before their germination (Fig. 7.1). This process,

which is commonly termed as **germling fusion**, was already described for numerous species in the early days of modern mycology (De Bary 1884; Laibach 1928; Köhler 1930). A more recent literature survey reported its occurrence in more than 70 species covering more than 20 different genera (Roca et al. 2005a).

Germling fusion can occur between germ tube tips but is commonly mediated by specialized cellular structures, so-called **conidial anastomosis tubes**, abbreviated as **CATs** (Roca et al. 2003). CATs had already been described in an early study, investigating germling fusion in *Botrytis allii* and *Fusarium* sp., where they were named with the German term "Fusionshyphen" (fusion hyphae) (Köhler 1930). "Fusionshyphen" or CATs are distinguished from germ tubes by a number of morphological and physiological characteristics, including a thinner diameter and limited linear extension. Most importantly, however, CATs exhibit positive tropism toward each other, while growing germ tubes tend to avoid each other, therefore probably optimizing the use of available substrate space (Roca et al. 2005b; Lichius et al. 2014).

It is still a matter of debate, if fusion via germ tube tips requires a prior differentiation into CATs. In *N. crassa,* fusing germ tube tips often appear narrowed and pointed, therefore supporting this hypothesis (Roca et al. 2005b). A mutant of this fungus has been described, which still undergoes CAT fusion but is highly restricted in germ tube tip merger (Schurg et al. 2012). However, it remains so far ambiguous, if this phenotype is caused by a specific inability for CAT differentiation from growing cell tips or by secondary effects. The molecular mechanisms and physiological roles of true CAT and germ tube fusion, however, appear to be comparable, rendering this discussion to be one of mostly theoretical matters.

During germling fusion, typically two cells merge to subsequently continue growth as one cellular unit. Consecutive fusion between such structures or with other still individual germlings results in the formation of supracellular networks, which further develop into the mycelial colony (Fig. 7.1). Joining originally individual cells into one functional unit appears to contribute to fitness and competitiveness (see below) (Richard et al. 2012; Bastiaans et al. 2015). However, the fusion frequency within a conidial population is influenced by environmental factors and can strongly vary between different clonal populations of the same species (see below) (Palma-Guerrero et al. 2013).

B. Hyphal Fusion

The network of germlings formed by CAT or germling fusion further develops into a mycelial colony by hyphal tip extension and branching. Mature colonies are typically spatially structured and comprise a combination of different hyphal types (Hickey et al. 2002; Bistis et al. 2003). Large and fast-extending leading hyphae grow outwards of the colony, while branches of different order fill in the spaces between the main filaments. Upward extending aerial hyphae might differentiate into conidiophores, and three-dimensional fruiting bodies might form in the older part of the mycelium. The different growth behaviors and functional differentiation of the various parts of the fungal colony are also reflected in physiological and gene transcriptional differences (Levin et al. 2007; Kasuga and Glass 2008). The increase of hyphal interconnectivity by the formation of anastomoses is typically restricted to the inner parts of the growing mycelium (Buller 1933; Hickey et al. 2002). Hyphal tips at the colony's periphery exhibit negative autotropism such that they avoid each other and actively adjust their growth direction if a neighboring cell is getting too close. This growth behavior likely results in an optimal utilization of space and substrate (Trinci 1984). In contrast, hyphal branches in the inner part of the colony frequently attract each other and form cross connections by fusion of their tips (Fig. 7.2). Insertion of such secondary hyphal links increases the interconnectivity of the hyphal network, thereby potentially promoting its growth and functionality as one living unit (see below) (Simonin et al. 2012; Roper et al. 2013).

An early study also indicated that hyphal fusion secures the maintenance of the hyphal network. In response to hyphal injury, septal pores are usually plugged by a specialized

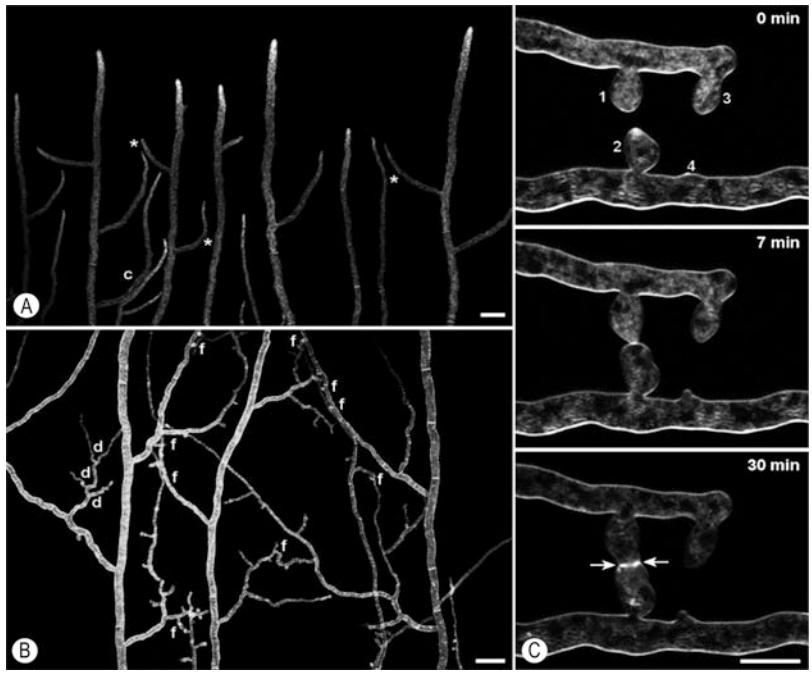


Fig. 7.2 Hyphal fusion occurs in the inner parts of a *Neurospora crassa* mycelial colony. Confocal images showing hyphal growth in different parts of the colony. (**a**) Hyphae at the periphery avoid contact and exhibit negative autotropism (*asterisks*). When hyphae make contact, they do not fuse (c). (**b**) In the inner parts of the colony, hyphal branches interact and form cross connections via hyphal fusion. *f* fusion, *d* dichotomous branching. Hyphae were stained with the membrane dye FM1-43. (**c**) Confocal images showing different stages of the fusion process. Hyphae were stained with the membrane dye FM4-64. Tips 1 and 2 establish physical contact and fuse. Tip 3 induces the formation of a peg (Tip 4) in the neighboring hypha. The *arrows* indicate formation of the fusion pore. All images are adapted from Hickey et al. (2002)

organelle, the Woronin body, and new tip growth is initiated at the closed septum (Buller 1933; Trinci and Collinge 1973; Collinge and Markham 1985). In *Sclerotium hydrophilum*, these newly formed tips grow through the dead compartment and fuse with the next living cell or a tip generated from the opposite septum, thereby restoring hyphal and network integrity (Rothert 1892). In addition to this repair mechanism, hyphal fusion is also employed for the formation of specialized three-dimensional structures. Nematode-trapping fungi, such as *Arthrobotrys oligospora*, form ringlike traps, in which the prey gets entangled. Formation of this ring requires fusion between a hyphal branch and a small peg formed by the parental hypha (Nordbring-Hertz et al. 1989). Anastomoses are also discussed to be involved in fruiting body formation. However, ultimate experimental proof of this hypothesis is still lacking (Lichius et al. 2012).

Mechanistically, hyphal fusion typically occurs in a **tip-to-tip mode** such that fusion always requires the merger of two cellular tip structures (Fig. 7.2) (Ward 1888; Hickey et al. 2002). The presence of an approaching hyphal branch often results in the formation of a newly lateral peg by an unbranched hypha, followed by fusion of the two tips. Fusion-competent branches are therefore able to induce the formation of new fusion structures in neighboring hyphae. Consistent with this hypothesis, anastomosis-rich regions can be distinguished from less intense connected parts within the mycelium, suggesting that the occurrence of anastomosis might induce additional fusion events in close proximity (Hickey et al. 2002).

During the tropic interaction of two fusion tips, both hyphae permanently adjust their

growth direction toward each other, indicated by the positioning of their Spitzenkörper closest toward the partner cell.

The Spitzenkörper is a three-dimensional structure formed by an accumulation of vesicles, which functions as supply center of these intracellular structures at growing hyphal tips (Howard 1981). Its relative position at the hyphal periphery controls tip extension by vesicle-membrane fusion and therefore growth directionality (Bartnicki-Garcia et al. 1995; Riquelme et al. 1998). The Spitzenkörper also appear to hold some function during cell-cell merger, since they remain present after the fusion cells have established physical contact. Once the two fusion cells touch, linear polar growth ceases and the tips become slightly swollen. Both Spitzenkörper become oriented directly opposite to each other, thereby marking the position of the future fusion pore, and remain in this position until cell-cell merger has been completed (Hickey et al. 2002).

Fusion of two hyphae is often followed by rapid **cytoplasmic mixing** or the torrential influx of one cellular content into the other. These dynamics are most likely driven by pressure differences between the fusion partners and might promote important physiological functions (see below).

III. Physiological Roles of Germling and Hyphal Fusion

A. Translocation of Nutrients and Maintaining Homeostasis

The prevalent occurrence of germling and hyphal fusion in filamentous fungi prompted already early assumptions, that anastomosis might benefit the **growth, development, and fitness** of mycelial colonies (Ward 1888). In an early, extensive study on fungal anastomoses, Köhler compares their function to plasmodesmata of plants such that they facilitate or promote **compound exchange** and **signal transduction** (Köhler 1929). However, even a century later, substantial experimental data supporting these hypotheses are scarce. Non-sexual anastomoses are certainly not essential for fungal propagation, since non-fusing mutants are usually still able to complete their vegetative life cycle (Fleißner et al. 2005; Prados Rosales and Di Pietro 2008), and fusion frequencies in different field isolates of the same species can vary significantly, even to a point of complete absence (Hyakumachi and Ui 1987; Palma-Guerrero et al. 2013). Comparison of non-fusing and fusing field isolates of the basidiomycete fungus *Rhizoctonia solani*, however, revealed a striking correlation between anastomoses and fitness (Hyakumachi and Ui 1987). While no significant growth differences between the two types of isolates were observed on potato dextrose agar, fusing strains grew also in sterile and unsterile soil, while non-fusing ones grew only in sterilized soil. Similarly, hyphae of non-fusing isolates did not survive a week after inoculation on organic debris, while strains undergoing anastomosis were still alive after two months. Together, these data suggest that in this specific case, the ability to fuse correlates with an **improved competitiveness** against biotic soil factors. In addition, the fusing strains were highly pathogenic on seedlings and mature roots of sugar beet, while the fusion-deficient strains were not (Hyakumachi and Ui 1987).

A more current study revealed that also in the ascomycete fungus *N. crassa*, germling fusion has the potential to promote growth and development. These benefits emerge, however, only under specific growth conditions (Richard et al. 2012). The authors compared the growth of a wild-type strain with an anastomosis-deficient mutant as radially or linearly growing cultures (the latter ones on long glass tubes, so-called “race tubes”). Starting the colonies from higher numbers of spores always proved beneficial for the wild type, while it was rather disadvantageous for the mutant. As radially growing cultures, the wild type grew larger, but in race tubes, the mutant grew more quickly.

Similarly, a current study found a positive correlation between the degree of fusion and spore yield in *N. crassa* (Bastiaans et al. 2015). An intuitive interpretation of these observations is that cooperation and resource pooling between genetically identical individuals promotes competitiveness. In contrast, establishment of numerous individual colonies from

non-fused spores would create competition for resources and space even between clonal colonies, which in total might hamper propagation of this genotype. Also, the higher competitiveness of fusing spore populations compared to non-fusing ones seems to be in part due to an increase in colony size per se such that larger colonies show in general a higher fitness than smaller ones (Bastiaans et al. 2015). Consistent with this notion, in confrontation assays employing colonies of different and identical basidiomycete species, commonly the larger one succeeded over its smaller competitor (Dowson et al. 1988; Holmer and Stenlid 1993).

Size and mutual competition, however, seem not to be the only issues faced by fusion-deficient fungal isolates. It is also commonly observed that the growth fronts of fusion mutants appear frayed and uneven in comparison to wild-type colonies, suggesting an uneven distribution of growth factors and nutrients within the mycelium (Fleißner et al. 2005; Richard et al. 2012). The hypothesis that anastomoses promote **homeostasis** within the mycelial colony exists since the early days of mycology. Marshall Ward, for example, stated already in 1888: "It might be asked whether the object served by fusions is to nourish the whole mycelium more equably, or to equilibrate certain differences which have unavoidably made themselves apparent in the metabolic processes." (Ward 1888).

Nutrient translocation within a mycelial colony is essential for the growth in heterogeneous environments or to supply nutritional sinks, such as forming fruiting bodies or resting structures such as sclerotia. In extreme cases, such as haustoria-forming biotrophic plant pathogens, tapping of the nutrient source is restricted to specialized structures, which support the entire developing mycelium. Although this nutrient translocation has well been demonstrated for the large and durable colonies of basidiomycete species (Jacobs et al. 2004; Harris and Boddy 2005), its experimental investigation in the comparably small and short-lived mycelia of ascomycete fungi is only just beginning. Analysis of the translocation of labeled nutrients in *N. crassa* wild-type and fusion-deficient colonies revealed that anastomoses in deed promote the transport of resources. The mutants' deficiencies became even more pronounced on heterogeneous nutrient sources, which are more likely to reflect the situation in nature (Simonin et al. 2012). Consistent with these findings, the rapid cytoplasmic flow, observed in the leading hyphae of *N. crassa* colonies, is strongly restricted in anastomosis-deficient mutants (Roper et al. 2013).

Translocation of nutrients and also genetic material via anastomoses is not only restricted to individual fungal colonies but can also occur between originally independent mycelia. In *N. crassa*, this inter-mycelial exchange, however, appears to be restricted to the very young stages of colony establishment, indicating that the interaction of individuals is finely regulated during the fungal life cycle and that only certain windows of cooperation exist (Simonin et al. 2012).

B. Genetic Exchange

In addition to resource pooling, anastomoses can also facilitate the **transfer of genetic material**. Germling or hyphal fusion between non-clonal individuals creates heterokaryotic mycelia, which can provide the basis for the emergence of new genotypes by non-meiotic **parasexual** recombination (Pontecorvo 1956). Karyogamy within heterokaryotic hyphae bears unstable diploid nuclei, which successively lose surplus chromosomes during mitotic divisions, until stable haploid states are restored. The initial diploid state together with the random chromosome loss allows genetic recombination. Parasexual recombination is considered to be a major force of evolution among asexual fungi, causing genetic diversity and the emergence of new pathotypes (Clay and Schardl 2002; Roper et al. 2011; Baskarathevan et al. 2012). It has, however, also been exploited as a tool for genetic analysis, such as genome mapping, and strain construction in asexual species, including the biotechnological workhorse *Aspergillus niger* (Debets et al. 1990, 1993; Bodie et al. 1994; Loera and Cordova 2003).

In nature, however, heterokaryon formation between genetically diverse individuals is often averted by genetic allorecognition mechanisms, such as **vegetative heterokaryon incompatibility.**

During the heterokaryon incompatibility reaction, fusion of two individuals carrying incompatible alleles of specific genomic loci, so-called *het* loci, is followed by sealing of the heterokaryotic compartments by septal plugging and subsequent destruction by programmed cell death reactions. Disjunction of the two fused but incompatible individuals by this post-fusion mechanism serves important functions for maintaining mycelial integrity and prevents the dissemination of infectious genetic elements, such as mycoviruses, or resource plundering by aggressive genotypes (Glass and Kaneko 2003; Glass and Dementhon 2006).

The potential takeover of cellular resources by an invading genotype is impressively illustrated in the plant and animal pathogenic fungus *F. oxysporum*. Here, even fusion of clonal conidial germlings results in the migration of one nucleus into the fusion partner and the subsequent degradation of the originally residing nucleus (Ruiz-Roldan et al. 2010).

Although fungi possess mechanisms to distinguish genetic "self" from "nonself," individual species appear to allow nonself interaction to different extent. Sexual propagation in heterothallic species always requires the physical interaction and nuclear exchange between different individuals and therefore the temporal suppression of incompatibility reactions. In *N. crassa*, the mating-type locus itself serves as a *het* locus (Glass and Kuldau 1992), illustrating the complexity of regulation in fungal interactions. Temporal suppression of incompatibility occurs also during vegetative growth. In *Colletotrichum lindemuthianum*, heterokaryon formation of incompatible strains is possible via germling fusion, resulting in strains whose vegetative progenies possesses features different from the parental strains, therefore indicating recombination (Ishikawa et al. 2012).

It is a current matter of debate to which extent anastomosis formation might contribute to interspecies, horizontal gene transfer. Although interspecies interactions appear to be rare (Köhler 1930), hybridization of two different *Colletotrichum* species via germling fusion has been reported (Roca et al. 2004). It will be of great interest to follow up on these questions not only under laboratory settings but also within the field.

IV. Molecular Bases of Germling/ Hyphal Fusion

Germling/hyphal fusion is a highly orchestrated cellular process, in which different sub-steps with specific molecular requirements can be distinguished (Fig. 7.3) (Hickey et al. 2002; Read et al. 2010). First, the cells need to gain a certain **fusion competence**. Not all germlings within a cell population will undergo fusion, and not all lateral branches within the mycelial colony will merge into hyphal bridges. Fusion, therefore, appears not to be part of the general cellular program but seems to require active induction. A competent cell then has to identify a potential fusion partner and establish a robust **communication** over the spatial distance. As a consequence, both fusion partners redirect their growth toward each other, in order to subsequently establish physical contact. Once the cells touch, the physical contact must be sensed in order for the cellular program to shift from "directed hyphal extension" toward "cell fusion." The cells will **adhere** tightly, and their walls are deconstructed in a careful and highly organized manner. Finally, the plasma membranes need to get into close physical contact to form a **fusion pore** by merger. In germ tube fusion, pore formation and subsequent cytoplasmic mixing are usually followed by the establishment of a new polarity center, which will serve as the origin of a newly growing tip. Despite this complexity, fungal anastomosis formation is an experimentally easily amenable model system for eukaryotic cell fusion. In *N. crassa*, conidia can be simply spread on a synthetic growth medium, and after 3–4 h of incubation, cell-cell communication and fusion are readily observable by simple light microscopy. This system therefore provides a simple model for addressing the various biological processes involved in germling fusion, including cell-cell communication, polar and directed growth, cell adhesion, cell wall remodeling, and plasma membrane merger.

In recent years, reverse and forward genetics approaches have revealed numerous molecular factors mediating the various steps of

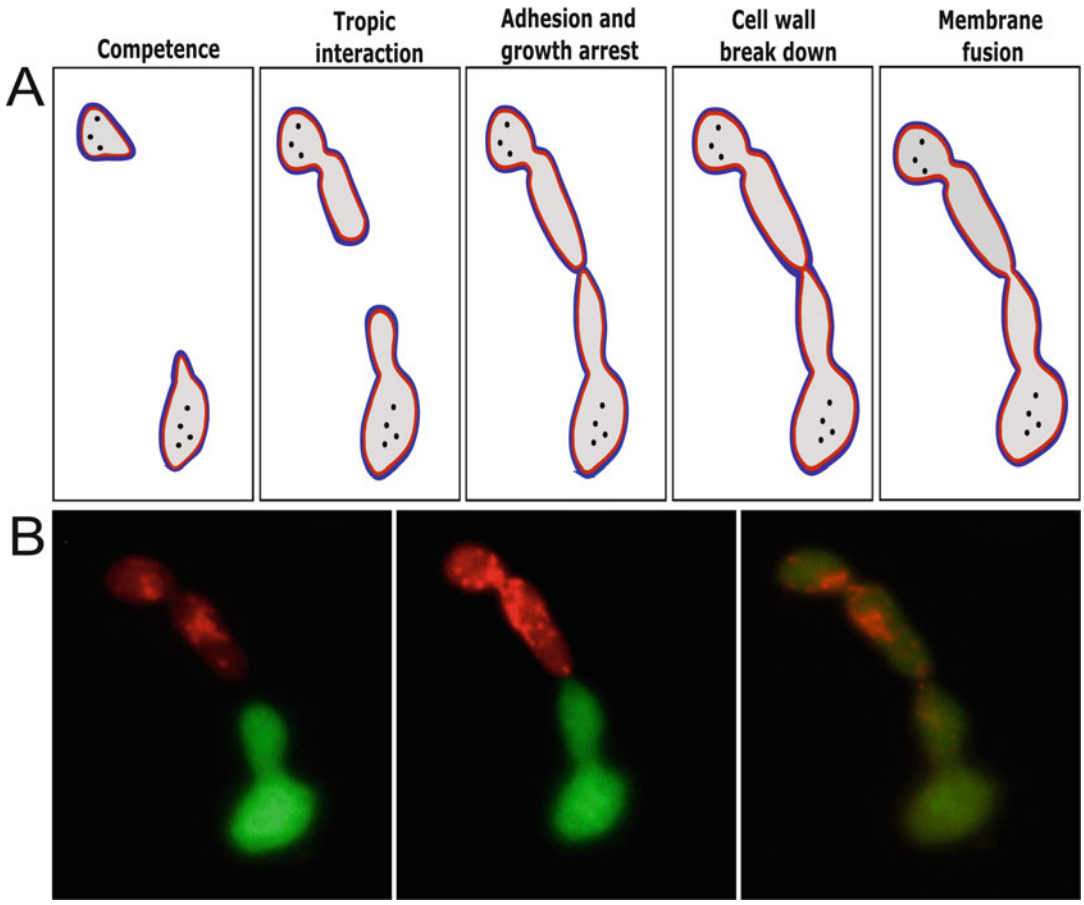


Fig. 7.3 Germling fusion is a multistep process. (**a**) After the germlings gained fusion competence, they establish a mutual tropic interaction. Ones physical contact is established, growth is arrested, and the two germlings tightly adhere to each other. The cell wall is deconstructed at the contact area and the plasma membranes merge, and the cytoplasm mixes. (**b**) Fusion of two germlings expressing either cytoplasmic GFP or cytoplasmic mCherry (images are merged). Note how the fluorescence mixes after fusion

anastomosis formation. It is becoming increasingly apparent that this biological process is controlled by an intricate signaling network, involving MAP kinase signaling cascades, reactive oxygen-generating systems, Ca^{2+} signaling, polarity factors, and other signaling complexes conserved in eukaryotic cells. Complete overviews of the so far reported factors involved in vegetative fusion have recently been provided by two different literature reviews (Lichius and Lord 2014; Weichert and Fleißner 2015). Here, we will focus on selected examples in an attempt to highlight some of the general molecular principles mediating vegetative cell fusion in filamentous ascomycete fungi.

A. Competence

The molecular basis of fusion competence is currently not understood. To some extent, the molecular machineries controlling competence seem to overlap with factors involved in the subsequent tropic interaction of the fusion partners. For example, strains lacking components of the MAK-2 MAP kinase module, which

is clearly involved in directed growth (see below), are unable to form CATs, suggesting that they might also be deficient in turning the developmental switch toward fusion (Pandey et al. 2004; Roca et al. 2005b). Deciphering these different functions of individual molecular factors poses a major challenge for future investigations.

It is, however, becoming increasingly clear that fusion competence is influenced by environmental factors and growth conditions, including nutrient availability, cell density, or the pH value of the growth substrate. Köhler showed already that germlings of *B. allii* readily fused on diluted potato juice or potato haulm extract agar, but that anastomoses were basically absent on the undiluted growth media (Köhler 1930). Similar observations were made for *Sclerotinia fructigena*, *Fusarium coeruleum*, and *Spicaria violacea* in the same study. In *Colletotrichum lindemuthianum*, conidial anastomosis tubes even only form when spores are cultivated in water, while the presence of nutrients fully suppresses this cellular differentiation (Ishikawa et al. 2010). A negative correlation between nutrient availability and fusion frequency has also been reported for the basidiomycete fungi *Schizophyllum commune* and *Rhizoctonia solani*, where the addition of nitrogen to the growth medium had the strongest effect (Ahmad and Miles 1970; Yokoyama and Ogoshi 1988). In the vascular wilt fungus *F. oxysporum*, the addition of ammonium to the growth medium repressed anastomosis formation. This repression requires the bZIP protein MeaB and the nitrogen regulator AreA and could be reversed by rapamycin, indicating an involvement of the Ser/Thr kinase TOR (Lopez-Berges et al. 2010). The addition of various amino acids, especially tryptophan, to the growth medium inhibited CAT fusion in *N. crassa*. Tryptophan appears to act intracellularly, since amino acid permease mutants are resistant against this inhibitory effect (Fischer-Harman et al. 2012). These negative effects of nutrients on anastomoses formation are consistent with the hypothesis that germling fusion, and therefore resource pooling, might increase the fungal fitness in response to starvation. If, in contrast, sufficient external resources are available, also the development of independent colonies within a restricted space is supported.

Induction of fusion within in spore populations also depends on cell density and appears to share characteristics of quorum-sensing behavior. In *Venturia inaequalis*, groups of spores readily initiate germling fusion, while two isolated cells rarely anastomose. Interestingly, fusion in isolated pairs could be induced by the addition of culture filtrates (Leu 1967), suggesting the presence of a chemical inducer. Similarly in *N. crassa*, the number of conidial anastomosis tubes negatively correlates with the conidial concentration (Roca et al. 2005b).

B. Cell-Cell Communication and Directed Growth

1. Signals

As non-motile cells, fungal fusion germlings or hyphae need to establish physical contact via directed growth. Typically both cell tips actively grow toward the partner, indicating **bidirectional signaling**, or as Marshall Ward put it in 1888: "(. . .) *it seems to me impossible to doubt that the hyphae exert an attractive influence upon one another* (. . .)" (Ward 1888). The early mycologists also speculated already on the nature of the signals involved, and Köhler stated in 1930 "*there should not be much doubt that these stimuli are of material nature*" (Köhler 1930). However, even a century later, the signals mediating germling and/or hyphal fusion are still unknown and have somewhat become the holy grail of this research field. A major obstacle in their identification is the lack of a robust bioassay to identify, for example, active fractions of a culture supernatant.

Fusion-related interactions are mostly species specific, suggesting that every species employs somewhat different signals (Köhler 1930). Oligopeptides, such as the pheromones involved in fungal sexual communications, are likely candidates. However, in *N. crassa*, the sexual pheromones and their receptors are dispensable for vegetative fusion processes (Kim and Borkovich 2004, 2006). In addition to small peptides, fungi produce a plethora of other

chemical substances which are involved in developmental regulatory processes (Leeder et al. 2011). Examples include the diterpenoid conidiogene inducing spore formation in *Penicillium cyclopium* (Roncal et al. 2002) or the sesquiterpene alcohol farnesol mediating quorum-sensing processes in *Candida albicans* (Hornby et al. 2001). Searching in various directions seems therefore advisable in efforts to identify the fusion-mediating signal.

2. MAP Kinase Signaling

Two different **MAP kinase** signaling cascades, homologous to the **pheromone response** and the **cell wall integrity** pathway of *Saccharomyces cerevisiae*, have conserved functions during germling and hyphal fusion in filamentous ascomycete fungi. In *N. crassa*, the two pathways are the MAK-2 and the MAK-1 cascade, respectively. The MAK-2 MAP kinase and its upstream activating kinases MEK-2 and NRC-1 are essential for the interaction of fusion cells. Mutants lacking one of these factors are deficient in cellular interactions related to fusion, and the cells appear to be blind toward each other (Pandey et al. 2004; Maerz et al. 2008; Fleißner et al. 2009b). Isolates of *F. oxysporum* lacking the MAK-2 homologous kinase Fmk1 exhibit comparable deficiencies (Prados Rosales and Di Pietro 2008). Similarly in *Aspergillus nidulans*, mutants in the respective MAP kinase MpkB and MAP kinase kinase kinase SteC are unable to undergo self and nonself fusion (Wei et al. 2003; Jun et al. 2011).

In *N. crassa*, subcellular localization of the three kinases of the MAK-2 module revealed an unusual signaling mechanism employed by the two fusion partners. In germlings undergoing positive autotropism, the three kinases assemble in membrane-associated complexes, in a dynamic, oscillating manner (Fig. 7.4) (Fleissner et al. 2009b; Dettmann et al. 2012). The membrane recruitment alternates between the two fusion partners with a phase of 6–12 min. Within one cell, recruitment of this signaling module alternates with the formation of membrane-associated complexes of the SO protein (see below) (Fleißner et al. 2009b). Together, these data suggest that the two fusion cells coordinately and continuously switch between two physiological stages during their interaction. An attractive hypothesis is that the cells alternate between signal sending and receiving and therefore establish a kind of **cellular dialog**. This mode of communication would allow both cells to employ the same signal/receptor pair while avoiding self-excitation (Fleißner et al. 2009b; Goryachev et al. 2012).

Plasma membrane recruitment and activation of MAP kinase modules commonly involve **scaffolding proteins**, which are often not well conserved. For example, regulation of the yeast pheromone response pathway, whose MAP kinase is homologous to MAK-2, strongly depends on the Ste5 scaffolding protein (Choi et al. 1994), which has no homolog in filamentous ascomycete fungi. Recently, however, two potential MAK-2 scaffolds were identified. The NDR kinase scaffold HYM-1 is essential for signal transduction through the kinase module. A physical fusion of the upstream kinases NRC-1 and MEK-2 reconstituted MAK-2 signaling in a Δ*hym-1* mutant, suggesting that HYM-1 functions in bridging the two kinases (Dettmann et al. 2012).

In addition, two recent studies independently identified HAM-5 as an additional scaffold for the MAK-2 module (Dettmann et al. 2014; Jonkers et al. 2014).

A Δ*ham-5* mutant is fully fusion deficient, and the HAM-5 protein co-localizes together with the three kinases of the MAK-2 module in the typical oscillating manner. While so far the alternating membrane recruitment of the various signaling proteins was only observed during germling fusion but not in hyphal fusion, HAM-5 shows robust oscillation in both processes. This finally supports the mutant phenotype-based hypothesis, that both processes are mediated by very similar molecular mechanisms (Jonkers et al. 2014). The complex of the MAK-2 module and HAM-5 also physically interacts with a number of additional signaling components, including the MAP kinase regulator STE50, two STE20-related kinases, the small GTPase RAS2, and factors of the STRIPAK signaling complex (see below), suggesting the presence of an intricate signaling network mediating anastomosis formation (Dettmann et al. 2014).

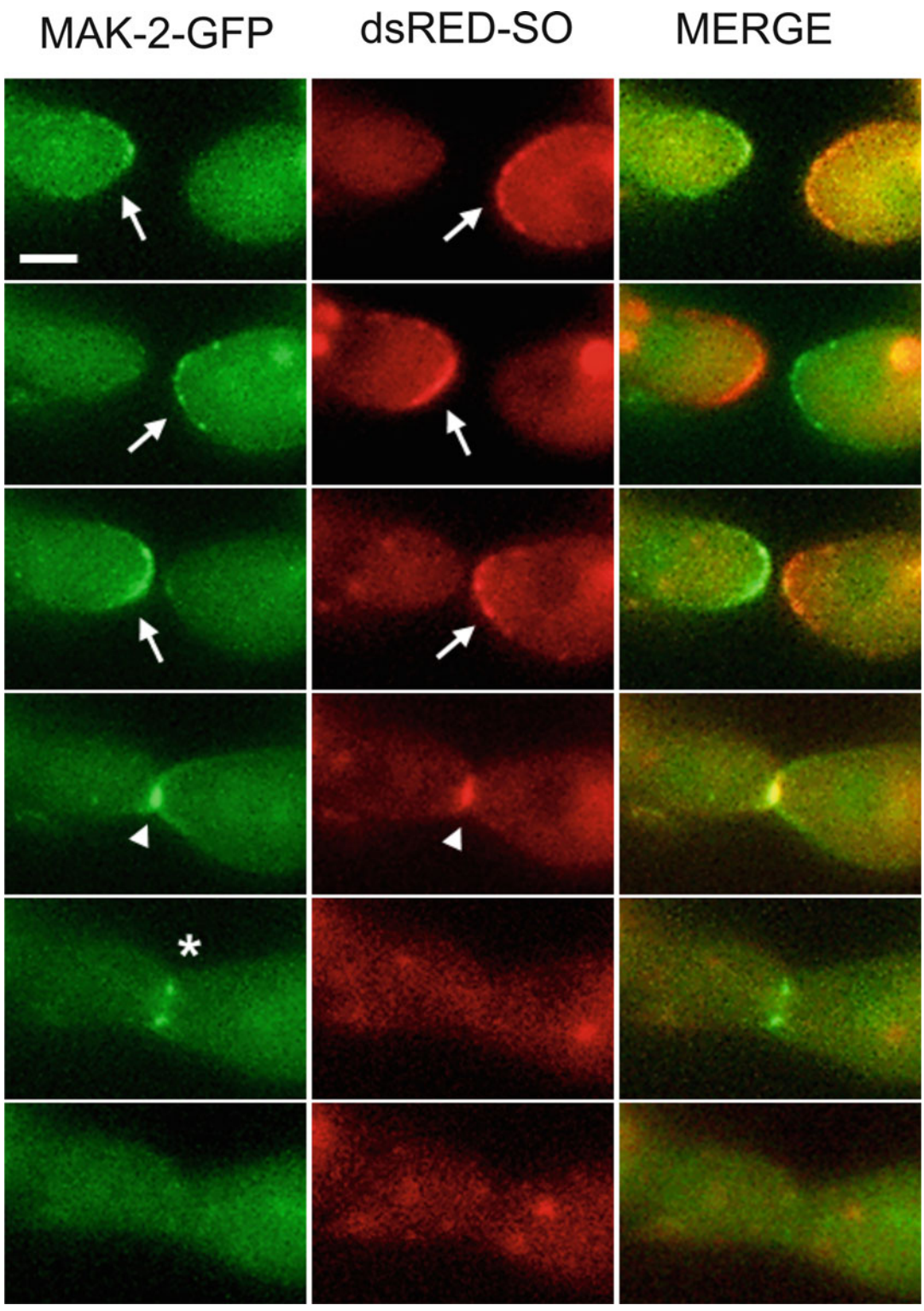


Fig. 7.4 Fusing germlings of *N. crassa* coordinately switch between two physiological stages during their interaction. These *switches* are indicated by the alternating recruitment of the MAP kinase MAK-2 (*green*)

The dynamic localization of the MAK-2-module within the cell likely reflects its various activation states and molecular functions. Activation of MAP kinases commonly occurs in membrane-associated complexes, since upstream activating factors are often membrane bound, such as transmembrane sensors (Pearson et al. 2001). Membrane recruitment might, however, also modulate the signal transmission process by concentrating the different interacting factors in a restricted space, thereby amplifying signaling through the cascade (Lamson et al. 2006). In addition to upstream activators, the complex-bound interactors can also involve MAP kinase targets. In *S. cerevisiae*, Fus3 phosphorylates the actin-organizing formin Bni1 resulting in polarization of the cytoskeleton (Matheos et al. 2004). A similar function of tip-localized MAK-2 in directing growth of the fusion tips of *N. crassa* seems more than likely.

In fusion germlings, MAK-2 localizes also to nuclei (Fleißner et al. 2009b). Common MAP kinase targets are transcriptional regulators linking cellular signaling to gene activity control. The MAK-2 kinase of *N. crassa* interacts with the transcription factor PP-1, a homolog of Ste12 of *S. cerevisiae*, and RCO-1, which forms together with RCM-1 a dimeric transcription factor. Both PP-1 and RCO-1/RCM-1 are essential for germling and hyphal fusion, indicating a role of the MAK-2 module in translating the cellular communication into transcriptional responses (Li et al. 2005; Aldabbous et al. 2010; Leeder et al. 2013).

The second MAP kinase module essential for germling and hyphal fusion is homologous to the yeast **cell wall integrity pathway** and consists of the three kinases MIK-1, MEK-1, and MAK-1. Mutants of *N. crassa*, *S. macrospora*, and *E. festucae* lacking the respective MAP kinase and its upstream activating kinases are fusion deficient and form no hyphal networks (Maerz et al. 2008; Teichert et al. 2014; Becker et al. 2015). A complex of the two transmembrane proteins HAM-6 and HAM-8 and the GPI-anchored HAM-7 potentially functions as an upstream sensor of the MAK-1 module in *N. crassa* (Maddi et al. 2012; Fu et al. 2014). The exact molecular function of this signaling cascade during the cellular interaction remains to be determined; however, in *N. crassa*, a potential link to the MAK-2 MAP kinase cascade appears to exist via the STRIPAK signaling complex (see below).

3. The SO Protein

SO is a protein specific only for filamentous ascomycete fungi and is not present in bakers or fission yeast (Fleißner and Glass 2007). Its molecular function during hyphal fusion is so far not fully understood. Mutants of *N. crassa*, *S. macrospora*, *F. oxysporum*, and *E. festucae* lacking the *so* gene are deficient in vegetative fusions (Fleißner et al. 2005; Engh et al. 2007; Prados Rosales and Di Pietro 2008; Charlton et al. 2012). As mentioned above, in *N. crassa* germlings undergoing tropic interactions, SO assembles at the plasma membrane of the cell tips. This recruitment alternates with the formation of MAK-2 module complexes, suggesting a role in the coordinated signaling behavior (Fig. 7.4) (Fleißner et al. 2009b). Based on the well-described functions of MAP kinases, the cell accumulating MAK-2 at the tip is the signal-receiving cell in the current working model. Hence, the cell carrying SO at its tip would be the signal-sending one, and a so far unknown role of SO in secretion of the signal is being discussed (Fleißner et al. 2009b; Goryachev et al. 2012). In *S. macrospora*, the SO homologous PRO40 functions as a scaffolding protein for the MAK-1 cell wall integrity MAP kinase cascade and links the kinase module to the upstream activator protein kinase C (PKC1) (Teichert et al. 2014). However, no membrane recruitment of MAK-1 and co-localization with

Fig. 7.4 (continued) and the SO protein (*red*) to the plasma membrane of the growing tips (*arrows*). MAK-2 remains at the contact zone after physical contact, while SO disappears (*arrowheads*). MAK-2 localizes around the forming fusion pore (*asterisk*) (Adapted from Weichert and Fleißner 2015)

SO are observed during germling fusion in *N. crassa* (our unpublished data). Therefore, SO has probably molecular functions beyond its role as a scaffold for MAP kinase modules. In the plant pathogenic fungus *A. brassicicola*, the SO homolog is a pathogenicity factor, while in the grass endophyte *Epichloe festucae*, the absence of SO shifts the mutualistic lifestyle toward pathogenic behavior (Craven et al. 2008; Charlton et al. 2012). The protein also accumulates at septal plugs in various fungi (Engh et al. 2007; Fleißner and Glass 2007; Maruyama et al. 2010) and in stress granules in *A. oryzae*, where it promotes the proper formation and localization of these structures (Huang et al. 2013), adding additional aspects to potential SO functions. Together, these data highlight the still somewhat cryptic SO protein as an important factor controlling growth and development of filamentous ascomycete fungi.

4. The STRIPAK Complex

Studies in *S. macrospora* first revealed the presence of a highly conserved multi-subunit signaling complex in filamentous fungi, homologous to the human **striatin-interacting phosphatase and kinase (STRIPAK) complex** (Poggeler and Kuck 2004; Bloemendal et al. 2012). In *S. macrospora* and *N. crassa*, STRIPAK signaling is involved in fruiting body formation during sexual development and anastomosis formation during vegetative growth (Simonin et al. 2010; Bernhards and Poggeler 2011; Bloemendal et al. 2012; Dettmann et al. 2013; Nordzieke et al. 2014). The complex localizes to the nuclear envelope and consists of six different proteins, HAM-3/PRO11/striatin, HAM2/PRO22/STRIP, HAM-4/SLAMP, MOB-3/SmMOB3/phocein, and protein phosphatase 2A subunits PPG-1/PP2A-C and SmPP2AA/PP2A-A. Striatin is a member of the calmodulin-binding protein family and is thought to serve as a scaffolding protein for signaling complexes. MOB-3 functions as a kinase activator.

In *N. crassa*, a link between STRIPAK and MAP kinase signaling exists. MOB-3/phocein is a target of the MAK-2 MAP kinase, and its phosphorylation influences the nuclear accumulation of MAK-1. As described above, both of these MAP kinases are essential for vegetative and hyphal fusion. Together with the STRIPAK complex, these signaling cascades appear to be linked into an intricate signaling network mediating vegetative cell-cell communication and fusion. The core STRIPAK complex can also associate with other regulatory proteins, thereby linking it to other signaling pathways involved in a plethora of eukaryotic cell functions, including signaling, cell cycle control, vesicular trafficking, cell polarity, and apoptosis (Hwang and Pallas 2014).

5. Reactive Oxygen Species-Generating Systems

Studies in *B. cinerea*, *N. crassa*, and *E. festucae* revealed a conserved function of **reactive oxygen species (ROS)**-generating systems in vegetative hyphal fusion (Takemoto et al. 2011; Read et al. 2012; Roca et al. 2012). In the gray mold *B. cinerea*, the NADPH oxidase BcNoxA and its regulator BcNoxR are essential for germling fusion, while a second NADPH oxidase BcNoxB is contributing to this process (Roca et al. 2012). All factors also play a role in the pathogenic development of this plant parasitic fungus, however, BcNoxB being the essential and BcNoxA the contributing factor. Interestingly, no germling fusion was observed, when spores germinated on plant surfaces, which induce pathogenic development, suggesting that fusion and infection might be mutually exclusive developmental routes for germinating conidia (Fleißner 2012; Roca et al. 2012).

Takemoto et al. identified the potential scaffolding protein Bem1 as part of NADPH oxidase complexes (Takemoto et al. 2011). Consistent with this finding, Δ*bem1* mutants of *N. crassa* and *B. cinerea* are fusion deficient (Schurg et al. 2012; Giesbert et al. 2014). Tropic interactions related to vegetative fusion are strongly reduced and instable in the *N. crassa* mutant, and signaling through the MAK-2 MAP kinase cascade is strongly affected in these isolates. The SO protein mislocalizes in Δ*bem1*

fusion germlings reminiscent to a pattern observed after specific chemical inhibition of MAK-2 (Fleißner et al. 2009b; Schurg et al. 2012). In *S. cerevisiae*, which lacks NADPH oxidases, Bem1 links the pheromone response MAP kinase pathway to cell polarity factors (Leeuw et al. 1995). In different fungi, Bem1 binds the small GTPases Rac-1 or Cdc42, the Guanine nucleotide exchange factor Cdc24, interacts with the actin organizing formin and is linked to the secretory pathway (Kozubowski et al. 2008; Li and Wedlich-Soldner 2009; Giesbert et al. 2014; Liu and Novick 2014). Rac-1 and Cdc24 are also part of the Nox complex of *E. festucae* and play a role in germling fusion in *N. crassa* (Fu et al. 2011; Takemoto et al. 2011; Lichius et al. 2014). Recently, further support for a regulatory role of specific ROS levels was provided by quantitative trait analysis. This study identified ARG-15, an acetylornithine-glutamate transacetylase, as a factor promoting cellular interactions related to anastomoses in *N. crassa*, which is potentially involved in ROS degradation (Palma-Guerrero et al. 2013). Together, these observations support a model, in which **ROS signaling**, **MAP kinase cascades**, and **cell polarity factors** form a regulatory network controlling cell-cell signaling and directed growth (Fig. 7.5).

6. Calcium Signaling

On media containing reduced amounts of Ca^{2+}, conidia of *N. crassa* germinate properly; however, their fusion-related interactions are significantly reduced (Palma-Guerrero et al. 2013). Ca^{2+}-mediated signaling might therefore be involved in anastomosis formation. In a genome-wide association study in *N. crassa*, *cse-1*, a homolog of a neuronal calcium sensor of vertebrates, was identified as a promoting factor of germling and hyphal fusion. In these processes, Ca^{2+} might be involved in secretory processes. Mutations in the two potential *cse-1*-interaction partners, *nfh-2* and *pik-1*, also result in fusion deficiencies (Palma-Guerrero et al. 2013). NFH-2, a 14-3-3 protein, could control the CSE-1 shuttle between the cytoplasm and the nucleus, while PIK-1 is a phosphatidylinositol kinase involved in vesicle transport from the Golgi to the plasma membrane. An attractive hypothesis is that these factors control **Ca^{2+}-mediated secretion** of the cell-cell communication signal and/or its cognate receptor (Palma-Guerrero et al. 2013). An essential role of secretory and/or endocytic processes for germling/hyphal fusion is further supported by the essential roles of the **exocyst** complex component SEC15 and the C2-domain-containing protein HAM-10 for vegetative fusion (Fu et al. 2011; Palma-Guerrero et al. 2013). C2-domain proteins mediate membrane targeting of proteins in an often Ca^{2+}-dependent manner. They are involved in various cellular processes, including signal transduction and vesicular trafficking (Cho and Stahelin 2006).

Interestingly, in mammalian cells, calcium binds to striatin via calmodulin. A potential role of the STRIATIN complex in Ca^{2+}-mediated signaling during vegetative hyphal fusion has therefore been proposed (Simonin et al. 2010), further expanding the proposed signaling network of MAP kinase cascades and STRIATIN by Ca^{2+}-mediated processes (Fig. 7.5).

C. Adhesion, Cell Wall Breakdown, and Plasma Membrane Fusion

Once the two fusion partners have established physical contact, they must tightly adhere in order to form a **fusion pore** by breaking down the cell walls and merging the plasma membranes. So far, molecular factors mediating adherence and cell wall deconstruction during vegetative fusion in filamentous fungi are unknown. The adhesins mediating cell-cell contact during yeast mating, Aga1, Aga2, Sag1 and Fig2 (Huang et al. 2009), are not conserved in filamentous ascomycete species. It has been hypothesized that the MAK-1 MAP cell wall integrity pathway is involved in cell wall remodeling during fusion in *N. crassa*; however, experimental proof is still lacking.

An important, basic hypothesis in the cell fusion research field is the existence of so-called

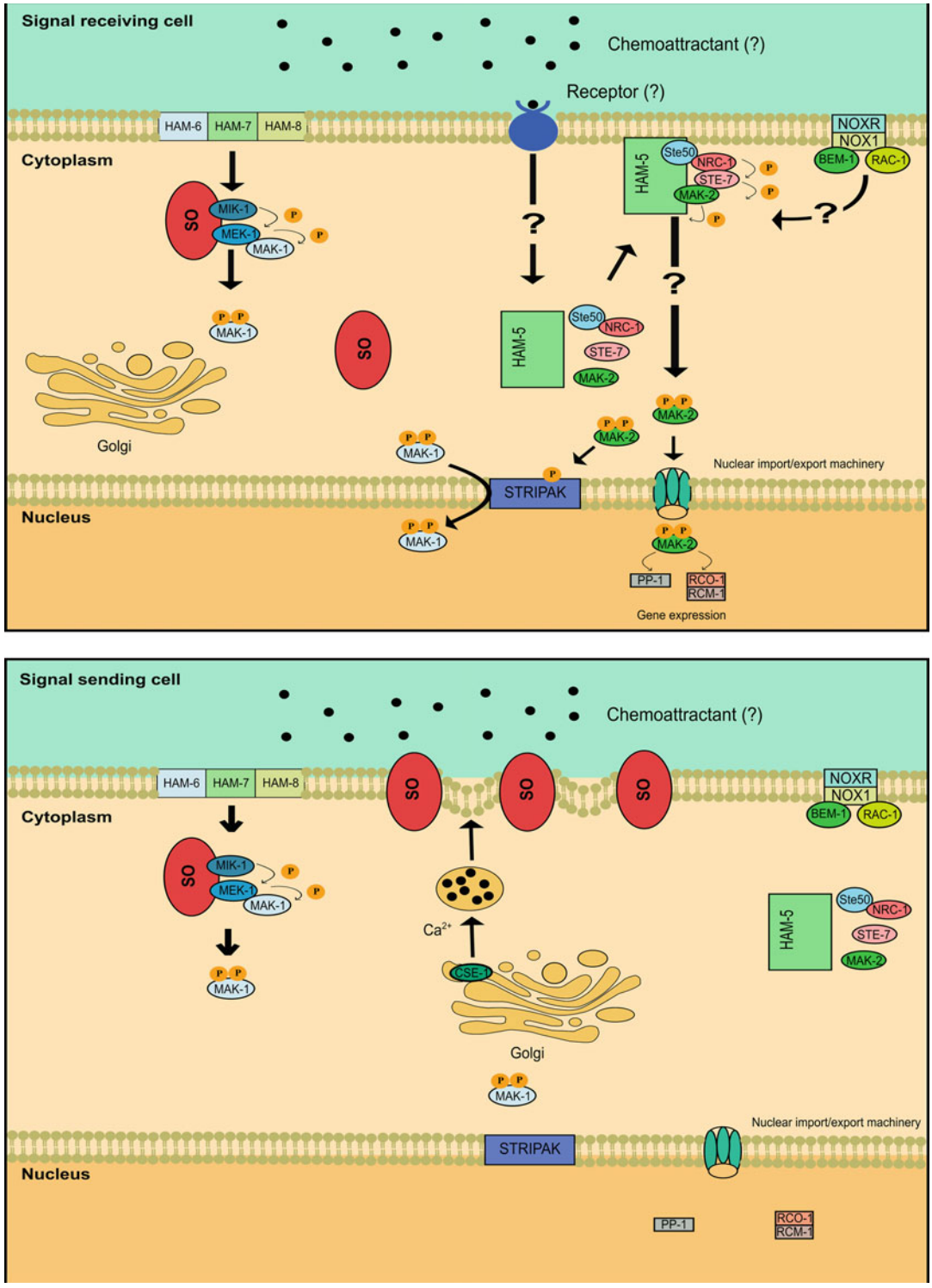


Fig. 7.5 Current working model describing the molecular bases of germling fusion in *N. crassa*. The cells alternate between signal receiving (*top*) and signal sending (*bottom*). Binding of the postulated signaling

fusogens. **Fusogens** are defined as cell surface proteins, which are essential and sufficient to mediate cell membrane merger (Aguilar et al. 2013). The hunt for this holy grail of cell fusion has so far only been successful in the nematode *Caenorhabditis elegans* (AFF-1, EFF-1) and for the mammalian placenta, where trophoblasts fuse (syncytin-1, syncytin-2) (Mohler et al. 2002; Blaise et al. 2003; Frendo et al. 2003; Sapir et al. 2007). So far, no fungal factor, meeting the criteria of a fusogen, has been identified. However, several proteins promoting plasma membrane merger are known. In a screen for potential fusogens mediating membrane fusion during mating of *S. cerevisiae*, the **Prm-1** protein was identified. Prm-1 is a transmembrane protein, whose expression is induced in response to mating pheromone. In a Δ*prm-1* mutant, about 50 % of mating pairs arrest as prezygotes at a stage where the cell wall had been degraded, but the plasma membranes did not fuse, yet (Heiman and Walter 2000). A comparable phenotype was observed in a Δ*prm-1* mutant in *N. crassa* in vegetative but also sexual trichogyne-conidium fusion, suggesting that PRM-1 is part of a general fusion machinery (Fleißner et al. 2009a). Recently, a protein with similar function to PRM-1 was identified. LFD-1 is a single-pass transmembrane protein, whose absence results in similar but less severe fusion defects as observed in Δ*prm-1*. Genetic analysis revealed that the defects of both mutants are additive, indicating that the two proteins function independently while sharing redundant functions (Palma-Guerrero et al. 2014).

V. Conclusion

While hyphal fusion in filamentous fungi caught already the early mycologists' attention, it was not until recent years that broader interest in this fascinating topic has been rekindled. Since modern techniques, such as life cell imaging, genome sequencing, transcriptomic analysis, or directed mutagenesis, are very well established for many filamentous ascomycete species, rapid progress in elucidating the molecular basis of anastomosis formation has been made. It is becoming increasingly evident that an intricate signaling network comprising many highly conserved molecular factors controls these processes. Vegetative hyphal fusion therefore provides an easily accessible model system to analyze eukaryotic cell-cell communication and fusion. Many vital questions, however, remain unanswered. What is the nature of the signal and its cognate receptor employed by interacting fusion cells? How can the cells coordinate and fine-tune their behavior over a spatial distance without physical contact? What is the role of germling and hyphal fusion in natural environments and for the different fungal life styles? Is vegetative fusion related to pathogenic development? To which extent does nonself fusion contribute to genetic recombination and the origin of new pathotypes? Does vegetative fusion offer new approaches in biotechnological applications? The currently rising interest of the fungal research community in hyphal fusion holds much potential for answering many of these questions in the near future.

Fig. 7.5 (continued) ligand to its cognate receptor results in the assembly of the MAK-2 signaling complex at the plasma membrane and activation of the MAP kinase. Activation involves or is promoted by reactive oxygen species produced by the NOX1 NADPH oxidase complex. Activation of MAK-2 results in control of the growth direction through a postulated interaction with cytoskeleton controlling factors. In addition, activated MAK-2 enters the nucleus, where it controls the transcription factors PP-1 and RCO-1/RCM-1, which regulate gene transcription. In addition, MAK-2 phosphorylates MOB-3 of the STRIPAK complex, resulting in translocation of the MAK-1 MAP kinase into the nucleus. In the signal-sending cell, the MAK-2 MAP kinase complex is disassembled, and the SO protein is present at the plasma membrane. The signal might be provided by vesicles, and their controlled fusion with the plasma membrane might result in a signal burst. Vesicle transport and fusion with the membrane is controlled by Ca^{2+}-controlled factors and might involve the SO protein. In addition, the SO protein functions as a scaffold for the MAK-1 MAP kinase module, which is activated by a membrane-bound complex of HAM6/7/8. If this activation is required for signal sending or receiving is so far unclear

Acknowledgment Work in our group is supported by funding from the German Research Foundation (FL706-2) and the European Union (PITN-GA-2013-607963) to A.F.

References

Aguilar PS, Baylies MK, Fleißner A, Helming L, Inoue N, Podbilewicz B, Wang H, Wong M (2013) Genetic basis of cell-cell fusion mechanisms. Trends Genet 29(7):427–437

Ahmad SS, Miles PG (1970) Hyphal fusions in Schizophyllum commune. 2. Effects of environmental and chemical factors. Mycologia 62:1008–1017

Aldabbous MS, Roca MG, Stout A, Huang IC, Read ND, Free SJ (2010) The ham-5, rcm-1 and rco-1 genes regulate hyphal fusion in *Neurospora crassa*. Microbiology 156(Pt 9):2621–2629

Bartnicki-Garcia S, Bartnicki DD, Gierz G, Lopez-Franco R, Bracker CE (1995) Evidence that Spitzenkorper behavior determines the shape of a fungal hypha: a test of the hyphoid model. Exp Mycol 19(2):153–159

Baskarathevan J, Jaspers MV, Jones EE, Cruickshank RH, Ridgway HJ (2012) Genetic and pathogenic diversity of Neofusicoccum parvum in New Zealand vineyards. Fungal Biol 116(2):276–288

Bastiaans E, Debets AJ, Aanen DK (2015) Experimental demonstration of the benefits of somatic fusion and the consequences for allorecognition. Evolution 69:1091–1099

Becker Y, Eaton CJ, Brasell E, May KJ, Becker M, Hassing B, Cartwright GM, Reinhold L, Scott B (2015) The fungal cell-wall integrity MAPK cascade is crucial for hyphal network formation and maintenance of restrictive growth of Epichloe festucae in symbiosis with Lolium perenne. Mol Plant Microbe Interact 28(1):69–85

Bernhards Y, Poggeler S (2011) The phocein homologue SmMOB3 is essential for vegetative cell fusion and sexual development in the filamentous ascomycete *Sordaria macrospora*. Curr Genet 57:133–149

Bistis GN, Perkins DD, Read ND (2003) Different cell types in *Neurospora crassa*. Fungal Genet Newslett 50:17–19

Blaise S, de Parseval N, Benit L, Heidmann T (2003) Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. Proc Natl Acad Sci USA 100(22):13013–13018

Bleichrodt RJ, van Veluw GJ, Recter B, Maruyama J, Kitamoto K, Wosten HA (2012) Hyphal heterogeneity in Aspergillus oryzae is the result of dynamic closure of septa by Woronin bodies. Mol Microbiol 86(6):1334–1344

Bloemendal S, Bernhards Y, Bartho K, Dettmann A, Voigt O, Teichert I, Seiler S, Wolters DA, Poggeler S, Kuck U (2012) A homologue of the human STRIPAK complex controls sexual development in fungi. Mol Microbiol 84(2):310–323

Bodie EA, Armstrong GL, Dunn-Coleman NS (1994) Strain improvement of chymosin-producing strains of Aspergillus niger var. awamori using parasexual recombination. Enzyme Microb Technol 16(5):376–382

Buller A (1933) Researches on fungi, vol V. Longman, London

Charlton ND, Shoji JY, Ghimire SR, Nakashima J, Craven KD (2012) Deletion of the fungal gene soft disrupts mutualistic symbiosis between the grass endophyte Epichloe festucae and the host plant. Eukaryot Cell 11(12):1463–1471

Cho W, Stahelin RV (2006) Membrane binding and subcellular targeting of C2 domains. Biochim Biophys Acta 1761(8):838–849

Choi KY, Satterberg B, Lyons DM, Elion EA (1994) Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in S. cerevisiae. Cell 78(3):499–512

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160(Suppl 4):S99–S127

Collinge AJ, Markham P (1985) Woronin bodies rapidly plug septal pores of severed *Penicillium chrysogenum* hyphae. Exp Mycol 9:80–85

Craven KD, Velez H, Cho Y, Lawrence CB, Mitchell TK (2008) Anastomosis is required for virulence of the fungal necrotroph *Alternaria brassicicola*. Eukaryot Cell 7(4):675–683

Dahlberg KR, Van Etten JL (1982) Physiology and biochemistry of fungal sporulation. Annu Rev Phytopathol 20:281–301

De Bary A (1884) Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien. Wilhelm Engelmann, Leipzig

Debets AJ, Swart K, Bos CJ (1990) Genetic analysis of Aspergillus niger: isolation of chlorate resistance mutants, their use in mitotic mapping and evidence for an eighth linkage group. Mol Gen Genet 221(3):453–458

Debets F, Swart K, Hoekstra RF, Bos CJ (1993) Genetic maps of eight linkage groups of Aspergillus niger based on mitotic mapping. Curr Genet 23 (1):47–53

Dettmann A, Illgen J, März S, Schürg T, Fleißner A, Seiler S (2012) The NDR kinase scaffold HYM1/MO25 is essential for MAK2 MAP kinase signaling in *Neurospora crassa*. PLoS Genet 8(9), e1002950

Dettmann A, Heilig Y, Ludwig S, Schmitt K, Illgen J, Fleißner A, Valerius O, Seiler S (2013) HAM-2 and HAM-3 are central for the assembly of the Neurospora STRIPAK complex at the nuclear envelope and regulate nuclear accumulation of the MAP

kinase MAK-1 in a MAK-2-dependent manner. Mol Microbiol 90(4):796–812

Dettmann A, Heilig Y, Valerius O, Ludwig S, Seiler S (2014) Fungal communication requires the MAK-2 pathway elements STE-20 and RAS-2, the NRC-1 adapter STE-50 and the MAP kinase scaffold HAM-5. PLoS Genet 10(11), e1004762

Dowson CG, Rayner ADM, Boddy L (1988) The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments. New Phytol 109:423–432

Engh I, Wurtz C, Witzel-Schlomp K, Zhang HY, Hoff B, Nowrousian M, Rottensteiner H, Kuck U (2007) The WW domain protein PRO40 is required for fungal fertility and associates with Woronin bodies. Eukaryot Cell 6(5):831–843

Fischer-Harman V, Jackson KJ, Munoz A, Shoji JY, Read ND (2012) Evidence for tryptophan being a signal molecule that inhibits conidial anastomosis tube fusion during colony initiation in Neurospora crassa. Fungal Genet Biol 49(11):896–902

Fleißner A (2012) Hyphal fusion. In: Perez-Martin J, Di Pietro A (eds) Morphogenesis and pathogenicity in fungi. Springer, Berlin, pp 43–60

Fleißner A, Glass NL (2007) SO, a protein involved in hyphal fusion in *Neurospora crassa*, localizes to septal plugs. Eukaryot Cell 6(1):84–94

Fleißner A, Sarkar S, Jacobson DJ, Roca MG, Read ND, Glass NL (2005) The so locus is required for vegetative cell fusion and postfertilization events in *Neurospora crassa*. Eukaryot Cell 4(5):920–930

Fleißner A, Diamond S, Glass NL (2009a) The *Saccharomyces cerevisiae* PRM1 homolog in *Neurospora crassa* is involved in vegetative and sexual cell fusion events but also has postfertilization functions. Genetics 181(2):497–510

Fleißner A, Leeder AC, Roca MG, Read ND, Glass NL (2009b) Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion. Proc Natl Acad Sci USA 106 (46):19387–19392

Frendo JL, Olivier D, Cheynet V, Blond JL, Bouton O, Vidaud M, Rabreau M, Evain-Brion D, Mallet F (2003) Direct involvement of HERV-W Env glycoprotein in human trophoblast cell fusion and differentiation. Mol Cell Biol 23(10):3566–3574

Fu C, Iyer P, Herkal A, Abdullah J, Stout A, Free SJ (2011) Identification and characterization of genes required for cell-to-cell fusion in Neurospora crassa. Eukaryot Cell 10(8):1100–1109

Fu C, Ao J, Dettmann A, Seiler S, Free SJ (2014) Characterization of the Neurospora crassa cell fusion proteins, HAM-6, HAM-7, HAM-8, HAM-9, HAM-10, AMPH-1 and WHI-2. PLoS ONE 9(10), e107773

Giesbert S, Siegmund U, Schumacher J, Kokkelink L, Tudzynski P (2014) Functional analysis of BcBem1 and its interaction partners in Botrytis cinerea: impact on differentiation and virulence. PLoS ONE 9(5), e95172

Glass NL, Dementhon K (2006) Non-self recognition and programmed cell death in filamentous fungi. Curr Opin Microbiol 9(6):553–558

Glass NL, Kaneko I (2003) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 2(1):1–8

Glass NL, Kuldau GA (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. Annu Rev Phytopathol 30:201–224

Glass NL, Jacobson DJ, Shiu PK (2000) The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu Rev Genet 34:165–186

Goryachev AB, Lichius A, Wright GD, Read ND (2012) Excitable behavior can explain the "ping-pong" mode of communication between cells using the same chemoattractant. Bioessays 34:259–266

Harris MJ, Boddy L (2005) Nutrient movement and mycelial reorganization in established systems of Phanerochaete velutina, following arrival of colonized wood resources. Microb Ecol 50(2):141–151

Heiman MG, Walter P (2000) Prm1p, a pheromone-regulated multispanning membrane protein, facilitates plasma membrane fusion during yeast mating. J Cell Biol 151(3):719–730

Hickey PC, Jacobson D, Read ND, Louise Glass NL (2002) Live-cell imaging of vegetative hyphal fusion in Neurospora crassa. Fungal Genet Biol 37(1):109–119

Holmer L, Stenlid J (1993) The importance of inoculum size for the competitive ability of wood decomposing fungi. FEMS Microbiol Ecol 12:169–176

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW (2001) Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol. Appl Environ Microbiol 67(7):2982–2992

Howard RJ (1981) Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkorper, cytoskeleton and endomembranes after freeze-substitution. J Cell Sci 48:89–103

Huang G, Dougherty SD, Erdman SE (2009) Conserved WCPL and CX4C domains mediate several mating adhesin interactions in Saccharomyces cerevisiae. Genetics 182(1):173–189

Huang HT, Maruyama J, Kitamoto K (2013) Aspergillus oryzae AoSO is a novel component of stress granules upon heat stress in filamentous fungi. PLoS ONE 8(8), e72209

Hwang J, Pallas DC (2014) STRIPAK complexes: structure, biological function, and involvement in human diseases. Int J Biochem Cell Biol 47:118–148

Hyakumachi M, Ui T (1987) Non-self-anastomosing isolates of *Rhizoctonia solani* obtained from fields of sugarbeet monoculture. Trans Br Mycol Soc 89 (2):155–159

Ishikawa FH, Souza EA, Read ND, Roca MG (2010) Live-cell imaging of conidial fusion in the bean pathogen, *Colletotrichum lindemuthianum*. Fungal Biol 114(1):2–9. doi:10.1016/j.funbio.2009.11.006, S1878-6146(09)00240-2 [pii]

Ishikawa FH, Souza EA, Shoji JY, Connolly L, Freitag M, Read ND, Roca MG (2012) Heterokaryon incompatibility is suppressed following conidial anastomosis tube fusion in a fungal plant pathogen. PLoS ONE 7(2), e31175

Jacobs H, Boswell GP, Scrimgeour CM, Davidson FA, Gadd GM, Ritz K (2004) Translocation of carbon by Rhizoctonia solani in nutritionally-heterogeneous microcosms. Mycol Res 108(Pt 4): 453–462

Jedd G (2007) Natural history of the fungal hypha: how Woronin bodies support a multicellular lifestyle. In: Gadd G, Watkinson SC, Dyer PS (eds) Fungi in the environment. Cambridge University Press, Cambridge, pp 22–37

Jonkers W, Leeder AC, Ansong C, Wang Y, Yang F, Starr TL, Camp DG 2nd, Smith RD, Glass NL (2014) HAM-5 functions as a MAP kinase scaffold during cell fusion in Neurospora crassa. PLoS Genet 10(11), e1004783, PGENETICS-D-14-01795 [pii]

Jun SC, Lee SJ, Park HJ, Kang JY, Leem YE, Yang TH, Chang MH, Kim JM, Jang SH, Kim HG, Han DM, Chae KS, Jahng KY (2011) The MpkB MAP kinase plays a role in post-karyogamy processes as well as in hyphal anastomosis during sexual development in Aspergillus nidulans. J Microbiol 49(3):418–430

Kasuga T, Glass NL (2008) Dissecting colony development of *Neurospora crassa* using mRNA profiling and comparative genomics approaches. Eukaryot Cell 7(9):1549–1564

Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52(6):1781–1798

Kim H, Borkovich KA (2006) Pheromones are essential for male fertility and sufficient to direct chemotropic polarized growth of trichogynes during mating in *Neurospora crassa*. Eukaryot Cell 5 (3):544–554

Köhler E (1929) Beiträge zur Kenntnis der vegetativen anastomosen der Pilze I. Planta 8:140–153

Köhler E (1930) Zur Kenntnis der vegetativen Anastomosen der Pilze (II. Mitteilung). Planta 10:495–522

Kozubowski L, Saito K, Johnson JM, Howell AS, Zyla TR, Lew DJ (2008) Symmetry-breaking polarization driven by a Cdc42p GEF-PAK complex. Curr Biol 18(22):1719–1726

Laibach F (1928) Über Zellfusionen bei Pilzen. Planta 5:340–359

Lamson RE, Takahashi S, Winters MJ, Pryciak PM (2006) Dual role for membrane localization in yeast MAP kinase cascade activation and its contribution to signaling fidelity. Curr Biol 16 (6):618–623

Leeder AC, Palma-Guerrero J, Glass NL (2011) The social network: deciphering fungal language. Nat Rev Microbiol 9(6):440–451

Leeder AC, Jonkers W, Li J, Glass NL (2013) Early colony establishment in Neurospora crassa requires a MAP kinase regulatory network. Genetics 195(3):883–898

Leeuw T, Fourest-Lieuvin A, Wu C, Chenevert J, Clark K, Whiteway M, Thomas DY, Leberer E (1995) Pheromone response in yeast: association of Bem1p with proteins of the MAP kinase cascade and actin. Science 270(5239):1210–1213

Leu LS (1967) Anastomosis in *Venturia inaequalis* (Cke.) Wint. PhD, University of Wisconsin, Madison

Levin AM, de Vries RP, Conesa A, de Bekker C, Talon M, Menke HH, van Peij NN, Wosten HA (2007) Spatial differentiation in the vegetative mycelium of Aspergillus niger. Eukaryot Cell 6(12):2311–2322

Li R, Wedlich-Soldner R (2009) Bem1 complexes and the complexity of yeast cell polarization. Curr Biol 19(5):R194–R195; author reply R195

Li D, Bobrowicz P, Wilkinson HH, Ebbole DJ (2005) A mitogen-activated protein kinase pathway essential for mating and contributing to vegetative growth in Neurospora crassa. Genetics 170(3): 1091–1104

Lichius A, Lord KM (2014) Chemoattractive mechanisms in filamentous fungi. Open Mycol J 8(Suppl-1, M2):28–57

Lichius A, Lord KM, Jeffree CE, Oborny R, Boonyarungsrit P, Read ND (2012) Importance of MAP kinases during protoperithecial morphogenesis in Neurospora crassa. PLoS ONE 7(8), e42565

Lichius A, Goryachev AB, Fricker MD, Obara B, Castro-Longoria E, Read ND (2014) CDC-42 and RAC-1 regulate opposite chemotropisms in Neurospora crassa. J Cell Sci 127(Pt 9):1953–1965

Liu D, Novick P (2014) Bem1p contributes to secretory pathway polarization through a direct interaction with Exo70p. J Cell Biol 207(1):59–72

Loera O, Cordova J (2003) Improvement of xylanase production by a parasexual cross between Aspergillus niger strains. Braz Arch Biol Technol 46 (2):177–181

Lopez-Berges MS, Rispail N, Prados-Rosales RC, Di Pietro A (2010) A nitrogen response pathway regulates virulence functions in *Fusarium oxysporum* via the protein kinase TOR and the bZIP protein MeaB. Plant Cell 22(7):2459–2475

Maddi A, Dettman A, Fu C, Seiler S, Free SJ (2012) WSC-1 and HAM-7 are MAK-1 MAP kinase pathway sensors required for cell wall integrity and hyphal fusion in Neurospora crassa. PLoS ONE 7 (8), e42374

Maerz S, Ziv C, Vogt N, Helmstaedt K, Cohen N, Gorovits R, Yarden O, Seiler S (2008) The nuclear Dbf2-related kinase COT1 and the mitogen-activated protein kinases MAK1 and MAK2 genetically interact to regulate filamentous growth, hyphal fusion and sexual development in *Neurospora crassa*. Genetics 179(3):1313–1325

Markham P (1994) Occlusions of septal pores in filamentous fungi. Mycol Res 98(10):1089–1106

Maruyama J, Escano CS, Kitamoto K (2010) AoSO protein accumulates at the septal pore in response to various stresses in the filamentous fungus Aspergillus oryzae. Biochem Biophys Res Commun 391 (1):868–873

Matheos D, Metodiev M, Muller E, Stone D, Rose MD (2004) Pheromone-induced polarization is dependent on the Fus3p MAPK acting through the formin Bni1p. J Cell Biol 165(1):99–109

Mohler WA, Shemer G, del Campo JJ, Valansi C, Opoku-Serebuoh E, Scranton V, Assaf N, White JG, Podbilewicz B (2002) The type I membrane protein EFF-1 is essential for developmental cell fusion. Dev Cell 2(3):355–362, doi: S1534580702001296 [pii]

Nordbring-Hertz B, Friman E, Veenhuis M (1989) Hyphal fusion during initial stages of trap formation in Arthrobotrys oligospora. Antonie Van Leeuwenhoek 55(3):237–244

Nordzieke S, Zobel T, Franzel B, Wolters DA, Kuck U, Teichert I (2014) A fungal SLMAP homolog plays a fundamental role in development and localizes to the nuclear envelope, ER, and mitochondria. Eukaryot Cell. doi:10.1128/EC.00241-14

Palma-Guerrero J, Hall CR, Kowbel D, Welch J, Taylor JW, Brem RB, Glass NL (2013) Genome wide association identifies novel loci involved in fungal communication. PLoS Genet 9(8), e1003669

Palma-Guerrero J, Leeder AC, Welch J, Glass NL (2014) Identification and characterization of LFD1, a novel protein involved in membrane merger during cell fusion in Neurospora crassa. Mol Microbiol 92(1):164–182

Pandey A, Roca MG, Read ND, Glass NL (2004) Role of a mitogen-activated protein kinase pathway during conidial germination and hyphal fusion in *Neurospora crassa*. Eukaryot Cell 3(2):348–358

Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 22 (2):153–183. doi:10.1210/edrv.22.2.0428

Poggeler S, Kuck U (2004) A WD40 repeat protein regulates fungal cell differentiation and can be replaced functionally by the mammalian homologue striatin. Eukaryot Cell 3(1):232–240

Pontecorvo G (1956) The parasexual cycle in fungi. Annu Rev Microbiol 10:393–400

Prados Rosales RC, Di Pietro A (2008) Vegetative hyphal fusion is not essential for plant infection by *Fusarium oxysporum*. Eukaryot Cell 7(1): 162–171

Read ND, Fleißner A, Roca MG, Glass NL (2010) Hyphal fusion. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Washington, DC, pp 260–273

Read ND, Goryachev AB, Lichius A (2012) The mechanistic basis of self-fusion between conidial anastomosis tubes during fungal colony initiation. Fungal Biol Rev 26:1–11

Richard F, Glass NL, Pringle A (2012) Cooperation among germinating spores facilitates the growth of the fungus, Neurospora crassa. Biol Lett 8 (3):419–422

Riquelme M, Reynaga-Pena CG, Gierz G, Bartnicki-Garcia S (1998) What determines growth direction in fungal hyphae? Fungal Genet Biol 24(1–2): 101–109

Riquelme M, Yarden O, Bartnicki-Garcia S, Bowman B, Castro-Longoria E, Free SJ, Fleißner A, Freitag M, Lew RR, Mourino-Perez R, Plamann M, Rasmussen C, Richthammer C, Roberson RW, Sanchez-Leon E, Seiler S, Watters MK (2011) Architecture and development of the Neurospora crassa hypha – a model cell for polarized growth. Fungal Biol 115(6):446–474

Roca MG, Davide LC, Mendes-Costa MC, Wheals A (2003) Conidial anastomosis tubes in *Colletotrichum*. Fungal Genet Biol 40(2):138–145

Roca MG, Davide LC, Davide LM, Mendes-Costa MC, Schwan RF, Wheals AE (2004) Conidial anastomosis fusion between *Colletotrichum* species. Mycol Res 108(Pt 11):1320–1326

Roca M, Read ND, Wheals AE (2005a) Conidial anastomosis tubes in filamentous fungi. FEMS Microbiol Lett 249(2):191–198

Roca MG, Arlt J, Jeffree CE, Read ND (2005b) Cell biology of conidial anastomosis tubes in *Neurospora crassa*. Eukaryot Cell 4(5):911–919

Roca MG, Weichert M, Siegmund U, Tudzynski P, Fleißner A (2012) Germling fusion via conidial anastomosis tubes in the grey mould Botrytis cinerea requires NADPH oxidase activity. Fungal Biol 116(3):379–387

Roncal T, Cordobes S, Sterner O, Ugalde U (2002) Conidiation in Penicillium cyclopium is induced by conidiogenone, an endogenous diterpene. Eukaryot Cell 1(5):823–829

Roper M, Ellison C, Taylor JW, Glass NL (2011) Nuclear and genome dynamics in multinucleate ascomycete fungi. Curr Biol 21(18):R786–R793

Roper M, Simonin A, Hickey PC, Leeder A, Glass NL (2013) Nuclear dynamics in a fungal chimera. Proc Natl Acad Sci USA 110(32):12875–12880

Rothert W (1892) Über *Sclerotium hydrophilum Sacc.* einen sporenlosen Pilz. Botanische Zeitung 50: 358–370

Ruiz-Roldan MC, Kohli M, Roncero MI, Philippsen P, Di Pietro A, Espeso EA (2010) Nuclear dynamics

during germination, conidiation, and hyphal fusion of *Fusarium oxysporum*. Eukaryot Cell 9 (8):1216–1224

Sapir A, Choi J, Leikina E, Avinoam O, Valansi C, Chernomordik LV, Newman AP, Podbilewicz B (2007) AFF-1, a FOS-1-regulated fusogen, mediates fusion of the anchor cell in C. elegans. Dev Cell 12(5):683–698

Schurg T, Brandt U, Adis C, Fleißner A (2012) The Saccharomyces cerevisiae BEM1 homologue in Neurospora crassa promotes co-ordinated cell behaviour resulting in cell fusion. Mol Microbiol 86(2):349–366

Simon UK, Bauer R, Rioux D, Simard M, Oberwinkler F (2005) The vegetative life-cycle of the clover pathogen Cymadothea trifolii as revealed by transmission electron microscopy. Mycol Res 109(Pt 7): 764–778

Simonin AR, Rasmussen CG, Yang M, Glass NL (2010) Genes encoding a striatin-like protein (*ham-3*) and a forkhead associated protein (*ham-4*) are required for hyphal fusion in *Neurospora crassa*. Fungal Genet Biol 47(10):855–868

Simonin A, Palma-Guerrero J, Fricker M, Glass NL (2012) Physiological significance of network organization in fungi. Eukaryot Cell 11(11): 1345–1352

Takemoto D, Kamakura S, Saikia S, Becker Y, Wrenn R, Tanaka A, Sumimoto H, Scott B (2011) Polarity proteins Bem1 and Cdc24 are components of the filamentous fungal NADPH oxidase complex. Proc Natl Acad Sci USA 108(7):2861–2866

Teichert I, Steffens EK, Schnass N, Franzel B, Krisp C, Wolters DA, Kuck U (2014) PRO40 is a scaffold protein of the cell wall integrity pathway, linking the MAP kinase module to the upstream activator protein kinase C. PLoS Genet 10(9), e1004582

Trinci APJ (1984) Regulation of hyphal branching and hyphal orientation. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, UK, pp 23–52

Trinci AP, Collinge AJ (1973) Structure and plugging of septa of wild type and spreading colonial mutants of Neurospora crassa. Arch Mikrobiol 91(4):355–364

Ward H (1888) A lily disease. Ann Bot 2(7):319–382

Wei H, Requena N, Fischer R (2003) The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus Aspergillus nidulans. Mol Microbiol 47(6):1577–1588

Weichert M, Fleißner A (2015) Anastomosis and heterokaryon formation. In: Van den Berg M, Maruthachalam K (eds) Genetic transformation systems in fungi, vol 2. Springer, Heidelberg, pp 3–21

Yokoyama K, Ogoshi A (1988) Studies on hyphal anastomosis of Rhizoctonia solani V. Nutritional conditions for anastomosis. Trans Mycol Soc Japan 29:125–132

8 Molecular Control of Fungal Senescence and Longevity

Matthias Wiemer[1], Carolin Grimm[1], Heinz D. Osiewacz[1]

CONTENTS

I. Introduction

Fungi are widely thought to be immortal. This view is supported by the description of a huge vegetation body of an *Armillaria bulbosa* individual in Michigan with an estimated weight of at least 10,000 kg and a current age of approximately 1500 years (Smith et al. 1992). However, there are also species described with a restricted lifespan and defined signs of age-related degeneration. Historically, the first detailed description of such a phenotype dates back to studies of Rizet in the early 1950s (Rizet 1953a, b). He reported that, during vegetative growth, cultures of the filamentous ascomycete *Podospora anserina* change their morphology, slow down growth until it completely ceases, and the hyphae die at their tips. Only some years later Mortimer and Johnson reported that the yeast *Saccharomyces cerevisiae* does only generate a finite number of daughter cells (Mortimer and Johnston 1959). Subsequently, over the last several decades many other age-related degenerative processes have been reported in fungi (Bertrand et al. 1985, 1986; Böckelmann and Esser 1986; Chan et al. 1991; Geydan et al. 2012; Lazarus et al. 1980; Lazarus and Küntzel 1981).

The time-dependent degenerative processes in fungi comply with the general definition of **biological aging** as a progressive decline of physiological functions (Kirkwood and Austad 2000). The understanding of the underlying mechanisms is of particular interest, because this knowledge is certainly a key toward the development of interventions into degenerative processes. For instance, this could be advantageous in biotechnology to increase yields of products (Scheckhuber et al. 2009) or in medicine to intervene in **age-related disabilities** and severe **diseases** in later age. Since experimental studies of human **aging** are complicated due to the complex organization, the long lifespan, and ethics considerations, such investigations are carried out in various model systems. Importantly, these organisms are short-lived and can be genetically manipulated (Osiewacz and Scheckhuber 2006; Partridge 2011). Among these models, the filamentous ascomycete *P. anserina* and the yeast *S. cerevisiae* have been extensively studied over decades. Both systems have special characteristics that make them ideal for the analysis of different aspects of

[1]Department of Biosciences, Institute of Molecular Biosciences and Cluster of Excellence Frankfurt Macromolecular Complexes, J W Goethe University, Frankfurt 60438, Germany; e-mail: osiewacz@bio.uni-frankfurt.de

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

aging. For instance, in *P. anserina*, aging can easily be followed macroscopically, since the multicellular individual is forming clearly visible colonies on the substrate. In addition, the fungus can genetically be modified and analyzed on different levels (Esser 1974; Osiewacz et al. 2013). *S. cerevisiae* is used as a model for aging of mitotic as well as post-mitotic cells (Jazwinski 2004; Longo et al. 2012). The first, **replicative aging**, is determined by the number of daughter cells that are generated by a mother cell in the process of budding. In contrast, **chronological aging** is the time-dependent survival of cells in stationary phase.

In this treatise, we will discuss the current view of fungal senescence in particular in *P. anserina*. We do not aim for a comprehensive description of what has been analyzed over decades of work. Instead, our special emphasis lies on the description of the emerging pathways and mechanisms that lead to age-related degenerations and the pathways involved in preventing molecular damage or getting rid of it. With this focus, we will also include work from yeast and other fungi. For other aspects of fungal senescence and earlier work, the reader is referred to chapters in previous editions of this series (Osiewacz and Hamann 1994) and other reviews (Esser and Tudzynski 1980; Griffiths 1992; Jazwinski 2004; Jazwinski and Kriete 2012; Osiewacz 1990, 2002).

II. Molecular Degeneration

The time-dependent accumulation of molecular damage leading to the progressive decline of physiological functions is the key of biological aging. In particular in fungi, early work has identified well-defined genetic changes in mitochondria during senescence. The following part of this chapter will summarize main conclusions from these studies and will discuss sources of molecular damage.

A. Mitochondrial DNA Instabilities

In the pre-genetic engineering area, formal genetic analysis revealed extrachromosomal traits to be effective in aging of *P. anserina*. When juvenile and senescent strains were used in reciprocal crosses, results were obtained that did not correspond to those expected for the segregation of Mendelian factors (Marcou 1961; Marcou and Schecroun 1959).

Fertilization of protoperithecia, the female gametangia, of juvenile *P. anserina* wild-type strains with spermatia from senescent strains gave rise to juvenile progeny. In contrast, the cultures derived from the fertilization of protoperithecia from senescent cultures let to the formation of mostly senescent progeny (Marcou 1961).

Attempts to identify the corresponding traits let to the isolation of a covalently closed circular DNA which, due to its structural similarities with prokaryotic plasmids, was termed **plDNA** (Stahl et al. 1978) and subsequently, due to its accumulation in senescence cultures, **αSenDNA** (Cummings et al. 1979). Subsequent analyses revealed that, in juvenile cultures, this DNA sequence is integrated as the first intron into cytochrome c oxidase subunit I gene within the circular **mitochondrial DNA** (mtDNA) of 95 kbp in size (Esser et al. 1981; Kück et al. 1981; Osiewacz and Esser 1984; Stahl et al. 1979). During aging, this intron (pl-intron) becomes liberated and accumulates as an oligomeric series of plDNA molecules (Esser et al. 1980). Due to reintegration into the *CoxI* gene ("homing integration") and into other ectopic sites in the mtDNA, iterated plDNA copies are generated in individual mtDNA molecules (Sellem et al. 1993). These sequences lead to recombination processes and gross **mtDNA reorganizations** with a loss of larger parts of the mtDNA (Sellem et al. 1993). As a consequence, older *P. anserina* cultures are impaired in mitochondrial functions because they are lacking essential sequences coding for respiratory chain components as well as RNAs of the mitochondrial protein biosynthesis apparatus. Since *P. anserina* is an obligate aerobic organism relying on mitochondrial ATP synthesis, these cultures turn to **senescence** and finally die.

The key role of the mtDNA reorganization in aging of *P. anserina* is supported by various data. Most importantly, mutants in which the mtDNA is stabilized are long-lived or even immortal (Maas et al. 2007; Osiewacz et al.

1989; Schulte et al. 1988, 1989). Further support stems from other *Podospora*, *Neurospora*, and *Aspergillus* species in which age-related degeneration was also found to be associated with mtDNA instabilities (Bertrand et al. 1980, 1985; Böckelmann and Esser 1986; Chan et al. 1991; Kuiper et al. 1990; Lazarus et al. 1980; Lazarus and Küntzel 1981; Myers et al. 1989; Nargang et al. 1992). For instance, in *Neurospora*, **linear plasmids**, which structurally resemble transposable elements with terminal inverted repeats, have been identified to act as **mtDNA mutators** and integrate into genes of the mtDNA. The affected, mutated mtDNA molecules become suppressive by a yet unknown mechanism and lead to functional impairments of mitochondria and senescence.

The analyses of various long-lived mutants of *P. anserina* unraveled a link of fungal senescence to respiration, the oxygenic generation of ATP in mitochondria.

Oxygenic respiration occurs in the mitochondrial inner membrane were electrons derived from breakdown products of nutrients like carbohydrates are transported via four protein complexes (I–IV) to molecular oxygen. In addition, protons are transported across the inner membrane at respiratory complexes I, III, and IV. The resulting electron motive force is the driving force to generate ATP at complex V.

Different long-lived or even immortal strains of *P. anserina* have been selected in which respiration proceeds via an alternative pathway with an **alternative terminal oxidase** termed AOX (Borghouts et al. 2001; Borghouts and Osiewacz 1998; Dufour et al. 2000; Schulte et al. 1988; Stumpferl et al. 2004). This protein carries electrons from the ubiquinol pool to oxygen and bypasses complexes III and IV. Since the latter two complexes are pumping protons across the inner mitochondrial membrane, ATP production in mutants lacking these complexes is lower than in mitochondria with a standard, COX-dependent respiration. Compared to the wild type, mutant strains are growing slower and are impaired in fertility. Most strikingly, these strains are long-lived (Fig. 8.1). This phenotype links aging of *P. anserina* to one prominent aging theory, the **mitochondrial free radical theory of aging** (MFRTA) which states that reactive oxygen species (ROS) cause the accumulation of molecular damage during aging and finally lead to death of the system (Harman 1956).

Free radicals are molecules or ions with unpaired valence electrons or an open electron shell, which renders them highly reactive. **Reactive oxygen species** (ROS) are defined as chemically reactive molecules containing oxygen. Therefore radicals that are composed of an oxygen molecule, like hydroxyl radicals ($HO\cdot$) belong to the group of ROS. On the other hand, hydrogen peroxide (H_2O_2) does not belong to the group of free radicals, but since it contains oxygen and is highly reactive, it is a ROS.

B. Generation and Function of Reactive Oxygen Species

The primary cellular ROS is the **superoxide anion** ($O_2\cdot^-$) which is not a strong oxidant, but able to oxidize iron-sulfur clusters and thereby inactivate proteins and release iron (Costa and Moradas-Ferreira 2001). A major portion of superoxide results from the transfer of a single electron to molecular oxygen at complex I and complex III of the mitochondrial respiratory chain (Dröse and Brandt 2008; Turrens and Boveris 1980). At complex III superoxide is released to the intermembrane space and the mitochondrial matrix, while superoxide generated by complex I is only released to the matrix. Other pathways and sites of superoxide generation are lipid metabolism in peroxisomes (Kawalek et al. 2013; Titorenko and Terlecky 2011), external NADH dehydrogenase (Fang and Beattie 2003), NADPH oxidase (Tudzynski et al. 2012), and glycerophosphate dehydrogenase (Drahota et al. 2002). Although superoxide is not able to cross pure lipid bilayers, it can cross the outer mitochondrial membrane via anion channels (Han et al. 2003). In contrast, the major portion of mitochondrial superoxide, which is produced at the matrix site of the inner membrane, cannot exit mitochondria. However, superoxide can be converted to **hydrogen peroxide** (H_2O_2), which is able to cross membranes. The efficiency of this transport depends on the composition of the membrane bilayer (Antunes and Cadenas 2000; Bienert et al. 2006; Branco et al. 2004;

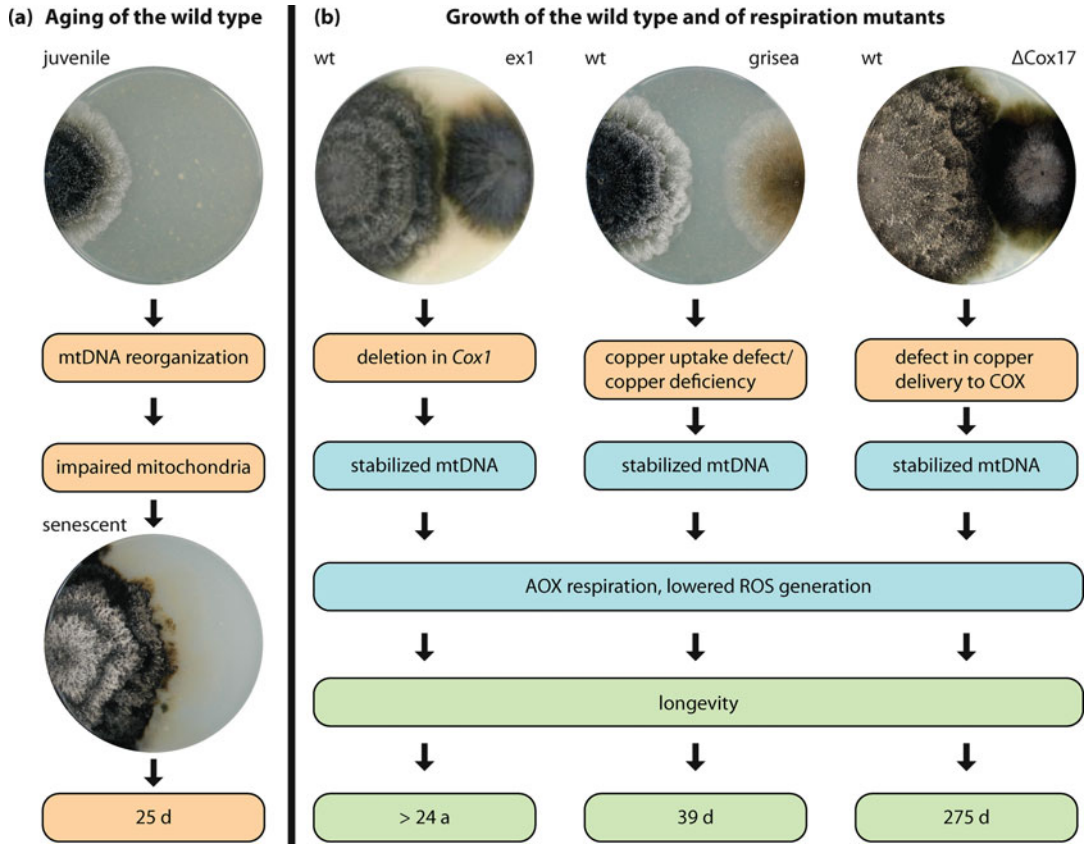


Fig. 8.1 Aging of the wild type and phenotypes of respiration mutants of *P. anserina*. (**a**) Juvenile and senescent culture of wild-type "s" of *P. anserina*. During aging, gross mtDNA reorganization is observed and functionally impaired mitochondria accumulate. The senescent culture dies at the hyphal tips. (**b**) Growth of respiratory mutants ex1, grisea, and ΔCox17. Each strain was inoculated for the same time as the wild type on the corresponding agar plate. In comparison to the wild type, the growth rate of the mutants is reduced, while lifespan is increased

Sousa-Lopes et al. 2004). Hydrogen peroxide is a weak oxidant, capable of inactivating enzymes with essential cysteines via the oxidation of their thiol groups. Since superoxide and hydrogen peroxide have a low reactivity at physiological concentrations, it is believed that their toxicity results from their conversion into the very reactive **hydroxyl radical** (HO·) which is a powerful oxidant reacting with proteins, lipids, and DNA at diffusion limit rates (Beauchamp and Fridovich 1970; Keyer et al. 1995). Hydroxyl radical generation from superoxide and hydrogen peroxide is catalyzed by metal ions (e.g., iron and copper) in the **Fenton reaction** (McCord and Day 1978). No detoxification enzyme exists for the hydroxyl radical.

Overall ROS can cause the oxidation of all kinds of biomolecules. Oxidatively modified proteins are characterized by temperature instability, increased hydrophobicity, decreased enzyme activity, and high susceptibility to proteolytic degradation (Baraibar et al. 2013; Levine et al. 1990). One type of **protein modification** is the irreversible formation of **carbonyl derivatives**, which are widely used to measure the extend of ROS-mediated damage (Stadtman and Berlett 1998). Utilizing immunological analyses techniques (i.e., "oxy blot analysis"), an age-dependent general bulk increase in **carbonylated proteins** is observed in some biological systems (Cabiscol et al. 2014; Jakubowski et al. 2000; Yasuda et al. 1999). However, utilization of

more specific methods like 2D electrophoresis and mass spectrometry, which allow the quantitative monitoring of carbonylated proteins at the proteome level, revealed that protein oxidation is more controlled than formerly believed and can lead to oxidation of specific key enzymes (Baraibar et al. 2013). Another kind of protein damage that can be amplified by oxidative stress is the reaction of proteins with reducing sugars or aldehydes like glyoxal or methyl glyoxal based on the Maillard reaction (Baynes 2001). This reaction causes the irreversible formation of **advanced glycation end products** (AGEs) which accumulate during aging and are considered to play a key role in aging and disease (Buée et al. 2000; da Cunha et al. 2011; Gerstbrein et al. 2005; Jeanmaire et al. 2001).

The deleterious effect of excess ROS in biological systems is impressively demonstrated in mutants of *P. anserina* in which, due to a reduction of ROS generation, a strong increase in lifespan is observed. Examples are the various mutants of *P. anserina* which respire via the alternative respiratory pathway. In one of these mutants, grisea, superoxide generation at the respiratory chain has been determined to be approximately 4-fold reduced and mitochondrial energy transduction is reduced 2.5-fold. Moreover, mutant grisea, which is a copper-uptake mutant, and other mutants of the cellular copper distribution pathway (Borghouts et al. 2002; Osiewacz and Stumpferl 2001; Stumpferl et al. 2004) also link the generation of ROS to aging and point to the importance of a regulated **copper metabolism** from uptake to the delivery to the enzymes, which require the metal for function. Unbalanced copper levels increase the risk of hydroxyl radical generation via Fenton chemistry. This topic has been extensively described in a chapter of the previous edition of Mycota I (Osiewacz and Hamann 1994).

Apart from their detrimental effect, ROS are also essential molecules in intracellular and extracellular **signaling**. This function is in particular relevant for proper **fungal differentiation** and **development** (Brun et al. 2009; Cano-Domínguez et al. 2008; Malagnac et al. 2004), recognition of other fungi (Silar 2005), and **host-pathogen interactions** (Heller and Tudzynski 2011). In *S. cerevisiae*, a response to hydrogen peroxide is a good example for the regulatory functions of hydrogen peroxide.

ScORP1 is a sensor for ROS and can be oxidized by H_2O_2 (Delaunay et al. 2002). Oxidized ScORP1 and the transcription factor ScYAP1 subsequently interact in the cytoplasm, which leads to the oxidation of ScYAP1 (Ma et al. 2007). Consecutive interaction of ScYAP1 with oxidized ScORP1 leads to the formation of two intracellular disulfide bonds in ScYAP1 (Delaunay et al. 2000). This conformational change masks the nuclear export signal of ScYAP1 and results in the nuclear localization of ScYAP1. Transcription of ScYAP1 target genes, including the peroxidase ScTRX2, is the consequence. The nuclear export of ScYAP1 is restored by reduction of the disulfide bonds by ScTRX2 (Marinho et al. 2014). *ScYap1* deletion strains are concordantly sensitive to H_2O_2 and paraquat (Schnell et al. 1992) and characterized by a reduced replicative lifespan (Kruegel et al. 2011). Chronological lifespan in *ScYap1* deletion strain is surprisingly unchanged or even prolonged, depending on the analyzed strain and the used carbon source, indicating that *ScYap1*-mediated gene induction is not advantageous under certain conditions and resources are better utilized for other surveillance pathways (Piper et al. 2006). The YAP1 homolog of the fungal pathogen *Aspergillus fumigatus* is required for defense against ROS (Lessing et al. 2007) and for protection against neutrophils during pathogenesis (Leal et al. 2012). Other studies showed that AfYAP1 is not necessary for virulence in a murine model (Kniemeyer et al. 2009). Ablation of the YAP1 homolog in *Aspergillus ochraceus* leads to disturbance in the redox balance that induces the synthesis of secondary metabolites (Reverberi et al. 2012), emphasizing the importance of YAP1 also in these filamentous fungi.

ROS have also been shown to be essential for fungal development. NADPH oxidase (NOX) enzymes localized in the cellular outer membrane have the exclusive function of generating superoxide by reduction of NADPH and are thereby involved in signaling (Lambeth 2004). For instance, a NOX-dependent effect has been shown for fruiting body formation in

Sordaria macrospora and *P. anserina* (Dirschnabel et al. 2014; Malagnac et al. 2004). Moreover, deletion of *Nox1* in *S. macrospora* and *Epichloë festucae* was found to result in defective hyphal fusion, a process depending on **ROS signaling** (Dirschnabel et al. 2014; Kayano et al. 2013). The defects in these mutants are attributed to differential expression of genes involved in cytoskeleton remodeling and hyphal fusion (Dirschnabel et al. 2014).

ROS signaling is also linked to the effect of **caloric restriction** (CR) which has been demonstrated to lead to lifespan extension in various biological systems including *P. anserina* and *S. cerevisiae*. For instance, in yeast, it was found that the inactivation of catalase or caloric restriction elevates the hydrogen peroxide abundance, leading to an activation of superoxide dismutase and to an extension of the chronological lifespan (Mesquita et al. 2010). This effect is an example for **hormesis**, which is a positive response to the exposure to low levels of toxins or stress. The induction of hormesis resulting from ROS with mitochondrial origin is termed **mitohormesis** (Tapia 2006). Longevity via hormesis is caused by signaling pathways that enhance the production of **ROS scavenging** and repair enzymes (Pan et al. 2011). Such a response can be induced by several stimuli like CR (Mesquita et al. 2010; Smith et al. 2007) or toxins (Antonenko et al. 2011; Wiemer and Osiewacz 2014b). In particular, the lifespan prolonging effect resulting from CR is conserved from fungi to mammals (Testa et al. 2014; van Diepeningen et al. 2010). CR has been shown to extend yeast chronological and replicative lifespan about 20–100 %, depending on the strain, when the glucose concentration was lowered from 2 to 0.5 % (Kaeberlein et al. 2004; Kennedy et al. 2007; Lin et al. 2000). CR in *P. anserina* was also reported to lead to lifespan extension. On average, lifespan was found to be doubled in 0.2 % glucose and 5 times prolonged in 0.02 % glucose compared to control medium with 2 % glucose. In one case, a lifespan extension was observed from 15 days to 3.5 years (van Diepeningen et al. 2010). While the growth rate and reproductive lifespan of *P. anserina* strains under CR are increased, the mycelium is less dense and less pigmented, pointing toward an adaption to search for more nutrients (van Diepeningen et al. 2010). For CR, it has been proposed that energetic deficits induce mitochondrial activity to counteract energy depletion, which in turn increases the level of ROS followed by the induction of antioxidant defense, leading to increased stress resistance (Ristow and Schmeisser 2011).

In order to avoid the negative effects of CR (e.g., fastening and unwanted weight reduction), substances are tested which mimic the effect of CR. These **CR mimetics** induce pathways that are active under CR conditions (Madeo et al. 2014). A number of compounds with lifespan extending properties are known. **Resveratrol** is one of them. This polyphenol is found in high concentrations in red wine and extends the lifespan of yeast, flies, worms, and fish (Guerrero et al. 2009). It also extends the life and health span of mice on a high-fat, high-sugar diet, but not of mice on a normal diet (Pearson et al. 2008). The lifespan extension resulting from resveratrol treatment is caused by an induction of autophagy (Morselli et al. 2010).

III. Counteracting the Accumulation of Molecular Degeneration

Biological systems contain a number of cellular pathways counteracting the accumulation of molecular damage at different levels. These pathways are linked to each other and represent a hierarchical network. In the following parts of this chapter, we will introduce and discuss these pathways and their role in fungal senescence in some detail.

A. ROS Scavenging

Cellular ROS abundance is dependent on the generation (see above) and the scavenging of these molecules. A number of nonenzymatic and enzymatic components constitute a network of ROS scavenging reactions (Fig. 8.2).

The primary cellular ROS **superoxide**, due to its negative charge, is basically not membrane permeable and therefore restricted to the site of generation. Since superoxide is generated at dif-

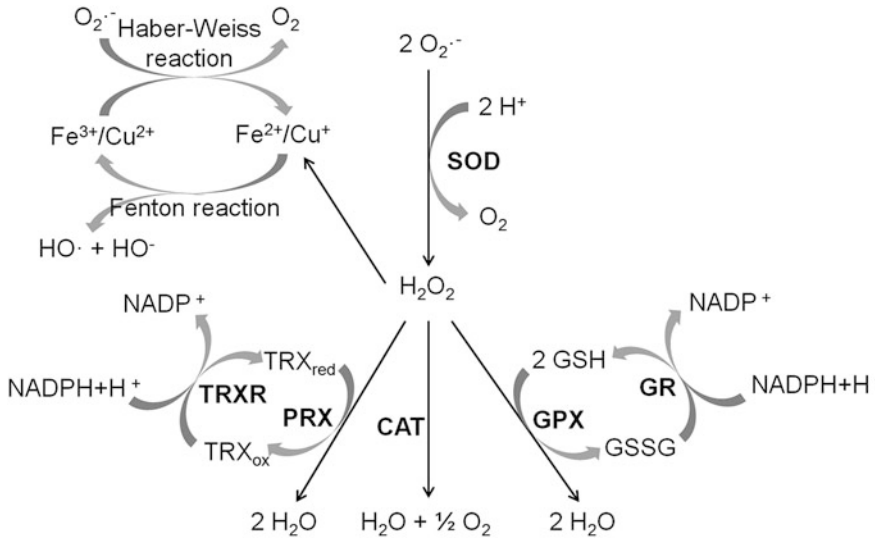


Fig. 8.2 ROS detoxification network. Balancing of cellular ROS abundance is performed by a network of reactions in which different enzymes participate. The first line of ROS defense is the dismutation of superoxide to hydrogen peroxide catalyzed by superoxide dismutases (SODs). The resulting hydrogen peroxide can be converted to water by, e.g., catalases (CAT), members of the peroxiredoxin family (Prx) or peroxidases. The most common representative of peroxidases is the glutathione peroxidase (GPX). But members of the peroxiredoxin family, as well as peroxidases, require further redox-active components like glutathione (GSH) to fulfill their catalytic cycle. If hydrogen peroxide is not efficiently degraded, it can also serve as basis for the Fenton and Haber-Weiss reaction in the presence of Fe^{2+} or Cu^{+} leading to the highly reactive hydroxyl radical. GR, glutathione reductase; GSSG, glutathione disulfide; TRXR, thioredoxin reductase; TRX_{ox}, oxidized thioredoxin; TRX_{red}, reduced thioredoxin

ferent cellular sites, it is necessary that superoxide-converting enzymes also localize to different cellular compartments. These enzymes are different isoforms of **superoxide dismutases** (SODs) which convert superoxide into molecular oxygen and hydrogen peroxide. Three types of SODs can be distinguished by their localization: a cytoplasmic (SOD1), a mitochondrial (SOD2), and an extracellular isoform (SOD3 or EC-SOD). Unfortunately, the nomenclature of the different SOD isoforms is not fully consistent between species. For example, in the ascomycete *P. anserina*, initially two SODs have been identified in total protein extracts and were termed PaSOD1 and PaSOD2 (Borghouts et al. 2001). Afterwards a third manganese-dependent SOD (PaSOD3) was found in mitochondria, which, due to low abundance in total proteins, was not recognized in the early studies. Microscopic analyses proved that PaSOD1 is the cytoplasmic SOD while PaSOD2 co-localizes with the endoplasmic reticulum and therefore appears to be a secreted MnSOD (Zintel et al. 2010). Beside the predominant localization of SOD1 in the cytoplasm, a small fraction of this SOD isoform is located in the mitochondrial intermembrane space or in the nucleus (Sturtz et al. 2001; Tsang et al. 2014; Zintel et al. 2010). Apart from their localization, SODs are characterized by the cofactor they require for catalytic activity. The cytoplasmic and the extracellular SODs usually bind copper and zinc (CuZnSODs) in their active center, while mitochondrial SODs are manganese-dependent (MnSODs). Besides the mitochondrial MnSOD, a secreted MnSOD has been found in the ericoid mycorrhiza species *Oidiodendron maius* as well as in *P. anserina* (Martino et al. 2002; Zintel et al. 2010). Furthermore, iron (FeSOD)- and nickel (NiSOD)-dependent SODs are known from prokaryotes (Culotta et al. 2006; Yost and Fridovich 1973; Youn et al. 1996). Recently, a copper-only extracellular SOD has been identified to be importantly involved in pathogen-host interactions of

the pathogenic fungus *Candida albicans* (Gleason et al. 2014). A potential gene encoding such an enzyme is also encoded in the genome of *P. anserina* but not analyzed so far.

The **dismutation** of superoxide by SODs results in the detoxification of this free radical. However, the reaction product is another ROS, namely, hydrogen peroxide. Although hydrogen peroxide is not a radical, it is the basis for production of the highly reactive hydroxyl radical in the **Fenton** and **Haber-Weiss reaction** as mentioned above. Since no enzymes are able to detoxify the hydroxyl radical, an efficient conversion of hydrogen peroxide to harmless water is indispensable to avoid access molecular damaging. Hydrogen peroxide detoxification is achieved by a variety of enzymes. The first class of hydrogen peroxide-degrading enzymes are **catalases** (CAT) which can be further divided into subclasses of monofunctional heme-catalases, bifunctional catalase-peroxidases, and non-heme manganese catalases. The latter are only known from bacteria (Vlasits et al. 2010; Zamocky et al. 2012). Catalases convert two molecules of hydrogen peroxide into molecular oxygen and two molecules of water. While catalases can only disproportionate hydrogen peroxide, peroxidases and members of the peroxiredoxin family can also use hydroperoxides and peroxynitrite as substrate. Peroxidases, the second class of hydrogen peroxide-detoxifying enzymes, convert their substrate along with a reduced donor, resulting in an oxidized donor and water. The most common representative of this class is the selenocysteine-containing **glutathione peroxidase** (GPx). Other peroxidases are NADH-peroxidases, haloperoxidases, or cytochrome c peroxidases.

Peroxidases and members of the **peroxiredoxin family** can convert hydrogen peroxide, organic peroxides and peroxynitrite together with a reduced donor resulting in the emergence of water, alcohol and nitrite, respectively, and an oxidized donor. Glutathione-peroxidases couple the reduction of their substrate to water with the oxidation of glutathione (GSH) to glutathione-disulfide (GSSG). A complex interplay of enzymes and cofactors is required to regenerate the oxidized glutathione for the next catalytic cycle (Weydert and Cullen 2010).

The third class of enzymes involved in hydrogen peroxide detoxification (also hydroperoxides and peroxynitrite) by simultaneously oxidating their co-substrates are proteins of the **peroxiredoxin family** (PRX). The PRXs are thiol-specific enzymes that exhibit a low-efficiency peroxidase activity (Hofmann et al. 2002), which is dependent on an active-site cysteine, called peroxidatic cysteine. Therefore these enzymes are also termed thioredoxin peroxidases and are specified as typical 2-Cys PRXs, atypical 2-Cys PRXs, and 1-Cys PRXs. Although the first reaction step of the different peroxyredoxins is the same, the subsequent mechanism of recycling varies.

In a first reaction step, the peroxidatic cysteine of the peroxiredoxin attacks the peroxide and is subsequently oxidized itself. For the typical and the atypical 2-Cys PRXs the next step is the formation of a stable disulfide bond of the oxidized cysteine with the remaining cysteine. To complete their catalytic cycle the resulting PRX needs to be reduced by a disulfide oxidoreductase. Since the 1-Cys PRXs contain only a single cysteine their peroxidatic cysteine is reduced by a donor which might be thiol- or glutathione-containing (Wood et al. 2003).

In addition to enzymes, small **nonenzymatic antioxidants** are active in ROS scavenging. Examples are vitamin C (Georgiou and Petropoulou 2001) or vitamin E (Emri et al. 2004), proline (Chen and Dickman 2005), carotenoids (Mandelli et al. 2012), flavonoids (Cos et al. 1998), polyamines (Valdes-Santiago and Ruiz-Herrera 2013), lipoic acid (Spalding and Prigge 2010), resveratrol (Jarolim et al. 2004), and many other antioxidants with the potential of quenching different free radicals. Beside its role in the enzymatic detoxification of ROS by glutathione peroxidase, glutathione itself can also act as an antioxidant due to the exposed sulfhydryl group that serves as ROS target (Jiang et al. 2015). Altogether, antioxidants are effective in lowering overall oxidative stress, reducing DNA damage or lipid peroxidation (auf dem Keller et al. 2006).

Due to the unique function of SODs in the disproportion of superoxide as the primary cellular ROS, much attention was paid to this class

of enzymes. Unfortunately, the results from studies in different systems did not clarify the precise effects of SODs on aging. In some investigations, results were obtained which are well compatible with expectations, while other studies led to unexpected and counterintuitive results. For instance, in *S. cerevisiae*, a deletion of the single mitochondrial *ScSod2* resulted in an expected shortened chronological and replicative lifespan (Longo et al. 1999; Unlu and Koc 2007). While the overexpression of the cytoplasmic and the mitochondrial *SOD*s led to the expected increase of the chronological lifespan and to an increased resistance to oxidative stress, a counterintuitive decrease in replicative lifespan was observed (Fabrizio et al. 2004). Also unexpectedly, in *P. anserina*, the deletion of the cytoplasmic or the mitochondrial SOD did not affect lifespan, while a deletion of the gene coding for the secreted PaSOD2 resulted in a moderate extension of the median lifespan. Moreover, overexpression of the gene coding for mitochondrial PaSOD3 led to a drastic shortened median and maximum lifespan of the corresponding strains. Subsequent analyses suggested that the latter effects result from increased hydrogen peroxide abundance arising from the elevated activity of PaSOD3 (Kowald et al. 2012) giving rise to damage of important proteins. For PaSOD3 overexpressors, an altered protein amount and pattern could be shown for the mitochondrial proteases PaLON and PaCLPP, the heat shock protein 60 (PaHSP60), and the hydrogen peroxide-detoxifying enzyme peroxiredoxin (PaPRX) (Zintel et al. 2010). A very recent study validated the suggestions from a computational model and found that the shortened lifespan of *PaSod3* overexpressors can be reverted to wild-type lifespan via the induction of a hydrogen peroxide detoxification system by manganese (Grimm and Osiewacz 2015).

In another study, the secreted catalase PaCATB was modified in abundance in *P. anserina*. The deletion resulted in the expected decreased resistance and the overexpression in an increased resistance for hydrogen peroxide. While the lifespans of these mutant strains were unaffected under standard growth conditions, cultivation on hydrogen peroxide-containing medium led to an increased and shortened lifespan for strains with an overexpression and deletion, respectively (Zintel et al. 2011). Also **thioredoxins** (Trxs), small proteins possessing a redox-active site, play an important role in protection against oxidative stress by the reduction of protein disulfide bonds. Together with NADPH, thioredoxins serve as hydrogen donors. *S. cerevisiae* encodes two cytoplasmic (*ScTRX1*, *ScTRX2*) and one mitochondrial TRX (*ScTrx3*). A single deletion of either *ScTrx1* or *ScTrx2* has no effect on morphology and cell growth, while Δ*ScTrx2* mutants are characterized by an elevated sensitivity and overexpressors of *ScTrx1* or *ScTrx2* by an elevated resistance to hydrogen peroxide (Garrido and Grant 2002). Besides, a deletion of both cytoplasmic thioredoxins affected the cell cycle (Muller 1991). *P. anserina* encodes a total of five thioredoxins whereof none is located in mitochondria. *PaTRX1*, the major thioredoxin, and *PaTRX3* were shown to possess a role in sexual reproduction. Additionally, Δ*PaTrx1* mutants lived longer than the wild type which could not be demonstrated for strains with a deletion of *PaTrx2* or *PaTrx3* (Malagnac et al. 2007). For *A. nidulans*, an elevated catalase activity and a reduced maturation of fruiting bodies have been observed in *AnTrxA* deletion strains (Thön et al. 2007). An additional Enzyme, known to be involved in the ROS-associated aging, is the mitochondrial methyltransferase MTH1 in *P. anserina*. A deletion of the corresponding gene results in a shortened lifespan of the fungus due to an impaired protection against ROS. The overexpression of *PaMth1* exhibited a better growth rate on hydrogen peroxide-containing medium than the wild type and a decreased level of oxidatively damaged proteins measured by protein carbonylation (Kunstmann and Osiewacz 2009).

Overall it is clear that the ROS scavenging system is very complex. Interventions into this system lead to multiple responses. This is not surprising because ROS do affect the function of various cellular components and in addition are active in signaling. Unraveling the role of the active molecular pathways and their impact on aging can only be successful if studies can be performed in a more holistic way, a way as it is followed in **Systems Biology**.

B. Repair and Degradation of Molecular Damage

ROS scavengers and antioxidants are able to lower the cellular abundance of ROS. However, since the system is limited in capacity, there will always be residual amounts of ROS in respiring organisms. Consequently, although the ROS-dependent processes can be slowed down, the accumulation of oxidative damage over time cannot be completely avoided. Cellular **repair** and **degradation processes** deal with this situation. In general, the repair of a damaged cell component is energetically more favorable than their degradation and **resynthesis**. Importantly, a complete degradation of damaged DNA is not possible, because this molecule represents the genetic information, the "blueprint," of a biological system that can only be produced from existing copies, while proteins can be resynthesized by **protein biosynthesis**. Therefore, DNA repair is very important for survival of biological systems, and since different types of DNA damage occur, a number of repair pathways are active. In contrast, **protein quality control** predominantly is controlled by degradation and remodeling pathways.

DNA repair pathways are highly conserved among species, stressing their importance in maintaining DNA stability and genome integrity (Aravind et al. 1999). **Nucleotide excision repair** (NER), **base excision repair** (BER) and **DNA mismatch repair** (MMR) pathways are the major systems for DNA repair. BER and NER detect damaged and oxidized nucleotides, while MMR substitutes misplaced nucleotides in newly synthesized DNA. NER is active in the nucleus. The NER pathway is capable of detecting and repairing bulky DNA lesions caused by ultraviolet light, but is also involved in repair of oxidatively damaged DNA. To induce NER, damaged DNA is first recognized by initial factors of transcription coupled repair or global genome repair. The DNA helix is unwound for the repair and incisions are made to remove a stretch of the damaged strain. Subsequent DNA synthesis and ligation reproduce the intact double strand (Melis et al. 2013).

The base excision repair pathway is active in the nucleus and in mitochondria, the major site of ROS generation, and is therefore particularly important during aging. BER is the principle pathway to remove 8-oxo-2'deoxyguanosine, the oxidized form of deoxyguanosine. Initiation begins with the recognition of oxidatively modified DNA bases by a glycohydrolase. The damaged base or a longer patch of bases are subsequently removed from the DNA backbone. This is followed by DNA incision by an apurinic endonuclease that results in a single strand break. The backbone is subsequently removed, which renders the site accessible to a DNA polymerase, that reinserts the missing nucleotides. The final ligation to complete the repair is realized by a DNA ligase (Bohr 2002; Liu et al. 2007).

The **oxidation of DNA** can lead to various forms of modifications. A prominent example is the oxidation of deoxyguanosine to 8-oxo-2'deoxyguanosine (8-oxo-dG). This type of oxidation occurs in the nucleus and in mitochondria and is 10 times more common in mtDNA (Nakamoto et al. 2007). The damaged deoxyguanosine can mispair with adenine during replication and transcription (Cheng et al. 1992). This leads to mutations if the mismatch is not repaired or recognized during replication or transcription (Sastre-Moreno et al. 2014). The portion of 8-oxo-dG in nucleus and mitochondria increases in senescent individuals, which is conserved from yeast to mammals (Gan et al. 2012; Hudson et al. 1998; Shigenaga et al. 1994), indicating an impaired repair mechanism during aging (Gredilla et al. 2012). Genetic manipulations of DNA repair pathways have subsequently been analyzed for their influence on lifespan.

Deletion of the genes *ScRad1* and *ScRad7* involved in NER in yeast do not have an effect on the replicative lifespan, suggesting that other pathways are able to compensate the impaired NER under laboratory conditions (Park et al. 1999). In contrast, the deletion of *ScRad2*, another gene involved in NER, results in decreased chronological lifespan (Marek and Korona 2013) indicating the importance of NER for survival during stress conditions that cannot be compensated completely. A defect of NER in humans is the cause of the **Cockayne syndrome** (Lehmann 1982). It is a congenital disorder characterized by impairments in growth and development and by **premature aging** (Cockayne 1936).

There are several reports of reduced chronological lifespan in different yeast deletion strains that have a defect in BER (Maclean et al. 2003; Marek and Korona 2013). For

instance, loss of glycosylases ScNTG1 and ScOGG1 results in reduced chronological lifespan, while single deletions have a normal lifespan, indicating a redundancy of these proteins (Maclean et al. 2003). The deletion of the AP endonucleases ScAPN2 and ScAPN1 has a similar effect (Maclean et al. 2003).

Poly(ADP-ribose) polymerase (PARP) is an enzyme associated with double- and single-strand repair and BER. Deletion of the homolog in *Neurospora crassa* results in an early onset of senescence (Kothe et al. 2010). Overexpression of *PaParp* in *P. anserina* has a negative effect on lifespan and paraquat resistance (Müller-Ohldach et al. 2011). This can be attributed to the increased PaPARP activity that leads to NADPH consumption and hence a depletion of ATP, which results in an **energetic crisis** (Müller-Ohldach et al. 2011). Wild-type strains of *P. anserina* show a decline of BER during aging (Soerensen et al. 2009). This may be linked to the mtDNA instability observed during aging of *P. anserina* (Borghouts et al. 1997; Scheckhuber and Osiewacz 2008). Taken together, although DNA repair in filamentous fungi is not so extensively investigated as in mammalian systems, the existing data indicate that regulated DNA repair is also important in this group of organisms to counteract degenerative processes.

Most **oxidative protein modifications** are irreversible and cannot be repaired. The exception is oxidative damage on sulfur-containing amino acids. Sulfur-containing amino acids can frequently be modified by weak oxidants. Thioredoxin and glutaredoxin reductases can in turn reverse the oxidations of disulfide bridges and cysteine sulfinic acids. **Methionine sulfoxide reductases** catalyze the reduction of oxidized methionine sulfoxide (Friguet 2006). Deletion of the gene coding for the methionine sulfoxide reductase ScMsrA in *S. cerevisiae* results in a 26 % shortened chronological lifespan, while *MsrA* overexpression increases the lifespan by 26 % (Koc et al. 2004). Ablation of both methionine sulfoxide reductases, *ScMSRA* and *ScMSRB*, decreases the lifespan even further than single deletion (Koc et al. 2004). The deletion of the methionine sulfoxide reductase gene *ScfRMsr* leads to a 20 % reduction in replicative lifespan (Le et al. 2009). Oxidations of other amino acids are irreversible. The oxidation of proteins can intervene with their stability and functionality and thereby lead to negative effects. Hence, it is important to identify, degrade, and resynthesize damaged proteins. Various systems are in place for this purpose to ensure the quality of the proteome.

The **ubiquitin proteasome system** (UPS) is the major system for protein degradation in the cytosol. It comprises a large number of different ubiquitin ligases that act jointly with the proteasome, a multi-protein complex with proteolytic activities. Ubiquitin ligases identify and mark proteins for removal. This modification occurs by formation of chains of ubiquitin on the target protein. Previous studies revealed that aging reduces the expression and the activity of the **proteasome** in several model systems (Carrard et al. 2002; Chondrogianni et al. 2000; Chondrogianni and Gonos 2005). High proteasome activity, in contrast, has a lifespan prolonging effect in several organisms. For example, the proteasome activity is elevated in human fibroblast cell cultures derived from centenarians (Chondrogianni et al. 2000) and in the liver of the naked mole rat (Perez et al. 2009; Rodriguez et al. 2014), a long-living rodent. The protein UMP1 is necessary for the assembly of the proteasome and *S. cerevisiae* overexpressing *ScUmp1* show increased lifespan and viability in response to oxidative stress (Chen et al. 2006). The transcription factor ScRPN4 is a positive regulator of proteasome genes. It is rapidly ubiquitinated and degraded by the proteasome under non-stress conditions. Increased abundance of ScRPN4 results in enhanced UPS capacity and resistance to proteotoxic stress and in a prolonged replicative lifespan (Kruegel et al. 2011). The role of the proteasome in filamentous fungi is hardly investigated at all. Proteasome inhibitor MG132 leads to an upregulation of proteasome subunits in *Trichoderma reesei* indicating a demand-dependent regulation (Kautto et al. 2013). Aging, on the other hand, does not affect the expression and protein abundance of selected 20S subunits in *P. anserina* (Wiemer and Osiewacz 2014a). Overall this indicates that high proteasome activity ensures a healthy

proteome and increases the healthy lifespan in mammals and yeast but may not be achieved in all biological systems.

Besides degradation of cytosolic proteins, the proteasome is able to degrade proteins from the endoplasmic reticulum and from mitochondria. The protein quality control in mitochondria is particularly important during aging, since a major fraction of ROS is generated in this organelle. A form of degradation of mitochondrial proteins described for *S. cerevisiae*, **mitochondria-associated protein degradation** (MAD), concordantly was reported to affect aging. MAD is dependent on VMS1, which translocates to mitochondria in response to stress conditions and promotes the recruitment of the UPS components CDC48 and NPL4 to mitochondria where they enable protein degradation via the proteasome. Matrix and inner membrane proteins may be retro-translocated to the outer membrane for ubiquitination and degradation (Taylor and Rutter 2011). A *S. cerevisiae ScVms1* deletion strain was found to accumulate ubiquitinated proteins at mitochondria. The strain is impaired in respiration and shows increased mitochondrial oxidative stress, increased protein oxidation, and a reduced chronological lifespan (Heo et al. 2010). Mitophagy is enhanced in this mutant, indicating transfer to a different protein quality control system. *Vms1* is highly conserved with orthologs in most eukaryotic species. A knockdown in *C. elegans* also results in a decreased lifespan (Heo et al. 2010).

The serine proteases LON and CLPXP, located in the mitochondrial matrix, are involved in degradation of mitochondrial proteins. **LON** is a homo-oligomeric protease and is probably the most important protein for degradation of damaged proteins in the mitochondrial matrix. Selectivity for damaged proteins was shown for PIM1, the LON homolog in *S. cerevisiae* (Major et al. 2006). Senescent *S. cerevisiae* have a decreased PIM1 activity accompanied by elevated levels of oxidized and aggregated proteins (Erjavec et al. 2013). The deletion of PIM1 in *S. cerevisiae* concordantly increases the amount of protein aggregates in mitochondria and shortens the replicative lifespan (Bender et al. 2011). High abundance of LON in *P. anserina* was consistently found to lead to low levels of oxidized mitochondrial proteins and an extended lifespan (Luce and Osiewacz 2009). In concordance, deletion of *PaLon* resulted in decreased lifespan, retarded growth at low and high temperatures, and defects in ascospore germination and sexual reproduction (Adam et al. 2012). These data identify LON as a positive factor in the regulation of lifespan that functions by degradation of proteins and prevention of **protein aggregation**, which is particularly important during temperature stress.

The mitochondrial protease **CLPP** is a protein complex consisting of two heptameric rings with the proteolytic sites facing the inner chamber. The structure of CLPP is conserved from bacteria to human, but *S. cerevisiae* does not have a CLPP homolog. CLPX forms a hexameric ring-shaped structure, interacting with the CLPP complex, and is responsible for substrate specificity, ATP-dependent unfolding, and transport of the substrate into the cylinder. The function of the assembled **CLPXP complex** in eukaryotes is poorly understood. CLPXP is thought to be a central part of mitochondrial **unfolded protein response** during which *ClpP* expression increases (Lionaki and Tavernarakis 2013). Deletion of *PaClpP* in *P. anserina* unexpectedly leads to an extension of lifespan (Fischer et al. 2013).

The ATP-dependent **i-AAA** and **m-AAA proteases** are located in the mitochondrial inner membrane. The i-AAA protease is facing the mitochondrial intermembrane space, while the m-AAA protease faces the matrix. Both complexes are associated with the extraction and degradation of improper folded membrane proteins at their respective site of the inner membrane (Baker et al. 2012). The simultaneous ablation of the two complexes in *S. cerevisiae* is lethal (Leonhard et al. 2000). Deletion of m-AAA protease alone causes respiratory deficiency (Arlt et al. 1998). The protease is involved in defense against ROS by processing cytochrome c peroxidase (Suppanz et al. 2009) and in mitochondrial dynamics by processing of MGM1 (Duvezin-Caubet et al. 2007). Deletion of the gene coding for the catalytic i-AAA homolog *ScYME1* in *S. cerevisiae* results in respiratory deficiency at high temperatures, mtDNA instability, mitochondrial morphology

defects (Thorsness et al. 1993), and decreased chronological lifespan (Francis et al. 2007). ScYME1 is involved in regulation of mitophagy by processing ATG32 (Wang et al. 2013) and in the assembly and disassembly of ATP synthase during stress conditions (Francis and Thorsness 2011). Ablation of the i-AAA homolog PaIAP in *P. anserina* decreases the lifespan, spore germination, and fruiting body formation, but only at elevated growth temperature, indicating a temperature-dependent role of this protein (Weil et al. 2011).

Overall, it is clear that various proteases are important components of the molecular quality control machinery. They are active in the regulation of **proteostasis** and as such of particularly importance to counteract processes leading to premature degeneration.

C. Mitochondrial Dynamics

Mitochondria are dynamic organelles which continuously undergo **fission** and **fusion**. Genetically regulated changes in the balance of these two processes allow to adjust mitochondrial morphology according to physiological constraints and developmental needs (Westermann 2010). Tubular mitochondria are associated with an active respiration and a healthy phenotype, while fragmented mitochondria accumulate in resting and aging cells (Merz et al. 2007). Mitochondrial dynamics is also involved in the control of the quality of this essential organelle. Fusion of mitochondria of different quality can lead to a kind of functional "repair" of affected mitochondria, while fission can separate strongly impaired parts of a mitochondrial network and facilitate the subsequent degradation of these units by the vacuolar system (in animals via lysosomes) by selective **autophagy**, termed **mitophagy**.

Mitochondrial fission is an active process in which a set of interacting proteins is involved. A key component is the GTPase DNM1. During fission DNM1 oligomers form a spiral on the mitochondrial outer membrane. The hydrolysis of GTP powers a conformational change in DNM1 that tightens the spiral until a part of the mitochondrion is constricted (Mears et al. 2011). **Mitochondrial fusion** proceeds via several stages. The contact of mitochondria is first established by the outer membrane protein FZO1. This is followed by a GTP dependent fusion of the outer membranes (Anton et al. 2011). UGO1 functions as linker between FZO1 and the GTPase MGM1 and is necessary for fusion of the inner membrane. MGM1 forms a lattice between the membranes and tethers them together. A GTP dependent conformational change is thought to trigger a membrane deformation that causes the fusion (Abutbul-Ionita et al. 2012).

A role of mitochondrial fission in aging and lifespan control has first been demonstrated in *P. anserina* and *S. cerevisiae*. An initial differential transcript analysis of juvenile and senescent wild type of *P. anserina* revealed an increase in *PaDnm1* transcript levels in senescent mitochondria (Scheckhuber et al. 2007). This increase is associated with an age-related fragmentation of filamentous mitochondria found in juvenile mycelia to punctate units in senescent ones. The deletion of *PaDnm1* coding for the **dynamin-related protein 1** (PaDNM1) of *P. anserina* led to a delay in mitochondrial fragmentation and release of hydrogen peroxide from cultures, a stabilization of the mtDNA, and an increased resistance to the induction of **programmed cell death** (PCD). The *PaDnm1* deletion strain displays an 11-fold increase in lifespan. In contrast to the respiration mutants described above, the lifespan increase is not accompanied by impairments in growth and fertility. Thus, it is the healthy lifespan, the health span, which is increased in this strain. A similar effect was obtained when the *Dnm1* gene of yeast was deleted. Mitochondrial fragmentation was delayed and replicative lifespan was increased (Scheckhuber et al. 2007).

Mitochondrial fusion has been found to be able to complement impaired mitochondrial function by fusion of mitochondria of different quality. This has been experimentally demonstrated in mouse embryonic fibroblasts, where the age of mitochondrial proteins can be displayed with a reporter protein that shifts in fluorescence from green to red after 24 h. Wild-type cells have an even distribution of old and new proteins, while cells lacking MFN1 and MFN2, the homologs of FZO1, have an increased heterogeneity in mitochondrial protein age, which means that mitochondria

with both mainly young and mainly old proteins are visible. This is caused by the missing mitochondrial fusion which results in a lack of commingling (Ferree et al. 2013). Damage in mtDNA can be resolved in a similar manner by fusion of damaged mitochondria with intact ones, which can result in complementation of the corrupted mtDNA (Ono et al. 2001). Concordantly, deletion of *ScFzo1* results in mtDNA loss and shortened chronological lifespan (Burtner et al. 2011; Hermann et al. 1998; Rapaport et al. 1998). Deletion of *ScMgm1* leads to strongly fragmented mitochondria and shortened replicative and chronological lifespan (Scheckhuber et al. 2011). In the filamentous ascomycete *N. crassa*, mutation in *NcFzo1* was found to be associated with fragmented mitochondria, accumulation of mtDNA deletions, and shortened lifespan (Kurashima et al. 2013).

Mechanistically a strong impact of the mitochondrial membrane potential on mitochondrial dynamics has been demonstrated (Twig et al. 2008). Mitochondria with a low membrane potential are unable to fuse with other units. Thus, the separation of functionally impaired parts of the mitochondrial network leads to the formation of mitochondrial units with a low membrane potential that remain separated and accessible to degradation via autophagy.

D. Autophagy

Apart from the proteasomal ubiquitin system and the activity of various specialized proteases, autophagy emerges as a new key player in cellular quality control. More exactly, under the term autophagy a number of processes are compiled which utilize the vacuole (in fungi and plants) or the lysosome (in animals) for degradation of cellular components. There are different types of autophagy active. **Nonselective autophagy** results in the degradation of bulk cellular material, while in **selective autophagy** specific targets, including whole organelles like mitochondria or peroxisomes, are delivered to the vacuole/lysosome for degradation. These types of selective autophagy are concordantly termed **mitophagy** and **pexophagy**, respectively. According to differences in cargo delivery, autophagy comes in three types: **microautophagy**, **macroautophagy**, and **chaperone-mediated autophagy**. Here, we will only refer to the most intensively studied form, macroautophagy, which we will subsequently term autophagy. For the other autophagy types, we refer to a number of recent reviews (Klionsky and Emr 2000; Parzych and Klionsky 2014; Voigt and Pöggeler 2013).

Autophagy is best known for its role under starvation, allowing cells to survive energetic stress conditions via the degradation of cellular material. The resulting metabolites are reused in energy transduction or biosynthetic processes (Yorimitsu and Klionsky 2005). Moreover, autophagy is active in the degradation of superfluous organelles and damaged components (Yang and Klionsky 2010) and as such part of the cellular quality control system.

A role of autophagy in aging is established in different systems including mammals, plants, and fungi (Alvers et al. 2009; Minina et al. 2013; Pyo et al. 2013). For instance, in *S. cerevisiae*, an unbiased screen searching for factors which influence chronological lifespan led to the identification of different short-lived mutants with defects in autophagy (Matecic et al. 2010). In *P. anserina* evidence for a role of autophagy in senescence is derived from a genome-wide transcriptome analysis (Philipp et al. 2013). Transcripts from genes coding for proteins related to autophagy were found to be enriched in strains of older age, while those coding for components of the proteasome were reduced. Subsequently, a role of autophagy in lifespan control was experimentally verified. In this and a subsequent study, a strong basal autophagic activity was identified even under non-induced conditions (Knuppertz et al. 2014; Wiemer and Osiewacz 2014a). Autophagy was found to be increased in older and in nitrogen-starved cultures. Lifespan in a strain ablated for the basal autophagy regulator PaATG1 is slightly but significantly reduced. Under nitrogen starvation conditions, the difference in lifespan of the wild-type and the *PaAtg1* deletion strain was much greater.

It is now of interest to elucidate the potential impact of mitophagy, the selective autophagic degradation of mitochondria, on aging and lifespan. A connection between mitochondrial

dynamics leading to the segregation of damaged parts of the mitochondrial network and the degradation of the resulting units via mitophagy has been suggested (Weber and Reichert 2010). However, currently experimental data proving this scenario are scarce. Recently, a computational model suggested such a role and provided experimentally testable predictions (Figge et al. 2012, 2013).

E. Replacement and Remodeling of Degraded Components

Degradation of affected proteins is clearly only one part of the responses to successfully counteract the processes of age-related molecular degeneration within the cell. After degradation of impaired proteins, replacement of these proteins and remodeling of the affected cellular units is required for functional integrity of the systems. The necessary molecular processes are of great importance to successfully counteract the molecular degeneration and keeping biological system functional over longer periods of time and thus for aging and lifespan. The fidelity of the pathways leading to the biosynthesis of proteins, their transport to the compartments of function, and in many cases the formation of complexes is of great significance. The necessity of a correct function of components involved in biosynthesis processes is part of the **error catastrophe theory** which states that aging is caused by an inaccurate gene expression (Orgel 1963). In more detail, it is not only necessary that the correct genetic information is available in a cell, but it is equally important that the biosynthesis machinery functions properly. Essential machines involved in protein biosynthesis are **ribosomes** in the cytoplasm as well as in mitochondria. The majority of all proteins required within the cell are encoded by nuclear DNA (nDNA) and translated in the cytoplasm. Subsequently, they are transported to their site of function. In the case of mitochondrial localization, proteins have to be kept in an unfolded conformation until they bind to specific membrane receptors and are transported via a sophisticated membrane transporter complex and delivered to the appropriate mitochondrial sub-compartments. For the generation of respiratory complexes I, III, IV, and V (F_0F_1ATP-synthase), proteins translated at cytoplasmic ribosomes interact with proteins encoded by the mtDNA, translated at mitochondrial ribosomes, and assemble into the macromolecular protein complexes of the respiratory chain. The coordinated synthesis of nuclear- and mtDNA-encoded proteins is of great importance.

In *P. anserina* only 14 of the required mitochondrial proteins are encoded by mtDNA and are thus directly transcribed and translated within this compartment. All other mitochondrial proteins are synthesized in the cytoplasm and subsequently imported into mitochondria (Cummings et al. 1990).

In fact, functional impairments of the biosynthesis and sorting machinery with impact on aging and lifespan have been reported in *P. anserina* and linked to senescence. For instance, the abundance and fidelity of the elongation factor-1α (EF-1α) can influence lifespan by enhancing the **accuracy of translation**. In *P. anserina* a high fidelity mutation in the EF-lα gene was found to result in an increased lifespan but led also to an impaired sporulation. The authors suggest various explanations for longevity mediated by EF-1α, like an altered error rate in translation per se or an increased protein synthesis efficiency (Silar and Picard 1994). Also in *P. anserina*, a mutation in the gene coding for the mitochondrial **TOM** (**translocase of the outer membrane**) transporter, which is involved in the import of cytosolic proteins into mitochondria, was found to lead to an elevated lifespan. This effect was suggested to result from lower amounts of αSenDNA compared to wild type (Jamet-Vierny et al. 1997).

A more recent longitudinal transcriptome analysis revealed evidence for the deterioration of ribosomes during aging. Comparisons of young and aged *P. anserina* wild type (6–14 days) clearly showed that, while autophagy associated transcripts increased during aging, transcript abundance linked to ribosomes and translation were lowered. Transcripts linked to **DNA maintenance** as well as to the proteasome were also decreased in the aged strain (Philipp et al. 2013).

Although different pathways contribute to the processes limiting lifespan, the most important one, the remodeling of the mitochondrial respiratory chain, is not available in senescent strains due to the mtDNA disintegration described at the beginning of this chapter. Regardless of any other approach to keep the system functional, this situation is finally lethal for *P. anserina*, an aerobic fungus that is depending on the generation of ATP via the mitochondrial respiratory chain.

VI. Programmed Cell Death

Despite the extensive attempts to prevent, repair, and eliminate molecular damage and to remodel affected functions, sooner or later molecular impairments pass thresholds and programs leading to cell death become induced. In mammalian systems, different forms of PCD (e.g., **apoptosis**) act as quality control systems, removing severely damaged cells from the body which otherwise could lead to the development of cancers. In these cases, damaged cells are removed for the sake of the individual. In yeast and *P. anserina*, the situation is different. In these unicellular or multicellular species, PCD is the final program bringing life to an end. In this way, functionally impaired individuals do not anymore compete with young individuals for restricted resources (Fröhlich and Madeo 2000).

The impact of PCD on lifespan has been demonstrated in yeast and *P. anserina*. In *P. anserina* first evidence for PCD is derived from in silico data. Different genes have been identified which encode putative apoptosis factors including **metacaspases** (MCA), **apoptosis-inducing factors** (AIF), **AIF-homologous mitochondrial inducer of death** (AMID), or **cyclophilin D** (CYPD). Subsequently, deletion studies of genes coding for the two metacaspases PaMCA1 and PaMCA2 as well as for PaAMID1 unraveled a lifespan extension in deletion strains (Hamann et al. 2007). Moreover, it was found that PaMCA1 becomes activated by hydrogen peroxide. In another study, members of the "apoptosis-inducing family" of proteins were investigated. It was found that ablation of PaAIF2 and PaAMID2, two proteins localized to mitochondria, leads to increased ROS tolerance and prolonged lifespan (Brust et al. 2010a).

In yeast, the single metacaspase ScMCA1 (YCA1) is necessary for induction of PCD and cleaves certain target proteins (e.g., glycerinaldehyde-3-phosphat dehydrogenase) (Silva et al. 2011) resulting in the accumulation of PCD markers and cell death (Madeo et al. 2002). Besides a role in the induction of PCD, ScMCA1 has an additional, vital, function in protein quality control. This is demonstrated by the buffering of negative effects in a HSP40 deletion strain by ScMCA1, suggesting that metacaspase is involved in preventing unfolded protein from aggregating (Shrestha et al. 2013). Concordantly, an increase in ScMCA1 levels leads to a prolonged replicative lifespan in a proteasome- and ScHSP104-dependent manner (Hill et al. 2014). In budding yeast, protein aggregates are asymmetrically distributed during budding of wild type, retaining most damaged proteins in the mother cell and thus ensuring daughter cells are basically free of aggregates (Liu et al. 2010). MCA1 removes aggregates that made it to daughter cells. When deleting *ScMca1*, the lack of this vital function leads to shortening of the chronological and replicative lifespan (Herker et al. 2004; Hill et al. 2014; Piper et al. 2006).

A more recent study in *P. anserina* unraveled details about age-related changes of mitochondrial ultrastructure and the molecular mechanisms controlling PCD. In an electron cryotomography study, age-related changes of the inner mitochondrial membrane were reported to occur in the wild type (Daum et al. 2013). While mitochondria from juvenile cultures contain only few lamellar cristae, mitochondria from senescent cultures typically contain a number of vesicles. These changes go along with a dissociation of F_1F_0-ATP synthase dimers, which, in young and middle-aged cultures, are located at the tips of the lamellar cristae (Davies et al. 2011). During aging, the dimers dissociate into monomers and the curvature of the inner membrane changes. In mitochondria from senescent cultures, the inner membrane occasionally comes

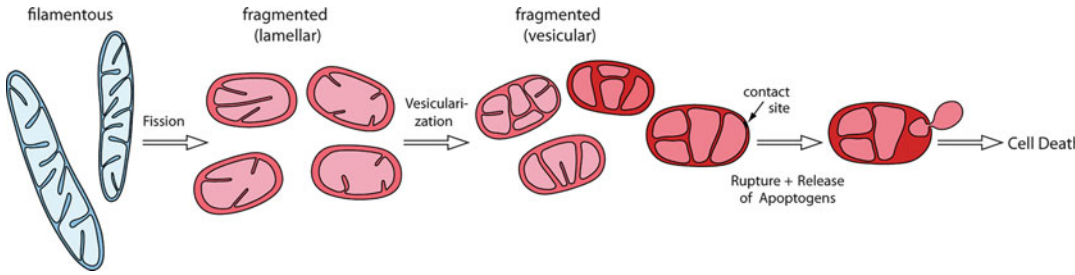


Fig. 8.3 Age-related changes in mitochondrial morphology and ultrastructure. Depicted is a series of events occurring during aging of *P. anserina*. In juvenile individuals, mitochondria are filamentous and functional (*blue color*). During aging, impairments in function accumulate (*light red*) and, due to a shift towards mitochondrial fission, mitochondria become fragmented. In earlier stages, they contain few lamellar cristae. Subsequently, the inner mitochondrial membrane becomes reorganized and vesicular units are formed. Occasionally, the inner membranes come into close contact with the outer membrane, and the outer membrane ruptures in the vicinity of these contact sites. As a consequence, components from the inside including apoptogens are released and lead to cell death

in close contact to the outer membrane which finally ruptures at the contact sites, releasing apoptotic factors to the cytoplasm (Fig. 8.3). Strikingly, the overexpression of the gene coding for **cyclophilin D**, a peptidyl prolyl cis-trans isomerase in the mitochondrial matrix, which is a known regulator of the **mitochondrial permeability transition pore** (mPTP), leads to acceleration of these processes. In *PaCypD* overexpressors, cultures of 6 days, that is young for the wild type, contain mainly vesicular mitochondria and display senescence characteristics like a lowered membrane potential, condensed nuclei, punctate mitochondrial morphotypes, and release of cytochrome c. Lifespan can be reverted by the addition of the CYPD inhibitor **cyclosporine A** (Brust et al. 2010b). Strikingly, during normal aging of the wild type, PaCYPD abundance was observed to increase in mitochondria (Groebe et al. 2007).

Components in fungi which induce PCD are likely not to be identical to mammalian counterparts since the PCD machineries differ considerably. In fungi, a role of cytochrome c in the induction of PCD is unlikely since there is no apoptosome and no procaspase 9, which are involved in the process in mammals. However, since mitochondria are known stores of **calcium** and the need of calcium to induce metacaspases has recently been demonstrated biochemically (Strobel and Osiewacz 2013), it is well possible that it is the release of this ion that is an important inductor of PCD in *P. anserina*. Other factors, like copper or cytochrome c which are also released from mitochondria, may only be **markers of mitochondrial rupture**, while AIF and other yet not characterized components may also contribute to the execution of PCD, the final step in the life cycle of *P. anserina*.

VI. Conclusions

Research on fungal senescence, in particular on *P. anserina* and *S. cerevisiae*, has unraveled a network of molecular pathways affecting aging and lifespan (Fig. 8.4). The physiological decline of biological systems is linked to impairments of cellular machines and to the generation of ROS which arise during normal metabolism (e.g., at the respiratory chain) and represent a “two-edged sword”. On the one hand, ROS signaling is essential for proper development. On the other hand, excess ROS levels lead to molecular damage. This situation, although the detailed underlying processes may differ from species to species, appears to be basically conserved between organisms.

In aerobic organisms, the main source of ROS is the mitochondrion in which superoxide is generated at the respiratory chain. In addition, phenolic components with vicinal hydroxyl groups can lead to the generation of

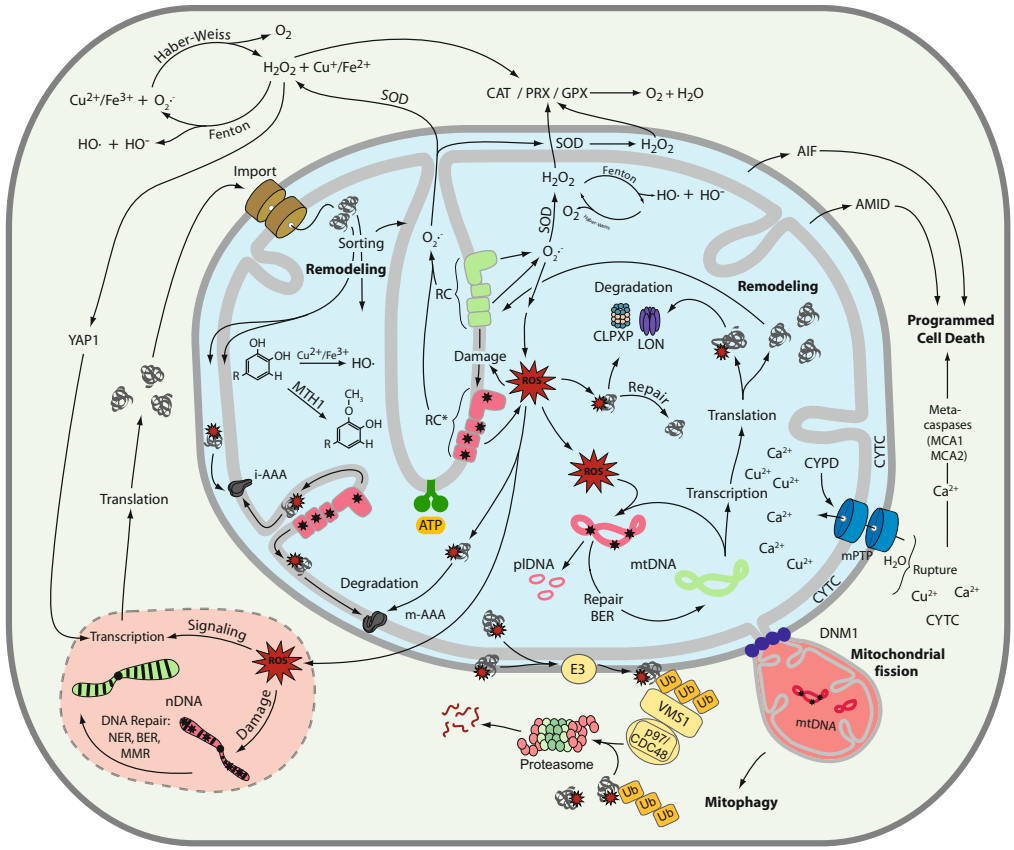


Fig. 8.4 Compilation of pathways affecting aging and lifespan in *P. anserina*. A major cellular generator of ROS (*large asterisks in black* or specific ROS as indicated) is the mitochondrion, in which superoxide is generated at the respiratory chain. In addition, phenolic components, the exact nature of which is not yet known, with vicinal hydroxyl groups lead to the generation of the hydroxyl radical. Different pathways (e.g., the methyltransferase MTH1, ROS scavengers like SOD, or catalase (CAT)) are active in balancing ROS levels. ROS are also acting in signaling allowing the control of gene expression. Due to limited capacity of this system, molecular impairments (*asterisks*) accumulate over time. This leads to the activation of the next level of the surveillance system: repair, refolding, degradation (e.g., at the proteasome or by specific proteases), resynthesis, and remodeling. Remodeling is largely dependent on the generation of nuclear-encoded proteins which are synthesized at cytoplasmic ribosomes, transported to mitochondria, and distributed to the site of function. In the case of the respiratory complexes, except of complex II, the proteins assemble by interactions of mitochondrial and nuclear-encoded proteins. After passing further thresholds, these systems also become overwhelmed and a response is activated. Mitochondrial dynamics (fission and fusion) and subsequently mitophagy can degrade severely damaged mitochondrial units (*red*) which were separated from functional units (*blue*) via fission (mediated by DNM1). Finally, if cellular impairments accumulate above limits which can be handled, mitochondria induce programmed cell death (PCD). Opening of mitochondria proceeds via the mitochondrial permeability transition pore (mPTP), which is controlled by cyclophilin D (CYPD), leading to the release of apoptogens like AIF, AMID, and most likely calcium. While the release of cytochrome c and of copper appears to be a marker for mitochondrial rupture, calcium is involved in the activation of cytoplasmic metacaspases

the highly reactive hydroxyl radical. Different pathways have evolved to balance cellular ROS levels. However, they cannot completely prevent molecular damage over time. After manifestation of damage, repair and refolding mechanisms can revert damage in DNA or of proteins. However, if the capacity of these surveillance pathways is overwhelmed, a next series of response pathways become activated and lead to the degradation of severely damaged components by proteases, the proteasome, or the vacuolar system. Also fission and fusion of mitochondria are effective processes in quality control. After removal of damaged proteins, it is essential that the corresponding proteins are resynthesized. This is, however, only possible if the genetic information is intact. In fact, this is the major problem in *P. anserina*, because the mtDNA coding for essential components of the respiratory chain is almost quantitatively rearranged. In senescent cultures of *P. anserina*, PCD is induced and the organism dies. The summarized scenario is based on experimental data and conclusions, some of which require more rigorous validation. However, the network of interactions of pathways as they occur currently is a good perspective for future investigations. What will now be specifically interesting to evaluate are the mechanisms controlling the **cross talk between pathways** and the ability of pathways to **compensate impairments** in other pathways. General conclusions about the mechanisms leading to fungal senescence and possibilities to intervene into these mechanisms leading to an extended **healthy lifespan** will certainly bear important information for transfer to other biological systems in which degenerative processes occur and may lead to a decrease in the biosynthesis of desired compounds or in the development of disabilities and severe diseases. Thus, use of fungi is not only of particular interest for the systematic evaluation of processes and pathways and their interactions to unravel the underlying mechanisms in the corresponding species. At the same time, these systems serve as organismal models and "living tools" to experimentally identify, modulate, and validate existing and novel components, pathways, and concepts with a general significance toward a holistic understanding of complex processes like biological aging.

References

Abutbul-Ionita I, Rujiviphat J, Nir I, McQuibban GA, Danino D (2012) Membrane tethering and nucleotide-dependent conformational changes drive mitochondrial genome maintenance (Mgm1) protein-mediated membrane fusion. J Biol Chem 287:36634–36638

Adam C, Picard M, Déquard-Chablat M, Sellem CH, Denmat SH, Contamine V (2012) Biological roles of the *Podospora anserina* mitochondrial Lon protease and the importance of its N-domain. PLoS ONE 7, e38138

Alvers AL, Wood MS, Hu D, Kaywell AC, Dunn WA Jr, Aris JP (2009) Autophagy is required for extension of yeast chronological life span by rapamycin. Autophagy 5:847–849

Anton F, Fres JM, Schauss A, Pinson B, Praefcke GJ, Langer T, Escobar-Henriques M (2011) Ugo1 and Mdm30 act sequentially during Fzo1-mediated mitochondrial outer membrane fusion. J Cell Sci 124:1126–1135

Antonenko YN, Avetisyan AV, Cherepanov DA, Knorre DA, Korshunova GA, Markova OV, Ojovan SM, Perevoshchikova IV, Pustovidko AV, Rokitskaya TI, Severina II, Simonyan RA, Smirnova EA, Sobko AA, Sumbatyan NV, Severin FF, Skulachev VP (2011) Derivatives of Rhodamine 19 as mild mitochondria-targeted cationic uncouplers. J Biol Chem 286:17831–17840

Antunes F, Cadenas E (2000) Estimation of H_2O_2 gradients across biomembranes. FEBS Lett 475:121–126

Aravind L, Walker DR, Koonin EV (1999) Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res 27:1223–1242

Arlt H, Steglich G, Perryman R, Guiard B, Neupert W, Langer T (1998) The formation of respiratory chain complexes in mitochondria is under the proteolytic control of the m-AAA protease. EMBO J 17:4837–4847

auf dem Keller U, Kumin A, Braun S, Werner S (2006) Reactive oxygen species and their detoxification in healing skin wounds. J Investig Dermatol Symp Proc 11:106–111

Baker MJ, Mooga VP, Guiard B, Langer T, Ryan MT, Stojanovski D (2012) Impaired folding of the mitochondrial small TIM chaperones induces clearance by the i-AAA protease. J Mol Biol 424:227–239

Baraibar MA, Ladouce R, Friguet B (2013) Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging. J Proteomics 92:63–70

Baynes JW (2001) The role of AGEs in aging: causation or correlation. Exp Gerontol 36:1527–1537

Beauchamp C, Fridovich I (1970) A mechanism for the production of ethylene from methional. The generation of the hydroxyl radical by xanthine oxidase. J Biol Chem 245:4641–4646

Bender T, Lewrenz I, Franken S, Baitzel C, Voos W (2011) Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. Mol Biol Cell 22:541–554

Bertrand H, Collins RA, Stohl LL, Goewert RR, Lambowitz AM (1980) Deletion mutants of *Neurospora crassa* mitochondrial DNA and their relationship to the "stop-start" growth phenotype. Proc Natl Acad Sci USA 77:6032–6036

Bertrand H, Chan BS, Griffiths AJ (1985) Insertion of a foreign nucleotide sequence into mitochondrial DNA causes senescence in *Neurospora intermedia*. Cell 41:877–884

Bertrand H, Griffiths AJ, Court DA, Cheng CK (1986) An extrachromosomal plasmid is the etiological precursor of kalDNA insertion sequences in the mitochondrial chromosome of senescent *Neurospora*. Cell 47:829–837

Bienert GP, Schjoerring JK, Jahn TP (2006) Membrane transport of hydrogen peroxide. Biochim Biophys Acta 1758:994–1003

Böckelmann B, Esser K (1986) Plasmids of mitochondrial origin in senescent mycelia of *Podospora curvicolla*. Curr Genet 10:803–810

Bohr VA (2002) Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells. Free Radic Biol Med 32:804–812

Borghouts C, Osiewacz HD (1998) GRISEA, a copper-modulated transcription factor from *Podospora anserina* involved in senescence and morphogenesis, is an ortholog of MAC1 in *Saccharomyces cerevisiae*. Mol Gen Genet 260:492–502

Borghouts C, Kimpel E, Osiewacz HD (1997) Mitochondrial DNA rearrangements of *Podospora anserina* are under the control of the nuclear gene grisea. Proc Natl Acad Sci USA 94:10768–10773

Borghouts C, Werner A, Elthon T, Osiewacz HD (2001) Copper-modulated gene expression and senescence in the filamentous fungus *Podospora anserina*. Mol Cell Biol 21:390–399

Borghouts C, Scheckhuber CQ, Werner A, Osiewacz HD (2002) Respiration, copper availability and SOD activity in *P. anserina* strains with different lifespan. Biogerontology 3:143–153

Branco MR, Marinho HS, Cyrne L, Antunes F (2004) Decrease of H_2O_2 plasma membrane permeability during adaptation to H_2O_2 in *Saccharomyces cerevisiae*. J Biol Chem 279:6501–6506

Brun S, Malagnac F, Bidard F, Lalucque H, Silar P (2009) Functions and regulation of the Nox family in the filamentous fungus *Podospora anserina*: a new role in cellulose degradation. Mol Microbiol 74:480–496

Brust D, Hamann A, Osiewacz HD (2010a) Deletion of *PaAif2* and *PaAmid2*, two genes encoding mitochondrial AIF-like oxidoreductases of *Podospora anserina*, leads to increased stress tolerance and lifespan extension. Curr Genet 55:225–235

Brust D, Daum B, Breunig C, Hamann A, Kühlbrandt W, Osiewacz HD (2010b) Cyclophilin D links programmed cell death and organismal aging in *Podospora anserina*. Aging Cell 9:761–775

Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof PR (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Brain Res Rev 33:95–130

Burtner CR, Murakami CJ, Olsen B, Kennedy BK, Kaeberlein M (2011) A genomic analysis of chronological longevity factors in budding yeast. Cell Cycle 10:1385–1396

Cabiscol E, Tamarit J, Ros J (2014) Protein carbonylation: proteomics, specificity and relevance to aging. Mass Spectrom Rev 33:21–48

Cano-Domínguez N, Álvarez-Delfín K, Hansberg W, Aguirre J (2008) NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in *Neurospora crassa*. Eukaryot Cell 7:1352–1361

Carrard G, Bulteau AL, Petropoulos I, Friguet B (2002) Impairment of proteasome structure and function in aging. Int J Biochem Cell Biol 34:1461–1474

Chan BS, Court DA, Vierula PJ, Bertrand H (1991) The kalilo linear senescence-inducing plasmid of *Neurospora* is an invertron and encodes DNA and RNA polymerases. Curr Genet 20:225–237

Chen C, Dickman MB (2005) Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. Proc Natl Acad Sci USA 102:3459–3464

Chen Q, Thorpe J, Dohmen JR, Li F, Keller JN (2006) Ump1 extends yeast lifespan and enhances viability during oxidative stress: central role for the proteasome? Free Radic Biol Med 40:120–126

Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA (1992) 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes G →T and A →C substitutions. J Biol Chem 267:166–172

Chondrogianni N, Gonos ES (2005) Proteasome dysfunction in mammalian aging: steps and factors involved. Exp Gerontol 40:931–938

Chondrogianni N, Petropoulos I, Franceschi C, Friguet B, Gonos ES (2000) Fibroblast cultures from healthy centenarians have an active proteasome. Exp Gerontol 35:721–728

Cockayne EA (1936) Dwarfism with retinal atrophy and deafness. Arch Dis Child 11:1–8

Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van PB, Pieters L, Vlietinck AJ, Vanden Berghe D (1998) Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod 61:71–76

Costa V, Moradas-Ferreira P (2001) Oxidative stress and signal transduction in *Saccharomyces cerevi-*

siae: insights into ageing, apoptosis and diseases. Mol Aspects Med 22:217–246

Culotta VC, Yang M, O'Halloran TV (2006) Activation of superoxide dismutases: putting the metal to the pedal. Biochim Biophys Acta 1763:747–758

Cummings DJ, Belcour L, Grandchamp C (1979) Mitochondrial DNA from *Podospora anserina*. II. Properties of mutant DNA and multimeric circular DNA from senescent cultures. Mol Gen Genet 171:239–250

Cummings DJ, McNally KL, Domenico JM, Matsuura ET (1990) The complete DNA sequence of the mitochondrial genome of *Podospora anserina*. Curr Genet 17:375–402

da Cunha FM, Demasi M, Kowaltowski AJ (2011) Aging and calorie restriction modulate yeast redox state, oxidized protein removal, and the ubiquitin-proteasome system. Free Radic Biol Med 51:664–670

Daum B, Walter A, Horst A, Osiewacz HD, Kühlbrandt W (2013) Age-dependent dissociation of ATP synthase dimers and loss of inner-membrane cristae in mitochondria. Proc Natl Acad Sci USA 110:15301–15306

Davies KM, Strauss M, Daum B, Kief JH, Osiewacz HD, Rycovska A, Zickermann V, Kühlbrandt W (2011) Macromolecular organization of ATP synthase and complex I in whole mitochondria. Proc Natl Acad Sci USA 108:14121–14126

Delaunay A, Isnard AD, Toledano MB (2000) H_2O_2 sensing through oxidation of the Yap1 transcription factor. EMBO J 19:5157–5166

Delaunay A, Pflieger D, Barrault MB, Vinh J, Toledano MB (2002) A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. Cell 111:471–481

Dirschnabel DE, Nowrousian M, Cano-Dominguez N, Aguirre J, Teichert I, Kück U (2014) New insights into the roles of NADPH oxidases in sexual development and ascospore germination in *Sordaria macrospora*. Genetics 196:729–744

Drahota Z, Chowdhury SK, Floryk D, Mracek T, Wilhelm J, Rauchova H, Lenaz G, Houstek J (2002) Glycerophosphate-dependent hydrogen peroxide production by brown adipose tissue mitochondria and its activation by ferricyanide. J Bioenerg Biomembr 34:105–113

Dröse S, Brandt U (2008) The mechanism of mitochondrial superoxide production by the cytochrome bc1 complex. J Biol Chem 283:21649–21654

Dufour E, Boulay J, Rincheval V, Sainsard-Chanet A (2000) A causal link between respiration and senescence in *Podospora anserina*. Proc Natl Acad Sci USA 97:4138–4143

Duvezin-Caubet S, Koppen M, Wagener J, Zick M, Israel L, Bernacchia A, Jagasia R, Rugarli EI, Imhof A, Neupert W, Langer T, Reichert AS (2007) OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. Mol Biol Cell 18:3582–3590

Emri T, Molnar Z, Pusztahelyi T, Rosen S, Pocsi I (2004) Effect of vitamin E on autolysis and sporulation of *Aspergillus nidulans*. Appl Biochem Biotechnol 118:337–348

Erjavec N, Bayot A, Gareil M, Camougrand N, Nystrom T, Friguet B, Bulteau AL (2013) Deletion of the mitochondrial Pim1/Lon protease in yeast results in accelerated aging and impairment of the proteasome. Free Radic Biol Med 56:9–16

Esser K (1974) *Podospora anserina*. In: King RC (ed) Handbook of genetics. Plenum, New York, pp 531–551

Esser K, Tudzynski P (1980) Senescence in fungi. In: Thimann KV (ed) Senescence in plants. CRC, Boca Raton, pp 67–83

Esser K, Tudzynski P, Stahl U, Kück U (1980) A model to explain senescence in the filamentous fungus *Podospora anserina* by the action of plasmid like DNA. Mol Gen Genet 178:213–216

Esser K, Kück U, Stahl U, Tudzynski P (1981) Mitochondrial DNA and senescence in *Podospora anserina*. Curr Genet 4:83

Fabrizio P, Pletcher SD, Minois N, Vaupel JW, Longo VD (2004) Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in *Saccharomyces cerevisiae*. FEBS Lett 557:136–142

Fang J, Beattie DS (2003) External alternative NADH dehydrogenase of *Saccharomyces cerevisiae*: a potential source of superoxide. Free Radic Biol Med 34:478–488

Ferree AW, Trudeau K, Zik E, Benador IY, Twig G, Gottlieb RA, Shirihai OS (2013) MitoTimer probe reveals the impact of autophagy, fusion, and motility on subcellular distribution of young and old mitochondrial protein and on relative mitochondrial protein age. Autophagy 9:1887–1896

Figge MT, Reichert AS, Meyer-Hermann M, Osiewacz HD (2012) Deceleration of fusion-fission cycles improves mitochondrial quality control during aging. PLoS Comput Biol 8, e1002576

Figge MT, Osiewacz HD, Reichert AS (2013) Quality control of mitochondria during aging: is there a good and a bad side of mitochondrial dynamics? Bioessays 35:314–322

Fischer F, Weil A, Hamann A, Osiewacz HD (2013) Human CLPP reverts the longevity phenotype of a fungal *ClpP* deletion strain. Nat Commun 4:1397

Francis BR, Thorsness PE (2011) Hsp90 and mitochondrial proteases Yme1 and Yta10/12 participate in ATP synthase assembly in *Saccharomyces cerevisiae*. Mitochondrion 11:587–600

Francis BR, White KH, Thorsness PE (2007) Mutations in the Atp1p and Atp3p subunits of yeast ATP synthase differentially affect respiration and fermentation in *Saccharomyces cerevisiae*. J Bioenerg Biomembr 39:127–144

Friguet B (2006) Oxidized protein degradation and repair in ageing and oxidative stress. FEBS Lett 580:2910–2916

Fröhlich KU, Madeo F (2000) Apoptosis in yeast - a monocellular organism exhibits altruistic behaviour. FEBS Lett 473:6–9

Gan W, Nie B, Shi F, Xu XM, Qian JC, Takagi Y, Hayakawa H, Sekiguchi M, Cai JP (2012) Age-dependent increases in the oxidative damage of DNA, RNA, and their metabolites in normal and senescence-accelerated mice analyzed by LC-MS/MS: urinary 8-oxoguanosine as a novel biomarker of aging. Free Radic Biol Med 52:1700–1707

Garrido EO, Grant CM (2002) Role of thioredoxins in the response of *Saccharomyces cerevisiae* to oxidative stress induced by hydroperoxides. Mol Microbiol 43:993–1003

Georgiou CD, Petropoulou KP (2001) Effect of the antioxidant ascorbic acid on sclerotial differentiation in *Rhizoctonia solani*. Plant Pathol 50:594–600

Gerstbrein B, Stamatas G, Kollias N, Driscoll M (2005) *In vivo* spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *Caenorhabditis elegans*. Aging Cell 4:127–137

Geydan TD, Debets AJ, Verkley GJ, van Diepeningen AD (2012) Correlated evolution of senescence and ephemeral substrate use in the Sordariomycetes. Mol Ecol 21:2816–2828

Gleason JE, Galaleldeen A, Peterson RL, Taylor AB, Holloway SP, Waninger-Saroni J, Cormack BP, Cabelli DE, Hart PJ, Culotta VC (2014) *Candida albicans* SOD5 represents the prototype of an unprecedented class of Cu-only superoxide dismutases required for pathogen defense. Proc Natl Acad Sci USA 111:5866–5871

Gredilla R, Garm C, Stevnsner T (2012) Nuclear and mitochondrial DNA repair in selected eukaryotic aging model systems. Oxid Med Cell Longev 2012:282438

Griffiths AJ (1992) Fungal senescence. Annu Rev Genet 26:351–372

Grimm C, Osiewacz HD (2015) Manganese rescues adverse effects on lifespan and development in *Podospora anserina* challenged by excess hydrogen peroxide. Exp Gerontol 63:8–17

Groebe K, Krause F, Kunstmann B, Unterluggauer H, Reifschneider NH, Scheckhuber CQ, Sastri C, Stegmann W, Wozny W, Schwall GP, Poznanovic S, Dencher NA, Jansen-Dürr P, Osiewacz HD, Schrattenholz A (2007) Differential proteomic profiling of mitochondria from *Podospora anserina*, rat and human reveals distinct patterns of age-related oxidative changes. Exp Gerontol 42:887–898

Guerrero RF, Garcia-Parrilla MC, Puertas B, Cantos-Villar E (2009) Wine, resveratrol and health: a review. Nat Prod Commun 4:635–658

Hamann A, Brust D, Osiewacz HD (2007) Deletion of putative apoptosis factors leads to lifespan extension in the fungal ageing model *Podospora anserina*. Mol Microbiol 65:948–958

Han D, Antunes F, Canali R, Rettori D, Cadenas E (2003) Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J Biol Chem 278:5557–5563

Harman D (1956) A theory based on free radical and radiation chemistry. J Gerontol 11:298–300

Heller J, Tudzynski P (2011) Reactive oxygen species in phytopathogenic fungi: signaling, development, and disease. Annu Rev Phytopathol 49:369–390

Heo JM, Livnat-Levanon N, Taylor EB, Jones KT, Dephoure N, Ring J, Xie J, Brodsky JL, Madeo F, Gygi SP, Ashrafi K, Glickman MH, Rutter J (2010) A stress-responsive system for mitochondrial protein degradation. Mol Cell 40:465–480

Herker E, Jungwirth H, Lehmann KA, Maldener C, Fröhlich KU, Wissing S, Buttner S, Fehr M, Sigrist S, Madeo F (2004) Chronological aging leads to apoptosis in yeast. J Cell Biol 164:501–507

Hermann GJ, Thatcher JW, Mills JP, Hales KG, Fuller MT, Nunnari J, Shaw JM (1998) Mitochondrial fusion in yeast requires the transmembrane GTPase Fzo1p. J Cell Biol 143:359–373

Hill SM, Hao X, Liu B, Nyström T (2014) Life-span extension by a metacaspase in the yeast *Saccharomyces cerevisiae*. Science 344:1389–1392

Hofmann B, Hecht HJ, Flohe L (2002) Peroxiredoxins. Biol Chem 383:347–364

Hudson EK, Hogue BA, Souza-Pinto NC, Croteau DL, Anson RM, Bohr VA, Hansford RG (1998) Age-associated change in mitochondrial DNA damage. Free Radic Res 29:573–579

Jakubowski W, Bilinski T, Bartosz G (2000) Oxidative stress during aging of stationary cultures of the yeast *Saccharomyces cerevisiae*. Free Radic Biol Med 28:659–664

Jamet-Vierny C, Contamine V, Boulay J, Zickler D, Picard M (1997) Mutations in genes encoding the mitochondrial outer membrane proteins Tom70 and Mdm10 of *Podospora anserina* modify the spectrum of mitochondrial DNA rearrangements associated with cellular death. Mol Cell Biol 17:6359–6366

Jarolim S, Millen J, Heeren G, Laun P, Goldfarb DS, Breitenbach M (2004) A novel assay for replicative lifespan in *Saccharomyces cerevisiae*. FEMS Yeast Res 5:169–177

Jazwinski SM (2004) Yeast replicative life span - the mitochondrial connection. FEMS Yeast Res 5:119–125

Jazwinski SM, Kriete A (2012) The yeast retrograde response as a model of intracellular signaling of mitochondrial dysfunction. Front Physiol 3:139

Jeanmaire C, Danoux L, Pauly G (2001) Glycation during human dermal intrinsic and actinic ageing: an *in vivo* and *in vitro* model study. Br J Dermatol 145:10–18

Jiang J, Qin C, Shu X, Chen R, Song H, Li Q, Xu H (2015) Effects of copper on induction of thiol-compounds and antioxidant enzymes by the fruiting body of *Oudemansiella radicata*. Ecotoxicol Environ Saf 111:60–65

Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol 2, E296

Kautto L, Grinyer J, Paulsen I, Tetu S, Pillai A, Pardiwalla S, Sezerman U, Akcapinar GB, Bergquist P, Te'o J, Nevalainen H (2013) Stress effects caused by the expression of a mutant cellobiohydrolase I and proteasome inhibition in *Trichoderma reesei* Rut-C30. N Biotechnol 30:183–191

Kawalek A, Lefevre SD, Veenhuis M, van der Klei IJ (2013) Peroxisomal catalase deficiency modulates yeast lifespan depending on growth conditions. Aging (Albany NY) 5:67–83

Kayano Y, Tanaka A, Akano F, Scott B, Takemoto D (2013) Differential roles of NADPH oxidases and associated regulators in polarized growth, conidiation and hyphal fusion in the symbiotic fungus *Epichloë festucae*. Fungal Genet Biol 56:87–97

Kennedy BK, Steffen KK, Kaeberlein M (2007) Ruminations on dietary restriction and aging. Cell Mol Life Sci 64:1323–1328

Keyer K, Gort AS, Imlay JA (1995) Superoxide and the production of oxidative DNA damage. J Bacteriol 177:6782–6790

Kirkwood TB, Austad SN (2000) Why do we age? Nature 408:233–238

Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. Science 290:1717–1721

Kniemeyer O, Lessing F, Brakhage AA (2009) Proteome analysis for pathogenicity and new diagnostic markers for *Aspergillus fumigatus*. Med Mycol 47 (Suppl 1):S248–S254

Knuppertz L, Hamann A, Pampaloni F, Stelzer E, Osiewacz HD (2014) Identification of autophagy as a longevity-assurance mechanism in the aging model *Podospora anserina*. Autophagy 10:822–834

Koc A, Gasch AP, Rutherford JC, Kim HY, Gladyshev VN (2004) Methionine sulfoxide reductase regulation of yeast lifespan reveals reactive oxygen species-dependent and -independent components of aging. Proc Natl Acad Sci USA 101:7999–8004

Kothe GO, Kitamura M, Masutani M, Selker EU, Inoue H (2010) PARP is involved in replicative aging in *Neurospora crassa*. Fungal Genet Biol 47:297–309

Kowald A, Hamann A, Zintel S, Ullrich S, Klipp E, Osiewacz HD (2012) A systems biological analysis links ROS metabolism to mitochondrial protein quality control. Mech Ageing Dev 133:331–337

Kruegel U, Robison B, Dange T, Kahlert G, Delaney JR, Kotireddy S, Tsuchiya M, Tsuchiyama S, Murakami CJ, Schleit J, Sutphin G, Carr D, Tar K, Dittmar G, Kaeberlein M, Kennedy BK, Schmidt M (2011) Elevated proteasome capacity extends replicative lifespan in *Saccharomyces cerevisiae*. PLoS Genet 7, e1002253

Kück U, Stahl U, Esser K (1981) Plasmid-like DNA is part of mitochondrial DNA in *Podospora anserina*. Curr Genet 3:151–156

Kuiper MT, Sabourin JR, Lambowitz AM (1990) Identification of the reverse transcriptase encoded by the Mauriceville and Varkud mitochondrial plasmids of *Neurospora*. J Biol Chem 265:6936–6943

Kunstmann B, Osiewacz HD (2009) The S-adenosylmethionine dependent O-methyltransferase PaMTH1: a longevity assurance factor protecting *Podospora anserina* against oxidative stress. Aging (Albany NY) 1:328–334

Kurashima K, Chae M, Inoue H, Hatakeyama S, Tanaka S (2013) A uvs-5 strain is deficient for a mitofusin gene homologue, *fzo1*, involved in maintenance of long life span in *Neurospora crassa*. Eukaryot Cell 12:233–243

Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4:181–189

Lazarus CM, Küntzel H (1981) Anatomy of amplified mitochondrial DNA in 'ragged' mutants of *Aspergillus amstelodami*: excision points within genes and a common 215 bp segment containing a possible origin of amplification. Curr Genet 4:99–107

Lazarus CM, Earl AJ, Turner G, Küntzel H (1980) Amplification of a mitochondrial DNA sequence in the cytoplasmically inherited 'ragged' mutant of *Aspergillus amstelodami*. Eur J Biochem 106:633–641

Le DT, Lee BC, Marino SM, Zhang Y, Fomenko DE, Kaya A, Hacioglu E, Kwak GH, Koc A, Kim HY, Gladyshev VN (2009) Functional analysis of free methionine-R-sulfoxide reductase from *Saccharomyces cerevisiae*. J Biol Chem 284:4354–4364

Leal SM Jr, Vareechon C, Cowden S, Cobb BA, Latge JP, Momany M, Pearlman E (2012) Fungal antioxidant pathways promote survival against neutrophils during infection. J Clin Invest 122:2482–2498

Lehmann AR (1982) Three complementation groups in Cockayne syndrome. Mutat Res 106:347–356

Leonhard K, Guiard B, Pellecchia G, Tzagoloff A, Neupert W, Langer T (2000) Membrane protein degradation by AAA proteases in mitochondria: extraction of substrates from either membrane surface. Mol Cell 5:629–638

Lessing F, Kniemeyer O, Wozniok I, Loeffler J, Kurzai O, Haertl A, Brakhage AA (2007) The *Aspergillus fumigatus* transcriptional regulator AfYap1 represents the major regulator for defense against reactive oxygen intermediates but is dispensable for pathogenicity in an intranasal mouse infection model. Eukaryot Cell 6:2290–2302

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, Ahn B-W, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186:464–478

Lin SJ, Defossez PA, Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science 289:2126–2128

Lionaki E, Tavernarakis N (2013) Oxidative stress and mitochondrial protein quality control in aging. J Proteomics 92:181–194

Liu Y, Prasad R, Beard WA, Kedar PS, Hou EW, Shock DD, Wilson SH (2007) Coordination of steps in single-nucleotide base excision repair mediated by apurinic/apyrimidinic endonuclease 1 and DNA polymerase beta. J Biol Chem 282:13532–13541

Liu B, Larsson L, Caballero A, Hao X, Oling D, Grantham J, Nystrom T (2010) The polarisome is required for segregation and retrograde transport of protein aggregates. Cell 140:257–267

Longo VD, Liou LL, Valentine JS, Gralla EB (1999) Mitochondrial superoxide decreases yeast survival in stationary phase. Arch Biochem Biophys 365:131–142

Longo VD, Shadel GS, Kaeberlein M, Kennedy B (2012) Replicative and chronological aging in *Saccharomyces cerevisiae*. Cell Metab 16:18–31

Luce K, Osiewacz HD (2009) Increasing organismal healthspan by enhancing mitochondrial protein quality control. Nat Cell Biol 11:852–858

Ma LH, Takanishi CL, Wood MJ (2007) Molecular mechanism of oxidative stress perception by the Orp1 protein. J Biol Chem 282:31429–31436

Maas MF, Sellem CH, Hoekstra RF, Debets AJ, Sainsard-Chanet A (2007) Integration of a pAL2-1 homologous mitochondrial plasmid associated with life span extension in *Podospora anserina*. Fungal Genet Biol 44:659–671

Maclean MJ, Aamodt R, Harris N, Alseth I, Seeberg E, Bjoras M, Piper PW (2003) Base excision repair activities required for yeast to attain a full chronological life span. Aging Cell 2:93–104

Madeo F, Herker E, Maldener C, Wissing S, Lachelt S, Herlan M, Fehr M, Lauber K, Sigrist SJ, Wesselborg S, Fröhlich KU (2002) A caspase-related protease regulates apoptosis in yeast. Mol Cell 9:911–917

Madeo F, Pietrocola F, Eisenberg T, Kroemer G (2014) Caloric restriction mimetics: towards a molecular definition. Nat Rev Drug Discov 13:727–740

Major T, von Janowsky B, Ruppert T, Mogk A, Voos W (2006) Proteomic analysis of mitochondrial protein turnover: identification of novel substrate proteins of the matrix protease pim1. Mol Cell Biol 26:762–776

Malagnac F, Lalucque H, Lepère G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserina*. Fungal Genet Biol 41:982–997

Malagnac F, Klapholz B, Silar P (2007) PaTrx1 and PaTrx3, two cytosolic thioredoxins of the filamentous ascomycete *Podospora anserina* involved in sexual development and cell degeneration. Eukaryot Cell 6:2323–2331

Mandelli F, Yamashita F, Pereira JL, Mercadante AZ (2012) Evaluation of biomass production, carotenoid level and antioxidant capacity produced by *Thermus filiformis* using fractional factorial design. Braz J Microbiol 43:126–134

Marcou D (1961) Notion de longevite et nature cytoplasmatique du determinant de senescence chez quelques champignons. Ann Sci Nat Biol Bot Veg 12:653–764

Marcou D, Schecroun J (1959) La senescence chez *Podospora* pourrait etre due a des particles cytoplasmatiques infectantes. Compte rendue des l'Academie des sciences 248:280–283

Marek A, Korona R (2013) Restricted pleiotropy facilitates mutational erosion of major life-history traits. Evolution 67:3077–3086

Marinho HS, Real C, Cyrne L, Soares H, Antunes F (2014) Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox Biol 2:535–562

Martino E, Franco B, Piccoli G, Stocchi V, Perotto S (2002) Influence of zinc ions on protein secretion in a heavy metal tolerant strain of the ericoid mycorrhizal fungus *Oidiodendron maius*. Mol Cell Biochem 231:179–185

Matecic M, Smith DL, Pan X, Maqani N, Bekiranov S, Boeke JD, Smith JS (2010) A microarray-based genetic screen for yeast chronological aging factors. PLoS Genet 6, e1000921

McCord JM, Day ED Jr (1978) Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. FEBS Lett 86:139–142

Mears JA, Lackner LL, Fang S, Ingerman E, Nunnari J, Hinshaw JE (2011) Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission. Nat Struct Mol Biol 18:20–26

Melis JP, van Steeg H, Luijten M (2013) Oxidative DNA damage and nucleotide excision repair. Antioxid Redox Signal 18:2409–2419

Merz S, Hammermeister M, Altmann K, Durr M, Westermann B (2007) Molecular machinery of mitochondrial dynamics in yeast. Biol Chem 388:917–926

Mesquita A, Weinberger M, Silva A, Sampaio-Marques B, Almeida B, Leao C, Costa V, Rodrigues F, Burhans WC, Ludovico P (2010) Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H_2O_2 and superoxide dismutase activity. Proc Natl Acad Sci USA 107:15123–15128

Minina EA, Sanchez-Vera V, Moschou PN, Suarez MF, Sundberg E, Weih M, Bozhkov PV (2013) Autophagy mediates caloric restriction-induced lifespan extension in *Arabidopsis*. Aging Cell 12:327–329

Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, Criollo A, Galluzzi L, Malik SA, Vitale I, Michaud M, Madeo F, Tavernarakis N, Kroemer G (2010) Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. Cell Death Dis 1, e10

Mortimer RK, Johnston JR (1959) Life span of individual yeast cells. Nature 183:1752

Muller EG (1991) Thioredoxin deficiency in yeast prolongs S phase and shortens the G1 interval of the cell cycle. J Biol Chem 266:9194–9202

Müller-Ohldach M, Brust D, Hamann A, Osiewacz HD (2011) Overexpression of *PaParp* encoding the poly(ADP-ribose) polymerase of *Podospora anserina* affects organismal aging. Mech Ageing Dev 132:33–42

Myers C, Griffiths AJF, Bertrand H (1989) Linear *kalilo* DNA is a *Neurospora* mitochondrial plasmid that integrates into the mitochondrial DNA. Mol Gen Genet 220:113–120

Nakamoto H, Kaneko T, Tahara S, Hayashi E, Naito H, Radak Z, Goto S (2007) Regular exercise reduces 8-oxodG in the nuclear and mitochondrial DNA and modulates the DNA repair activity in the liver of old rats. Exp Gerontol 42:287–295

Nargang FE, Pande S, Kennell JC, Akins RA, Lambowitz AM (1992) Evidence that a 1.6 kilobase region of *Neurospora* mtDNA was derived by insertion of part of the LaBelle mitochondrial plasmid. Nucleic Acids Res 20:1101–1108

Ono T, Isobe K, Nakada K, Hayashi JI (2001) Human cells are protected from mitochondrial dysfunction by complementation of DNA products in fused mitochondria. Nat Genet 28:272–275

Orgel LE (1963) The maintenance of the accuracy of protein synthesis and its relevance to ageing. Proc Natl Acad Sci USA 49:517–521

Osiewacz HD (1990) Molecular analysis of aging processes in fungi. Mutat Res 237:1–8

Osiewacz HD (2002) Aging in fungi: role of mitochondria in *Podospora anserina*. Mech Ageing Dev 123:755–764

Osiewacz HD, Esser K (1984) The mitochondrial plasmid of *Podospora anserina*: a mobile intron of a mitochondrial gene. Curr Genet 8:299–305

Osiewacz HD, Hamann A (1994) Senescence and longevity. In: Wessels JGH, Meinhardt F (eds) The mycota I, 2nd edn. Springer, Berlin, pp 189–200

Osiewacz HD, Scheckhuber CQ (2006) Impact of ROS on ageing of two fungal model systems: *Saccharomyces cerevisiae* and *Podospora anserina*. Free Radic Res 40:1350–1358

Osiewacz HD, Stumpferl SW (2001) Metabolism and aging in the filamentous fungus *Podospora anserina*. Arch Gerontol Geriatr 32:185–197

Osiewacz HD, Hermanns J, Marcou D, Triffi M, Esser K (1989) Mitochondrial DNA rearrangements are correlated with a delayed amplification of the mobile intron (plDNA) in a long-lived mutant of *Podospora anserina*. Mutat Res 219:9–15

Osiewacz HD, Hamann A, Zintel S (2013) Assessing organismal aging in the filamentous fungus *Podospora anserina*. Methods Mol Biol 965:439–462

Pan Y, Schroeder E, Ocampo A, Barrientos A, Shadel G (2011) Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. Cell Metab 13:668–678

Park PU, Defossez PA, Guarente L (1999) Effects of mutations in DNA repair genes on formation of ribosomal DNA circles and life span in *Saccharomyces cerevisiae*. Mol Cell Biol 19:3848–3856

Partridge L (2011) Some highlights of research on aging with invertebrates, 2010. Aging Cell 10:5–9

Parzych KR, Klionsky DJ (2014) An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal 20:460–473

Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le CD, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8:157–168

Perez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, Andziak B, Yang T, Edrey Y, Friguet B, Ward W, Richardson A, Chaudhuri A (2009) Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proc Natl Acad Sci USA 106:3059–3064

Philipp O, Hamann A, Servos J, Werner A, Koch I, Osiewacz HD (2013) A genome-wide longitudinal transcriptome analysis of the aging model *Podospora anserina*. PLoS ONE 8, e83109

Piper PW, Harris NL, MacLean M (2006) Preadaptation to efficient respiratory maintenance is essential both for maximal longevity and the retention of replicative potential in chronologically ageing yeast. Mech Ageing Dev 127:733–740

Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, Jung S, Jung YK (2013) Overexpression of *Atg5* in mice activates autophagy and extends lifespan. Nat Commun 4:2300

Rapaport D, Brunner M, Neupert W, Westermann B (1998) Fzo1p is a mitochondrial outer membrane protein essential for the biogenesis of functional mitochondria in *Saccharomyces cerevisiae*. J Biol Chem 273:20150–20155

Reverberi M, Gazzetti K, Punelli F, Scarpari M, Zjalic S, Ricelli A, Fabbri AA, Fanelli C (2012) Ao*yap1* regulates OTA synthesis by controlling cell redox balance in *Aspergillus ochraceus*. Appl Microbiol Biotechnol 95:1293–1304

Ristow M, Schmeisser S (2011) Extending life span by increasing oxidative stress. Free Radic Biol Med 51:327–336

Rizet G (1953a) Sur l'impossibilité d'obtenir la multiplication végétative ininterrompue illimitée de l'ascomycète *Podospora anserina*. C R Acad Sci Paris 237:838–855

Rizet G (1953b) Longevity of strains of *Podospora anserina*. C R Hebd Seances Acad Sci 237:1106–1109

Rodriguez KA, Osmulski PA, Pierce A, Weintraub ST, Gaczynska M, Buffenstein R (2014) A cytosolic protein factor from the naked mole-rat activates proteasomes of other species and protects these from inhibition. Biochim Biophys Acta 1842 (11):2060–72

Sastre-Moreno G, Sanchez A, Esteban V, Blanco L (2014) ATP insertion opposite 8-oxo-deoxyguanosine by Pol4 mediates error-free tolerance in *Schizosaccharomyces pombe*. Nucleic Acids Res 42:9821–9837

Scheckhuber CQ, Osiewacz HD (2008) *Podospora anserina*: a model organism to study mechanisms of healthy ageing. Mol Genet Genomics 280:365–374

Scheckhuber CQ, Erjavec N, Tinazli A, Hamann A, Nyström T, Osiewacz HD (2007) Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models. Nat Cell Biol 9:99–105

Scheckhuber CQ, Mitterbauer R, Osiewacz HD (2009) Molecular basis of and interference into degenerative processes in fungi: potential relevance for improving biotechnological performance of microorganisms. Appl Microbiol Biotechnol 85:27–35

Scheckhuber CQ, Wanger RA, Mignat CA, Osiewacz HD (2011) Unopposed mitochondrial fission leads to severe lifespan shortening. Cell Cycle 10:3105–3110

Schnell N, Krems B, Entian KD (1992) The *PAR1* (*YAP1/SNQ3*) gene of *Saccharomyces cerevisiae*, a c-*jun* homologue, is involved in oxygen metabolism. Curr Genet 21:269–273

Schulte E, Kück U, Esser K (1988) Extrachromosomal mutants from *Podospora anserina*: permanent vegetative growth in spite of multiple recombination events in the mitochondrial genome. Mol Gen Genet 211:342–349

Schulte E, Kück U, Esser K (1989) Multipartite structure of mitochondrial DNA in a fungal longlife mutant. Plasmid 21:79–84

Sellem CH, Lecellier G, Belcour L (1993) Transposition of a group II intron. Nature 366:176–178

Shigenaga MK, Hagen TM, Ames BN (1994) Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA 91:10771–10778

Shrestha A, Puente LG, Brunette S, Megeney LA (2013) The role of Yca1 in proteostasis. Yca1 regulates the composition of the insoluble proteome. J Proteomics 81:24–30

Silar P (2005) Peroxide accumulation and cell death in filamentous fungi induced by contact with a contestant. Mycol Res 109:137–149

Silar P, Picard M (1994) Increased longevity of EF-1 alpha high-fidelity mutants in *Podospora anserina*. J Mol Biol 235:231–236

Silva A, Almeida B, Sampaio-Marques B, Reis MI, Ohlmeier S, Rodrigues F, Vale A, Ludovico P (2011) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a specific substrate of yeast metacaspase. Biochim Biophys Acta 1813:2044–2049

Smith ML, Bruhn JN, Anderson JB (1992) The fungus *Armillaria bulbosa* is amongst the largest and oldest living organisms. Nature 356:428–431

Smith DL Jr, McClure JM, Matecic M, Smith JS (2007) Calorie restriction extends the chronological lifespan of *Saccharomyces cerevisiae* independently of the Sirtuins. Aging Cell 6:649–662

Soerensen M, Gredilla R, Muller-Ohldach M, Werner A, Bohr VA, Osiewacz HD, Stevnsner T (2009) A potential impact of DNA repair on ageing and lifespan in the ageing model organism *Podospora anserina*: decrease in mitochondrial DNA repair activity during ageing. Mech Ageing Dev 130:487–496

Sousa-Lopes A, Antunes F, Cyrne L, Marinho HS (2004) Decreased cellular permeability to H_2O_2 protects *Saccharomyces cerevisiae* cells in stationary phase against oxidative stress. FEBS Lett 578:152–156

Spalding MD, Prigge ST (2010) Lipoic acid metabolism in microbial pathogens. Microbiol Mol Biol Rev 74:200–228

Stadtman ER, Berlett BS (1998) Reactive oxygen-mediated protein oxidation in aging and disease. Drug Metab Rev 30:225–243

Stahl U, Lemke PA, Tudzynski P, Kück U, Esser K (1978) Evidence for plasmid like DNA in a filamentous fungus, the ascomycete *Podospora anserina*. Mol Gen Genet 162:341–343

Stahl U, Tudzynski P, Kück U, Esser K (1979) Plasmid-like DNA in senescent cultures of the ascomycetous fungus *Podospora anserina*. Z Physiol Chem 360:1045

Strobel I, Osiewacz HD (2013) Poly(ADP-ribose) polymerase is a substrate recognized by two metacaspases of *Podospora anserina*. Eukaryot Cell 12:900–912

Stumpferl SW, Stephan O, Osiewacz HD (2004) Impact of a disruption of a pathway delivering copper to mitochondria on *Podospora anserina* metabolism and life span. Eukaryot Cell 3:200–211

Sturtz LA, Diekert K, Jensen LT, Lill R, Culotta VC (2001) A fraction of yeast Cu, Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. J Biol Chem 276:38084–38089

Suppanz IE, Wurm CA, Wenzel D, Jakobs S (2009) The m-AAA protease processes cytochrome c peroxidase preferentially at the inner boundary membrane of mitochondria. Mol Biol Cell 20:572–580

Tapia PC (2006) Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: "Mitohormesis" for health and vitality. Med Hypotheses 66:832–843

Taylor EB, Rutter J (2011) Mitochondrial quality control by the ubiquitin-proteasome system. Biochem Soc Trans 39:1509–1513

Testa G, Biasi F, Poli G, Chiarpotto E (2014) Calorie restriction and dietary restriction mimetics: a strategy for improving healthy aging and longevity. Curr Pharm Des 20:2950–2977

Thön M, Al-Abdallah Q, Hortschansky P, Brakhage AA (2007) The thioredoxin system of the filamentous fungus *Aspergillus nidulans*: impact on development and oxidative stress response. J Biol Chem 282:27259–27269

Thorsness PE, White KH, Fox TD (1993) Inactivation of YME1, a member of the *ftsH-SEC18-PAS1-CDC48* family of putative ATPase-encoding genes, causes increased escape of DNA from mitochondria in *Saccharomyces cerevisiae*. Mol Cell Biol 13:5418–5426

Titorenko VI, Terlecky SR (2011) Peroxisome metabolism and cellular aging. Traffic 12:252–259

Tsang CK, Liu Y, Thomas J, Zhang Y, Zheng XF (2014) Superoxide dismutase 1 acts as a nuclear transcription factor to regulate oxidative stress resistance. Nat Commun 5:3446

Tudzynski P, Heller J, Siegmund U (2012) Reactive oxygen species generation in fungal development and pathogenesis. Curr Opin Microbiol 15:653–659

Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427

Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27:433–446

Unlu ES, Koc A (2007) Effects of deleting mitochondrial antioxidant genes on life span. Ann N Y Acad Sci 1100:505–509

Valdes-Santiago L, Ruiz-Herrera J (2013) Stress and polyamine metabolism in fungi. Front Chem 1:42

van Diepeningen AD, Slakhorst SM, Koopmanschap AB, Ikink GJ, Debets AJ, Hoekstra RF (2010) Calorie restriction in the filamentous fungus *Podospora anserina*. Exp Gerontol 45:516–524

Vlasits J, Jakopitsch C, Bernroitner M, Zamocky M, Furtmüller PG, Obinger C (2010) Mechanisms of catalase activity of heme peroxidases. Arch Biochem Biophys 500:74–81

Voigt O, Pöggeler S (2013) Self-eating to grow and kill: autophagy in filamentous ascomycetes. Appl Microbiol Biotechnol 97:9277–9290

Wang K, Jin M, Liu X, Klionsky DJ (2013) Proteolytic processing of Atg32 by the mitochondrial i-AAA protease Yme1 regulates mitophagy. Autophagy 9:1828–1836

Weber TA, Reichert AS (2010) Impaired quality control of mitochondria: aging from a new perspective. Exp Gerontol 45:503–511

Weil A, Luce K, Dröse S, Wittig I, Brandt U, Osiewacz HD (2011) Unmasking a temperature-dependent effect of the *P. anserina* i-AAA protease on aging and development. Cell Cycle 10:4280–4290

Westermann B (2010) Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol 11:872–884

Weydert CJ, Cullen JJ (2010) Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 5:51–66

Wiemer M, Osiewacz HD (2014a) The proteasome activity reporter GFP-Cl1 is degraded by autophagy in the aging model *Podospora anserina*. F1000Res 3:230

Wiemer M, Osiewacz HD (2014b) Effect of paraquat-induced oxidative stress on gene expression and aging of the filamentous ascomycete *Podospora anserina*. Microbial Cell 1:225–240

Wood ZA, Schroder E, Robin HJ, Poole LB (2003) Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 28:32–40

Yang Z, Klionsky DJ (2010) Eaten alive: a history of macroautophagy. Nat Cell Biol 12:814–822

Yasuda K, Adachi H, Fujiwara Y, Ishii N (1999) Protein carbonyl accumulation in aging dauer formation-defective (daf) mutants of *Caenorhabditis elegans*. J Gerontol A Biol Sci Med Sci 54:B47–B51

Yorimitsu T, Klionsky DJ (2005) Autophagy: molecular machinery for self-eating. Cell Death Differ 12 (Suppl 2):1542–1552

Yost FJ Jr, Fridovich I (1973) An iron-containing superoxide dismutase from *Escherichia coli*. J Biol Chem 248:4905–4908

Youn HD, Kim EJ, Roe JH, Hah YC, Kang SO (1996) A novel nickel-containing superoxide dismutase from *Streptomyces* spp. Biochem J 318:889–896

Zamocky M, Gasselhuber B, Furtmuller PG, Obinger C (2012) Molecular evolution of hydrogen peroxide degrading enzymes. Arch Biochem Biophys 525:131–144

Zintel S, Schwitalla D, Luce K, Hamann A, Osiewacz HD (2010) Increasing mitochondrial superoxide dismutase abundance leads to impairments in protein quality control and ROS scavenging systems and to lifespan shortening. Exp Gerontol 45:525–532

Zintel S, Bernhardt D, Rogowska-Wrzesinska A, Osiewacz HD (2011) PaCATB, a secreted catalase protecting *Podospora anserin*a against exogenous oxidative stress. Aging (Albany NY) 3:768–781

Signals in Growth and Development

9 Autoregulatory Signals in Mycelial Fungi

Unai Ugalde[1], Ana Belén Rodriguez-Urra[2]

CONTENTS

I. Introduction

The mycelium is a colony form which combines the ability to adapt its morphology to the underlying substrate and conditions while maintaining functional unity through interconnectedness. Thus, regions that access nutrient-rich substrates may support other's undertaking exploration or differentiation processes. This display of versatility has led to the realisation that the mycelium is governed by a sophisticated chemo-sensitive system.

Many of the elements of this system have probably been already inventoried after many decades of research, but the functional connections between them remain largely unknown. On the one hand, many reported colony functions have been ascribed to as yet unidentified chemical signals. On the other, many thousands of molecules have been purified and characterised, of which only a small proportion have been credited with a biological role.

[1]Biochemistry Laboratory II, University of the Basque Country, 1072, 20080 Donostia-San Sebastian, Spain; e-mail: unaiona.ugalde@ehu.es
[2]Biofungitek, SL, Parque Científico y Tecnológico de Bizkaia, Ibaizabal Bidea (Edificio 800-2), 48170 Zamudio, Spain

Those signals recognised so far comprise two large categories. Firstly, those directed at relations with other organisms, such as hosts and include elicitors and inhibitors, competitors involving antibiotics and predators where mycotoxins are implicated (for further information see Mycota vols V (2nd edn), X (2nd edn) and XI (2nd edn)).

The second category consists of self-directed signals that formerly fell under the generic term *hormones* (Gooday 1994). They include *pheromones,* which facilitate the interaction of compatible gametes, and *developmental hormones,* which regulate the formation or maintenance of differentiated fungal tissues or proto-organs, such as ascomata or basidiomata. These will be dealt with in Wöstemeyer et al. (2016), Dyer et al. (2016), Pelkmans et al. (2016), respectively.

In addition to hormones, other intracolonial cues convey information on the status of each cell within the mycelium. They often involve extracellular metabolites and results in adaptations which are advantageous to the colony as a living unit. In the past, these signals have been referred to as autoinducers, autoinhibitors, quorum-sensing factors and morphogens. From a functional viewpoint they all encompass the regulation of transitions between morphogenetic programmes; hence, they may be collectively termed as *autoregulators*. Signalling molecules performing autoregulatory roles had already been assigned this term when first discovered in filamentous bacteria (Horinouchi and Beppu 1992).

This review will focus on those autoregulatory signals which are involved in germination, colony morphogenesis and asexual and sexual

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

development in mycelial fungi. Finally, the incidence of autoregulators in dimorphism will also be covered.

II. Germination

The programmed outcome of every fungal spore is to land on an appropriate habitat and successfully establish a new colony. A number of mechanisms (temperature changes, physical stress and chemical sensing) are involved in gauging the feasibility of germination at a given environmental condition. Chemosensory mechanisms report on the chemical characteristics of the substrate, be it the wax composition of a leaf surface, as in the case of the obligate biotroph rust *Blumeria graminis* on perennial ryegrass (Ringelmann et al. 2009) or the sugar and amino acid composition of a ripe fruit or seed, as in the case of the saprophyte *Aspergillus niger* (Hayer et al. 2013, 2014). In addition to these cues, autoregulatory signals play an important role in preventing futile competition between sister spores.

The first demonstration that spores sense and respond to an overcrowded environment through self-produced chemical signals came from studies with the rust *Puccinia graminis* (Allen 1955). These autoregulators were specifically termed *autoinhibitors* or *self-inhibitors* (Macko and Staples 1973) and were reported to exist in more than 60 fungal species (Allen 1976). A representative collection is shown in Table 9.1.

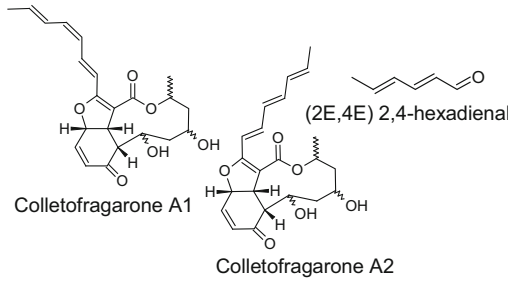
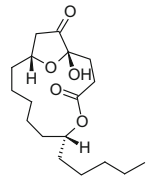
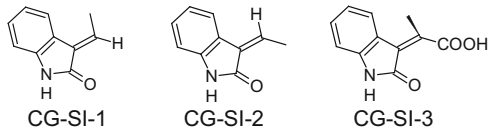
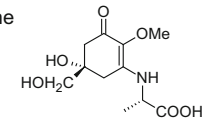
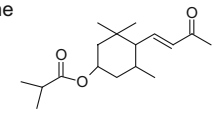
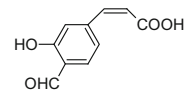
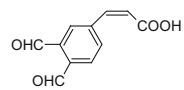
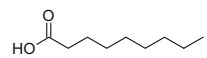
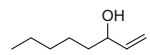
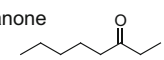
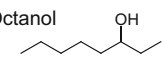
Germination autoinhibitors are thought to be produced at the time of sporulation and deposited at the outer wall layers of spores. Whether they found in diffusible form or progressively pass from a bound to soluble form is still a subject of speculation (Macko and Staples 1973). Once in contact with an aqueous medium, they diffuse through the bulk liquid surrounding the spore but also commonly partition to the gas phase and carry the signal to other spores in the vicinity. The signal is extremely effective, and 50 % inhibition was reported at nanomolar concentrations in those cases in which it was measured (Allen 1972).

Germination autoinhibitors of plant rusts show structural diversity and remarkable specificity. In the case of the bean rust *Uromyces phaseoli*, the active agent is methyl-*cis*-3,4-dimethoxycinnamate (Table 9.1), while the wheat stem rust counterpart *Puccinia graminis* is methyl-*cis*-ferulate (methyl-*cis*-4-hydroxy-3-methoxycinnamate, Table 9.1). In both cases, it has been shown that the *cis* isomer is the active inhibitor, although even low UV radiations result in a conversion into *trans* isomers with both forms found at equilibrium under natural conditions (Allen 1972). Other plant pathogens, such as *Colletotrichum gloeosporioides,* have been reported to produce CG-SI 1 and 2 ((E)- and (Z)-3-ethylidine-1, 3-dihydroindol-2-one) and CG-SI 3 ((2R)-(3-indoyl) propionic acid, Table 9.1, Tsurushima et al. 1995) and the less active molecule gloeosporone (Lax et al. 1985; Table 9.1). The related *C. fragariae* has been reported to produce several germination autoinhibitors as colletofragarone A1 and A2, the volatile (2E, 4E) 2,4-hexadienal and the previously reported molecules CG-SI 1, CG-SI-2 and CG-SI-3 (Inoue et al. 1996; Miyagawa et al. 2001; Table 9.1). The tobacco blight fungus *Peronospora tabacina*, in turn, uses quiesone (5-Isobutyroxy-β-ionone, Table 9.1) as germination autoinhibitor (Leppik et al. 1972).

In contrast with the chemical specificity displayed by plant pathogens, saprophytes appear to use a more generalised signalling cue. Nonanoic acid (Table 9.1) has been shown to be produced and sensed by spores of many soil fungi (Garret and Robinson 1969). Related compounds such as 1-octen-3-ol (Table 9.1) have been reported to act in the cereal pathogen *Penicillium paneum* (Chitarra et al. 2004) and *Aspergillus nidulans* (Herrero-García et al. 2011). Herrero-García et al. (2011) found two other related autoinhibitors, 3-octanone and 3-octanol, that could inhibit germination to a lesser degree (Table 9.1).

The marked differences in specificity between biotrophic pathogens and saprophytes may originate from the fact that the former occupy a specific niche in which the host may also generate molecules which play an important role in the infection process. These often comprise volatile alcohols of various chain

Table 9.1 Germination inhibition autoregulatory signals

Germination autoinhibitors		
Species	Molecule/structure	References
Colletotrichum fragariae		Inoue et al. (1996) Miyagawa et al. (2001)
Colletotrichum gloeosporioides	Gloeosporone 	Lax et al. (1985)
C. fragariae *C. gloeosporioides*		Tsurushima et al. (1995) Inoue et al. (1996)
Colletotrichum graminicola	Mycosporine-Alanine 	Leite and Nicholson (1992)
Peronospora tabacina	Quiesone 	Leppik et al. (1972)
Puccinia graminis	Methyl-cis-4-hydroxy -3-methoxycinnamate 	Macko et al. (1971a) Allen (1972)
Uromyces phaseoli *Puccinia helianthi* and *Puccinia antirrhini*	Methyl-cis-3, 4-dimethoxycinnamate 	Macko et al. (1970, 1971b)
Fusarium oxysporum *Syncephalastrum racemosum*	Nonanoic acid 	Garret and Robinson (1969) Hobot and Gull (1980)
Penicillium *paneumAspergillus* *nidulans*	1-Onten-3-ol 	Chitarra et al. (2004) Herrero-García et al. (2011)
Aspergillus nidulans	3-Octanone  3-Octanol 	Herrero-García et al. (2011)

lengths, such as nonyl alcohol (Allen 1972), *trans*-2-hexen-1-ol (Collins et al. 2001) and surface waxes (Podila et al. 1993). These stimulatory signals share molecular and physicochemical characteristics with germination autoinhibitors and must, therefore, be

clearly distinguished by the spore. The general consensus is that monounsaturated aliphatic chain-containing self-inhibitors are *cis* isomers, whereas the stimulators are mostly in the *trans* conformation. In addition, the positioning of the unsaturated bonds is important for differentiation between stimulators and inhibitors (Wang et al. 2002).

Saprophytes need not differentiate necessarily between self and non-self but may preferentially need to determine the extent of occupancy of the substratum in a first instance, independently of the nature of the occupants. This may be the principal reason for a less varied array of reported germination autoinhibitors in saprophytic fungi. Nevertheless, as work progresses on the chemical ecology of these signals, this current understanding may be confirmed or reformed by the weight of the evidence.

The precise mechanism of action of germination autoinhibitors remains little understood, although it has been shown that spores remain blocked at the isotropic growth stage (swelling), prior to germ tube formation (Herrero-García et al. 2011). Different molecular mechanisms have been attributed with a role in this effect. Nonanoic acid has been postulated to provoke alterations in membrane permeability and, consequently, in intracellular pH (Breewer et al. 1997). Other studies point to a direct involvement of K^+ channel activation and consequent loss of intracellular potassium (Wang et al. 2002) or interference with germination signalling pathways involving RAS and cAMP signalling pathways (Fillinger et al. 2002). Autoinhibitors have been shown to block calmodulin gene expression (Liu and Kalattukudy 1999). Whether these are directly or indirectly exerted by the germination autoinhibitors awaits verification.

Although mycelial fungi are mostly known to produce germination inhibitors, the higher fungi possess self-produced germination *autoinducers*. These are also volatile compounds, mostly short-chain fatty acids, induce germination up to a concentration threshold and become inhibitory over that level (Lösel 1967). Amongst them, isovaleric acid is the best known autoinducer, and it is produced by spores and mycelia of *Agaricus bisporus* (Rast and Stäuble 1970).

The currently accepted interpretation of the role of germination autoinducers is that they could maximise the opportunity of compatible spores to form a dikaryon (an important feature of the life cycle of basidiomycetes).

In all, the information available on germination autoinhibitors and autoinducers is still limited and dated. An interdisciplinary approach to the study of these molecules and their mode of action should yield important new knowledge for the understanding of fungal behaviour in natural conditions.

III. Colony Morphogenesis

The germ tubes emerging from spores may produce short specialised hyphae called conidial anastomosis tubes (CATs, they are also known to emerge directly from conidia, Read et al. 2012). CATs grow towards each other and fuse by complex signalling mechanisms (Fig. 9.1). Diffusible signals are consecutively released and perceived through converging CAT tips (Roca et al. 2005; Read et al. 2012). Genetically identical conidia can produce germ tubes and CATs that exhibit negative and positive chemotropism, respectively. This fact indicates that, from the very beginning of the colony formation, the presence of other cells is sensed and controlled through autoregulatory signals (Lichius et al. 2014). The identity of the extracellular signals remains unknown, although they are species specific (Read et al. 2009, 2010). Read and collaborators have recently demonstrated that RAC-1 is essential for CAT formation and cell fusion, in contrast with germ tube development, where CDC-42 is required. The authors propose a model in which the local assembly of plasma-membrane-associated GTPase–PAK–MAPK signalling elements regulates chemoattractant perception and secretion in order to synchronise oscillatory cell–cell communication and directional CAT tip growth. This mode of action explains the differences between CAT fusion and germ tube growth (Lichius et al.

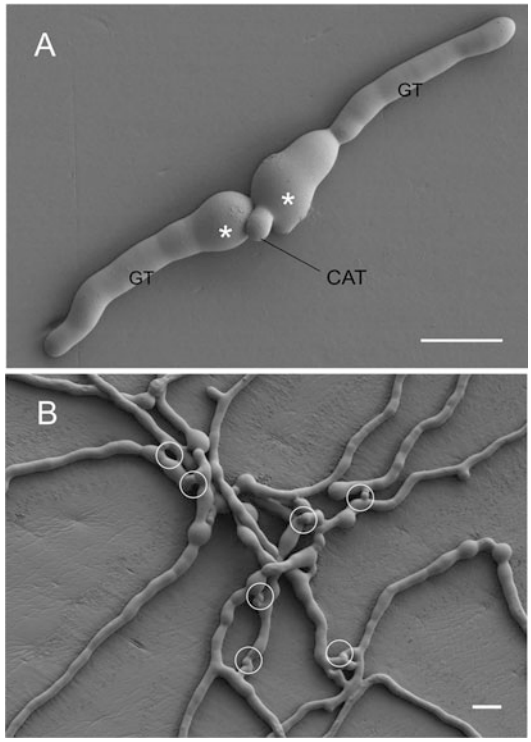


Fig. 9.1 CAT fusion between conidia and conidial germlings in *Neurospora crassa* imaged by low-temperature scanning electron microscopy. (**a**) Most CATs emerge directly from conidia homing towards other conidia (GT: germ tube). (**b**) CAT can emerge from germ tubes and fuse with other CATs, forming an interconnected, supracellular germling network (cell fusion connections are *circled*). Scale bars = 10 μm (Adapted from Lichius et al. 2014)

2014). The biological significance of CAT fusion may include the rapid formation of a multicellular platform from identical individuals that would otherwise compete for nutrients and space. Its occurrence has been reported in many ascomycetes and has been also observed in uredospores (Read et al. 2012; Read and Roca 2006).

Having completed the germination process, emerging hyphae extend away from the germling, eventually giving rise to a radial colony. The development of a colony from a germling to a mycelium has been recognised as a complex process (Fig. 9.2a, Buller 1933; Simonin et al. 2012). At the advancing edge, apical extension growth is clearly dominant with few ramifications. Older subapical zones display lateral branches which fuse with already existing leading hyphae or amongst themselves. This transforms the initial radial colony into a web-shaped network, with important functional consequences. At distal regions near the origin of the colony, living vacuolated cells support intercellular transport and the formation of long-term survival structures. There is no record of backward hyphal growth from the periphery into these regions. Hence, the colony appears to undergo a self-organising plan which can be modified in response to environmental cues such as nutrient availability, physical damage and predation (Fricker et al. 2007). The autoregulatory signals that direct mycelium development are little understood, but some elements have been reported.

Newly formed hyphae elongate by apical extension growth, and the process by which they grow away from the colony origin, as well as each other, has been the subject of study (Leeder et al. 2013). A clear depiction of this pattern is clearly provided by Hickey et al. (2002) in *Neurospora crassa* (Fig. 9.2b), but the chemical signals driving this guiding are the subject of speculation. Chemotropism driving growth towards nutrients (and therefore away from lower nutrient containing zones) has found little supporting evidence (Gooday 1975). Some specialised examples involving parasitic or symbiotic fungi and their hosts (Brand and Gow 2009 and references therein) cannot be extrapolated to mycelium development. However, positive aerotropism (tropism towards higher oxygen concentrations) has been shown to occur in early studies (Robinson 1973a, b, c). This guiding cue would not only direct hyphae away from the centre of the colony but also away from each other. No evidence has yet emerged on the existence of autoregulatory signals guiding this growth pattern, but they cannot be ruled out at this time.

In contrast to the outward tropism shown by leading hyphae at the colony edge, subapical regions generate lateral branches which fuse with each other (Gooday 1999; Fleißner and Serrano 2016). Detailed studies with fluorophore-labelled *Neurospora crassa* hyphae (Hickey et al. 2002) showed that the formation of lateral branches is induced by the remote

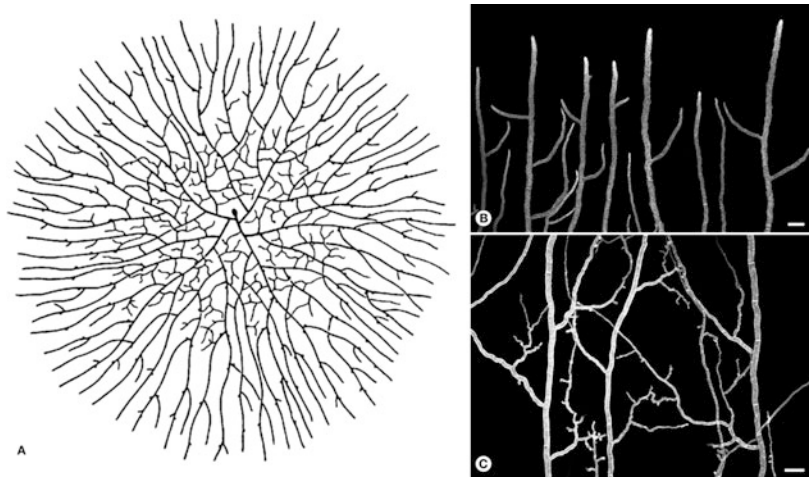


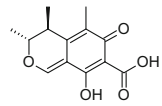
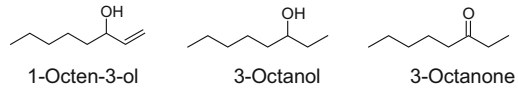
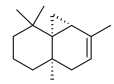
Fig. 9.2 (**a**) Drawing showing the typical pattern of a fungal colony resulting from a single germinated spore. The peripheral zone contains no anastomosis of hyphae, whereas the central zone shows anastomosis and the formation of a matrix (Adapted from Buller 1933). (**b, c**) Confocal images showing hyphal organisation at the periphery (**b**) and at distal regions (**c**) in a colony of *Neurospora crassa* (Adapted from Hickey et al. 2002)

presence of other hyphae, and moreover, once formed, they attract each other (Fig. 9.2c).

Despite these advances, the chemical signals which drive the formation of lateral branches and their homing and fusion remain unidentified. Early analyses on autoregulator-mediated positive autotropism by Müller and Jaffe (1965) in *Botrytis cinerea* postulated that a diffusible growth stimulator, with a half-life of 10 s, acting at a radius of 10 μm around each hypha, would fit in with their observations. This projection resembles other independently conducted studies (Gooday 1975). Flow cell studies using *Aspergillus oryzae* showed that substitution of fresh medium with medium which was recirculated resulted in increased apical extension growth and increased branching (Spohr et al. 1998). This supported the suggestion that a relatively stable self-produced growth and branching inducer or inducers were present in the medium. Further studies along these lines should clarify the nature of the endogenous signals.

In addition to positive autotropism (perpendicular to the growth axis) at subapical zones, a negative tropism (along the growth axis) inhibiting back growth towards older parts of the colony has also been reported in mycelial colonies. Studies in static agar cultures by Bottone et al. (1998) showed that cultures of *Mucor* spp. and *Aspergillus fumigatus* on membrane filter over agar plates, upon removal of the membranes, left clear circular patches of agar, into which back growth could not occur. Nutrient depletion was ruled out by several controls with supplementation. Back growth occurred only when the patches were replaced by fresh medium and separated from the surrounding colony by a "moat". This measure avoided back diffusion of an unidentified inhibitor from the surrounding biomass. This finding supported earlier theoretical models which could only emulate colony morphogenesis by incorporating the effect of a self-produced inhibitor of back growth in fungal colonies (Indermitte et al. 1994). A chemical signal fulfilling the requirements was later identified as extracellular bicarbonate, likely resulting from respiratory metabolism, in *Aspergillus nidulans* (Rodríguez-Urra et al. 2009). These authors demonstrated that a bicarbonate concentration gradient formed from the periphery to the centre of the colony in agar minimal medium (Table 9.2). When the bicarbonate concentration gradient reached 25 mM in subperipheral regions, it acted as a morphogen by increasing the branching frequency. At distal regions, increasing concentration exerted pro-

Table 9.2 Colony morphogenesis autoregulatory signals

Colony morphogenesis autoregulators		
Species	Molecule/structure	References
Aspergillus nidulans	Bicarbonate 	Rodríguez-Urra et al. (2009)
Aspergillus niger	Citrinin 	Robinson and Park (1966)
Aspergillus nidulans *Penicillium paneum*	 1-Octen-3-ol 3-Octanol 3-Octanone	Chitarra et al. (2004) Herrero-García et al. (2011)
Penicillium decumbens	(+)-Thujopsene 	Polizzi et al. (2011)

gressive growth inhibition, in addition to increased branching (Fig. 9.3a). At the oldest regions, growth eventually ceased, leaving cells in a viable quiescent state (Fig. 9.3b,c).

The mechanism by which bicarbonate exerts this overlapping biphasic effect on hyphal morphology and growth rate has been associated with the activation of adenylyl cyclase (Bahn and Mühlschlegel 2006). In *Candida albicans*, the enzyme (Cyr1p) which is the highly conserved Lys 1373 residue is essential for CO_2/bicarbonate binding, resulting in increased cAMP levels and filamentation within the colony (Hall et al. 2010). No such effects have been demonstrated in filamentous fungi, although earlier reports do confirm increased branching effects with upon addition of cAMP to the growth medium (Robson et al. 1991).

Another example of an endogenous factor exerting effects on colony morphology is that of citrinin in *Aspergillus niger* where it has been attributed the role of inducing vacuolation and thin hyphae formation at the centre of the colony (Robinson and Park 1966, Table 9.2).

Volatile organic compounds (VOCs) have already been attributed a role as quorum-sensing factors in germination (see above). In addition, they also act as hyphal extension rate inhibitors with no apparent role on branching in *Penicillium paneum* (Chitarra et al. 2004) and *A. nidulans* (Herrero-García et al. 2011). Although these studies describe an inhibitory effect between colonies, an intra-colonial effect may not be ruled out. The volatile sesquiterpene (+)-thujopsene has also been postulated to exert an autoregulatory function on hyphal extension in *Penicillium decumbens* (Polizzi et al. 2011; Table 9.2).

It is generally accepted that specific signals direct positive autotropism and self-fusion (anastomosis) between intra-colonial hyphae behind the peripheral region. The nature of the chemical signals involved in these processes remains to be elucidated (Kües and Navarro-González 2009). Small extracellular peptides sharing molecular features with pheromones remain plausible candidates, given the similarity between both processes (Read et al. 2012).

IV. Asexual Development

Behind the peripheral growth region, where cells are no longer engaged in colony expansion, aerial hyphae differentiate into mitospore-producing structures, suitable for dispersal. Conidia are a common form of mitospore present in Ascomycetes and Basidiomycetes (Webster and Weber 2007). For more details on conidiation induction, see the review by Park and Yu (2012). The mechanism by which hyphae trigger the process involves the sensing of several environmental cues, amongst which low water activity, light, contact with the

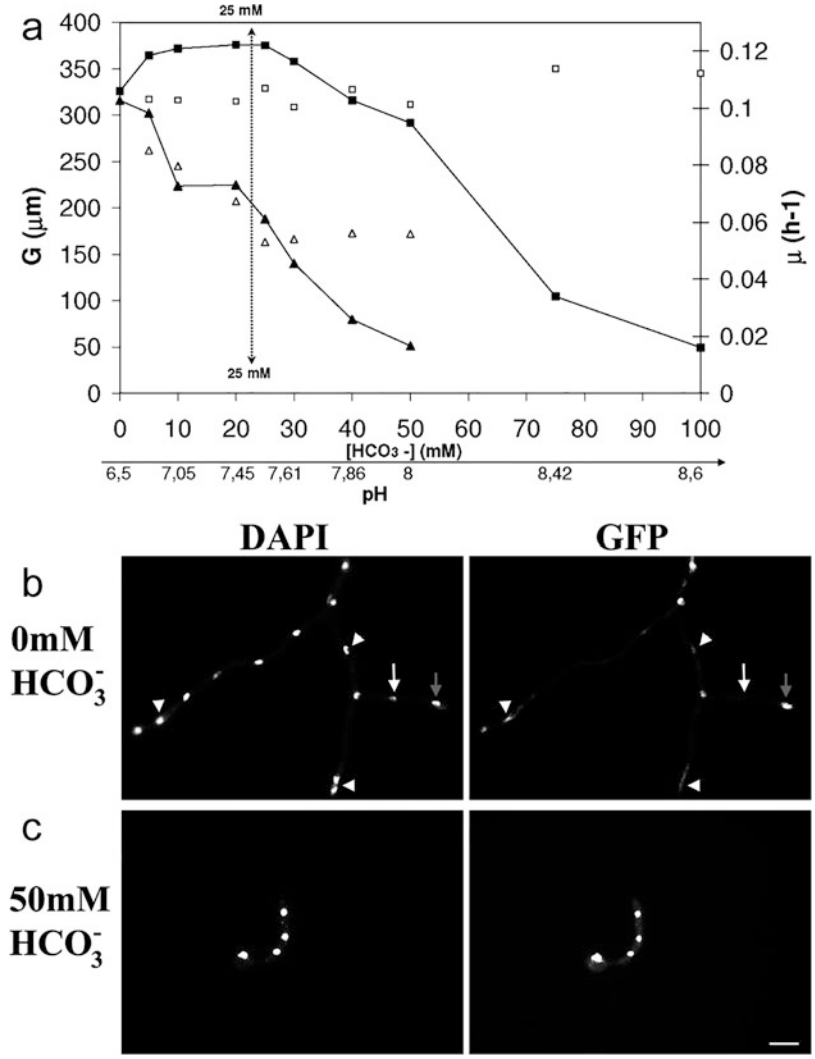
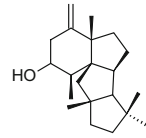
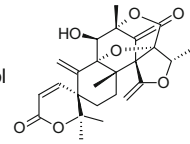
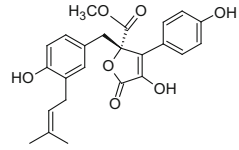
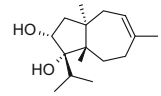
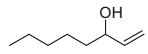
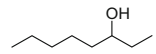
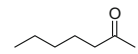


Fig. 9.3 Bicarbonate modulates kinetic parameters G and μ and induces quiescence in cells at the colony centre. (**a**) $KHCO_3$ was incorporated into MMA plates, emulating the intra-colonial bicarbonate concentration and pH. Parallel bicarbonate-free MMA controls were set at the appropriate pH values for each treatment. Two growth kinetics parameters were analysed in *Aspergillus nidulans* cultures: G, the length of the hyphal growth unit, which represents the average length of a hypha which supports the extension of each tip in the mycelium (high values of G are indicative of low mycelial branching and vice versa). And μ, the specific growth rate, which indicates the rate of biomass production per unit time. Low concentrations of bicarbonate (0–25 mM) affected the hyphal growth unit, G, resulting in increased hyphal branching. At concentrations above 25 mM, hyphal branching continued to increase, and specific growth rate (μ) was affected. (*filled triangle*) G in HCO_3^- cultures, (*open square*) G in control HCO_3^--free cultures (at the same pH as the former); (*closed square*) μ in HCO_3^- cultures; (*open square*) μ in control HCO_3^--free cultures (at the same pH as the former). Standard deviations (not shown) are lower than ±10 %. (**b, c**) The stage of the cell cycle at which cells remained under bicarbonate culture conditions was investigated. Sporelings (6 h) exposed to conditions emulating those at the centre of a 72 h colony (MMA diluted to 1/10, pH 9, and 50 mM $KHCO_3$) were analysed by observing their nuclei after DAPI (4',6-diamidino-2-phenylindole) staining. The strain used also had the B type cyclin NimE tagged with green fluorescent protein (GFP). NimE is localised in the cytosol and gradually accumulates in the nucleus during G1. This process proceeds through S phase (DNA synthesis) and reaches maximal accumulation at G2 prior to mitosis. When mitosis begins, NimE is degraded. (**b**) Control cells grown in the absence of bicarbonate show varied NimE distribu-

Table 9.3 Asexual development autoregulatory signals

Asexual development autoregulators					
Species	Molecule/structure				References
Penicillium cyclopium	Conidiogenone				Roncal et al. (2002)
Aspergillus nidulans	Dehydroaustinol				Rodríguez-Urra et al. (2012)
Aspergillus terreus	Butyrolactone I				Schimmel et al. (1998)
Trichoderma virens	Carot-4-en9,10-diol				Wang et al. (2013)
Aspergillus nidulans *Trichoderma atroviride*	 1-Octen-3-ol	 3-Octanol	 3-Octanone		Nemčovič et al. (2008) Herrero-García et al. (2011)

atmosphere and the proximity of other fungi have been described. Endogenous factors have been attributed important roles in this process (Etxebeste et al. 2010).

Early studies of asexual spore production point to the involvement of endogenous factors in calcium-induced submerged sporulation in *Penicillium notatum* (Hadley and Harrold 1958). Four decades later, the diterpene conidiogenone was identified and attributed a conidiation-inducing role in the closely related *P. cyclopium*. Calcium ions were shown to act as enhancers in the induction process (Roncal et al. 2002). The authors proposed that when the hyphae grow in a liquid environment, conidiogenone is diluted in the bulk medium. On emergence to the air, however, conidiogenone is believed to accumulate at the surface of hyphae, thus attaining threshold levels (350 pico M) which activate the conidiation through as yet unknown sensory processes (Table 9.3).

In order to avoid long-term signal accumulation which would result in miss-scheduled conidiation induction, conidiogenone is continuously converted to an inactive derivative conidiogenol by the reduction of a single keto group (Roncal et al. 2002).

In *Aspergillus nidulans*, a secreted factor was attributed a role in early induction of conidiation, which was not produced in mutants defective in the *fluG* gene (Lee and Adams 1994). Later studies showed that exogenous addition of the meroterpenoid dehydroaustinol, obtained from culture extracts of a wild-

Fig. 9.3 (continued) tions, due to the asynchronous occurrence of different cell cycle stages. NimE::GFP accumulates in nuclei in G2 (*grey arrow*), is absent in mitosis (*white arrow*) and resynthesised in the cytoplasm after the completion of mitosis (early G1) and partially accumulating in nuclei in mature G1 (*white arrowhead*). (**c**) In the presence of bicarbonate, NimE::GFP protein accumulates in nuclei as determined by co-staining with DAPI, with some protein remaining in the cytoplasm. DAPI-stained nuclei appeared compact, which is normally ascribed to cells in G_1. Scale bar = 10 μm (Adapted from Rodríguez-Urra 2012)

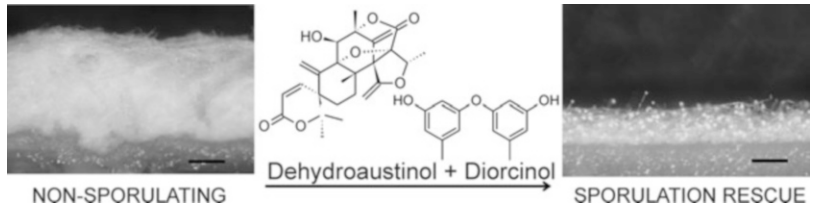


Fig. 9.4 *Aspergillus nidulans* wild-type culture extracts containing dehydroaustinol and diorcinol restore aerial sporulation in ΔfluG colonies. Diorcinol prevents dehydroaustinol crystal formation. Moreover, wild-type extract-treated colonies rescue the sporulating phenotype and do not show masses of undifferentiated hyphae. Scale bar = 1.5 mm (Adapted from Rodríguez-Urra et al. 2012)

type strain, restored the conidial phenotype in a null *fluG* mutant (Table 9.3; Rodríguez-Urra et al. 2012). The signalling compound was found forming an adduct with diorcinol, which prevented the formation of dehydroaustinol crystals, thus enabling the signalling function at the aerial hypha surface (Fig. 9.4; Rodríguez-Urra et al. 2012).

A second secreted factor acting downstream of the FluG signal was also identified using other mutants defective in the formation of the conidium-bearing structure (conidiophore). Its synthesis requires a set of four genes (*flbA-D*; Flb, standing for fluffy with low *brlA* expression), and it is required for the formation of the conidiophore (Wieser et al. 1994). In contrast to the FluG signal, which is secreted by growing hyphae at all times (Cordobés 2006), the Flb signal is only produced by hyphae that are about to become conidiophores (Iradi and Ugalde, unpublished results). This would support the view that the Flb signal may determine those cells destined to form conidiophores, as opposed to other developmental paths.

Miscellaneous examples of other self-produced secondary metabolites exerting a role as autoregulators in asexual spore production have been reported in different fungal species. The partially elucidated diterpene sporogen-PF-1 is produced by *Penicillium funiculosum* under blue light and promotes the production of conidia (Katayama et al. 1989). The sesquiterpene sporogen-AO1 ((1aR,6R,7bR)-5,6,7,7a-tetrahydro-6-hydroxy-7,7a-dimethyl-1a-(prop-1-en-2-yl)naphtho[2,1-b]oxiren-2(1aH,4H,7bH)-one) has been reported to exert sporogenic effects in *Aspergillus oryzae* (Tanaka et al. 1984). Butyrolactone I (methyl 2-(4-hydroxy-3-(3-methylbut-2-enyl) benzyl)-tetrahydro-3-(4-hydroxy phenyl)-4,5-dioxofuran-2-carboxylate; Table 9.3), a small γ-butyrolactone-containing metabolite, has been found in *Aspergillus terreus* cultures, with significant effects on branching and asexual spore production (Schimmel et al. 1998). Moreover, butyrolactone I addition to liquid cultures of *A. terreus* triggered a significant increase in the butyrolactone I levels associated with an increase in the production of the secondary metabolite lovastatin. This suggests that butyrolactone I is capable to induce its own synthesis following a similar pattern as reported for several quorum-sensing molecules (Raina et al. 2012). Interestingly, small γ-butyrolactone-containing molecules are important quorum-sensing factors in gram-negative bacteria and also regulate cellular differentiation in filamentous bacteria (McCormick and Flärdh 2012).

Quorum-sensing signals that modulate germination or growth (see earlier sections) have also been shown to regulate the formation of asexual spores. 8COs, which inhibit germination and colony growth, are capable of enhancing conidiation response in *A. nidulans* and *Trichoderma atroviride* (Herrero-García et al. 2011; Nemčovič et al. 2008; Table 9.3). These compounds were shown regulate asexual development at very early stages (Herrero-García et al. 2011). The multiple mode of action of 8COs is coherent with the biological responses required under growth restrictive conditions. The mechanism of action remains unclear.

Another example of an autoregulator involved in stress-related asexual development has been recently described example, in *Tricho-*

derma virens, where production of the sesquiterpenoid carot-4-en-9,10-diol has been reported under cathecol-induced stress, also exerting an autoregulatory conidiation-inducing role (Wang et al. 2013; Table 9.3).

V. Sexual Development

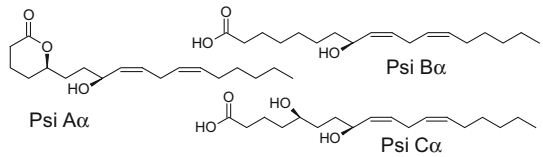
In addition to the formation of mitospores, which often serve as short-term dispersal propagules, mycelial fungi may also produce sexual organs bearing meiospores. These, in turn, are endowed with the capacity to endure long periods with minimal loss of viability (Webster and Weber 2007). Soil-dwelling species often produce sclerotia, survival structures containing specialised hyphae, which may have derived from meiospore development in ascomycetes (Geiser et al. 1996). Sexual development and its regulation are covered in Peraza-Reyes and Malagnac (2016).

In the Aspergilli, sexual development involves the formation of asci-bearing structures called cleistothecia. The process is often favoured in dark, nutritionally poor and enclosed conditions (for a full review, consult Dyer and O'Gorman 2012). Endogenous signals have shown to play a role in the triggering of sexual development, as well as on the balance between asexual and sexual development within the colony.

Pioneering work on the role of autoregulatory signals in sexual development by Sewell P. Champe and collaborators represents an example of the importance of a multidisciplinary studies in biology. A series of mutants of *Aspergillus nidulans* which showed low conidiation levels, premature production of cleistothecia (sexual development structures) and the secretion of the antibiotic diorcinol (3,3′-dihydroxy-5,5′-dimethyldiphenyl ether) led the investigators to conclude that asexual and sexual development were controlled by a common modulating pathway (Butnick et al. 1984a, b). It was later observed that the overproduction of a solvent extractable chemical factor (psi factor, standing for premature sexual induction) was responsible for the phenotype. Even when administered to wild-type strains, the factor could inhibit conidiation and promote sexual development (Champe et al. 1987). Three types of hydroxylated derivatives of C18 unsaturated fatty acids (also called oxylipins) were identified based on the starting fatty acid molecule subject to hydroxylation. Those based on oleic acid (18:1) were termed psiβ, those derived from linoleic (18:2), psiα, and those from linolenic (18:3), psiγ. The placing of the hydroxyl groups gave rise to the terms psiB(hydroxyl substitution in position 8), psiC (position 5 and 8) and psiA (a lactoniester of psiC; Champe and El-Zayat 1989; Mazur et al. 1990, 1991).

Dosage studies with psiα oxylipins indicated that marked differences in biological activity could be obtained between applications of psiBα (8-hydroxylinoleic acid, Table 9.4) and psiCα (5,8-dihydroxylinoleic acid, Table 9.4), which stimulated sexual spore development to the detriment of conidiation, and psiAα (a lactonized ester of psiCα, Table 9.4) which acted as an antagonist which shifted the balance towards asexual spore development.

Table 9.4 Sexual development autoregulatory signals

Sexual development autoregulators		
Species	Molecule/structure	References
Aspergillus nidulans	Nitric Oxide NO	Baidya et al. (2011) Marcos et al. (2011)
Aspergillus spp.		Champe and El-Zayat (1989) Mazur et al. (1990, 1991)

Later studies by Tsitsigiannis et al. (2004a, b, 2005) and Brodhun and Feussner (2011) further identified and characterised two *A. nidulans* linoleate diol synthases (dioxygenases), PpoA and PpoC, which are required for the biosynthesis of psiBα and psiBγ, respectively. The former oxylipin was confirmed to provoke a decrease in the ratio of conidia to ascospores, while the latter provoked the contrary. The concerted regulation of both enzymatic activities through gene expression was shown to be complex, and dependent on, but also modulating the expression of *brlA* and *nsdD*, central regulator genes of conidiogenesis and ascogenesis, respectively. Moreover, detailed transcriptional and biochemical analysis showed that psiBα and psiBγ possibly act as antagonist signals in regulatory feedback loops which ultimately control lipid biosynthesis.

The above studies appear as the initial unveiling of a sophisticated regulation system involving an array of oxylipin derivatives as modulators of the balance between asexual and sexual development.

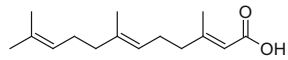
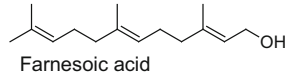
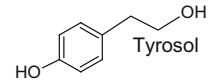
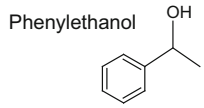
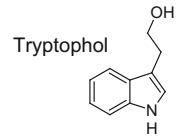
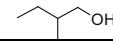
The formulation of such endogenous autoregulatory signals is also known to integrate exogenous stimuli, and one environmental cue which affects the equilibrium between sexual and asexual development in wild-type cultures of *Aspergillus nidulans* is light, which stimulates conidiation and represses sexual development (Bayram and Braus 2011). An active *veA* gene, encoding for light sensitivity in this organism (Yager 1992), is essential for sensitivity to externally added linoleic acid derivatives (Champe et al. 1987) and even affects fatty acid content and composition (Calvo et al. 1999).

The above cases may be considered as examples of metabolites which regulate the balance sexual and asexual development. Other investigations have revealed a role of self-generated reactive oxygen species (ROS) as triggering signals of sexual development. Endogenously produced nitric oxide has been shown to promote cleistothecial production in *A. nidulans* (Baidya et al. 2011; Marcos et al. 2011; Table 9.4). Indeed, the overall oxidation state of the cell appears to determine various steps of sexual development. Lara-Ortiz and collaborators (2003) demonstrated that a NADPH oxidase (NoxA) which is a member of a family of enzymes ubiquitous to lower eukaryotes plays a relevant role in cleistothecial development in *Aspergillus nidulans*. Expression of *NoxA* is specifically induced in Hülle cells and the outer layers of cleistothecia initials, and its deletion blocks maturation of cleistothecia at an early stage. Cleistothecia and associated Hülle cells both produce ROS (peroxide which is probably dismutated to H_2O_2) during development, and treatment with DPI (diphenyleneiodonium sulphate), a substrate inhibitor of NADPH oxidases, blocks the process. The authors therefore proposed that H_2O_2 regulates the differentiation of ascogenous and peridial tissues. A homologue of *NoxA* in *Podospora anserina* (*PaNox1*) has also been shown to be involved in differentiation of fruiting bodies (Malagnac et al. 2004), showing that ROS act as sexual development signals across a wide range ascomycete fungi. Whether they are specific regulators of this morphogenetic process or act as a signal within the wider context of the ageing colony remains to be clarified (Bernhardt et al. 2014).

VI. Dimorphism

The transition between yeast and mycelial forms is a common feature in some genera and has traditionally been associated with virulence in pathogens such as *Candida albicans*, *Histoplasma capsulatum* and *Penicillium marneffei* (see The Mycota Vol XII (2nd edn) and a review by Albuquerque and Casadevall 2012). In *C. albicans,* cellular density determines the morphological transition between yeast and mycelial forms. At high cell densities ($>10^6$ cells mL^{-1}), the yeast form predominates, while low cell densities favour the production of germ tubes (Hornby et al. 2001). Two molecules which were responsible for the maintenance of the yeast form at high cell densities were identified simultaneously as farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol, Table 9.5; Hornby et al. 2001) and farnesoic acid (3,7,11-trimethyl-2,6,10-dodecatrienoate, Table 9.5; Oh et al. 2001). Both compounds are produced by yeast cells and are effective in

Table 9.5 Dimorphism and quorum-sensing autoregulatory signals

Dimorphism and quorum-sensing autoregulators		
Species	Molecule/structure	References
Candida albicans	 Farnesol  Farnesoic acid  Tyrosol	Hornby et al. (2001) Oh et al. (2001) Chen et al. (2004)
Saccharomyces cerevisiae	 Phenylethanol  Tryptophol	Chen and Fink (2006)
Ophiostoma ulmi	2-Methyl-1-Butanol 	Berrocal et al. (2012)

the micromolar concentration range. Farnesol has also been described to inhibit biofilm formation (Ramage et al. 2002), and an array of genes affected by hyphae exposed to farnesol has been reported (Cao et al. 2005), many of them related to oxidative stress (Shirtliff et al. 2009).

The mycelial form of *C. albicans*, predominant at low-density cultures ($<10^6$ cells mL^{-1}), produces tyrosol (2-(4-hydroxyphenyl) ethanol, Table 9.5). This autoregulatory signal is capable of stimulating germ tube formation and extension at concentrations around 3 μM (Chen et al. 2004). Aromatic alcohols phenylethanol and tryptophol have been reported as quorum-sensing molecules that induce filamentation of *Saccharomyces cerevisiae* under nitrogen stress (Chen and Fink 2006, Table 9.5). The authors showed that these alcohols did not have any effects on the dimorphic switch in *C. albicans*, although the latter can produce them organism (Ghosh et al. 2008). Recent studies by Berrocal et al. (2012) on the Dutch elm disease-causing pathogen *Ophiostoma ulmi* have shown that 2-methyl-1-butanol was produced by high-density yeast-form cultures and the quorum-sensing factor-inhibited germ tube formation, a feature of low-density cultures.

Taken together, the contributions that are emerging in the literature point to an increasing number of quorum-sensing molecules acting as intercelular communication signals that also participate in other processes, such as endurance of host attack during infection or abiotic stress (Albuquerque and Casadevall 2012). The spectrum of these signals may expand beyond aromatic alcohols. A quorum-sensing peptide which is derived from the cleavage of a larger precursor has been described in the human pathogen *Cryptococcus neoformans* (Lee et al. 2007).

VII. Conclusions

The information presented in this chapter reflects on the participation of autoregulatory signals in a wide range of mycelial processes. The various examples of this participation also indicate that the action of autoregulatory signals is combined with other environmental cues. Autoregulatory signals serve to integrate the responses of single cells into a higher level of organisation, represented by the colony. The versatile responses observed in mycelia or their parts under different conditions may anticipate the future unveiling of a number of known endogenous fungal compounds as autoregulators. Others may simply assist as medium conditioners or adjuvants necessary for proper function of the former.

One evident avenue to pursue in order to assign biological roles to candidate molecules is to define a functional assay, obtain biological extracts from cultures and test extracts against the assay through a purification procedure.

This approach has enabled the discovery of many autoregulatory signals and should doubtlessly continue to prove fruitful in the future.

However, there are other approaches which can be beneficially followed which take advantage of our current knowledge of molecular biology and natural product's chemistry. For instance, autoregulators fulfilling comparable biological roles show marked similarities in their molecular structure and physico-chemical properties. These properties provide some clues to the possible functions of other similar molecules encountered within extracts of the same organism or from different species. In this regard, new disciplines like metabolomics should contribute to the discovery of new autoregulatory signals when combined with classical approaches.

If autoregulatory signals are relevant for the understanding of functional aspects of fungal cells and colonies, they also offer a potential for the development of new biological products. One attractive aspect of autoregulatory signals arising from their fundamental role is that their long-term application cannot result in a resistance by the target organism, since it is also the producer of the compound in nature.

Germination inhibitors could find a place in biological agriculture or the control of post-harvest pathogens, by reducing the need for conventional antifungal agents which often present unwanted hazards. Staling factors indicating senescent regions of colonies could potentially be used to limit the spread of certain unwanted fungal pathogens, in combination with chemical treatments, or biological control agents. Conidiation autoinducers could be employed to attain high titres of conidia from large-scale fermenter cultures, where conidiation is rarely achieved. Much the same application could be found for autoregulators predictably involved in the production of microsclerotia.

Acknowledgements The authors wish to acknowledge those who pioneered the study of chemical signals in mycelial fungi, particularly Professor Paul J. Allen, from the Department of Botany, University of Wisconsin, who first described fungal autoínhibitors in rusts, and Professor Sewell P. Champe from The Waksman Institute, Rutgers University, New Jersey, for the discovery of *psi* factors through genetic studies.
Most of the cited publications by the first author's research group were financed by the Department of Education, Universities and Research of The Basque Government and The Ministry of Education and Science of Spain. Continued support received by the University of The Basque Country through equipment, student grants and postdoctoral contracts is gratefully acknowledged.

References

Albuquerque P, Casadevall A (2012) Quorum sensing in fungi- a review. Med Mycol 50(4):337–345. doi:10.3109/13693786.2011.652201

Allen PJ (1955) The role of a self-inhibitor in the germination of rust uredospores. Phytopathology 45:259–266

Allen PJ (1972) Specificity of the *cis*-isomers of inhibitors of uredospore germination in the rust fungi. Proc Natl Acad Sci USA 69:3497–3500

Allen PJ (1976) Spore germination and its regulation. In: Heitefuss R, Williams PH (eds) Encyclopedia of plant physiology, vol 4. Springer, New York, pp 51–85

Bahn YS, Mühlschlegel FA (2006) CO_2 sensing in fungi and beyond. Curr Opin Microbiol 9:572–578

Baidya S, Cary JW, Grayburn WS, Calvo AM (2011) Role of nitric oxide and flavohemoglobin homologous genes in *Aspergillus nidulans* sexual development and mycotoxin production. Appl Environ Microbiol 77:5524–5528. doi:10.1128/AEM.00638-11

Bayram Ö, Braus GH (2011) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol Rev 36:1–24. doi:10.1111/j.1574-6976.2011.00285.x

Bernhardt D, Hamann A, Osiewacz HD (2014) The role of mitochondria in fungal aging. Curr Opin Microbiol 22:1–7. doi:10.1016/j.mib.2014.09.007

Berrocal A, Navarrete J, Oviedo C, Nickerson KW (2012) Quorum sensing activity in *Ophiostoma ulmi*: effects of fusel oils and branched chain amino acids on yeast-mycelial dimorphism. J Appl Microbiol 113(1):126–134. doi:10.1111/j.1365-2672.2012.05317.x

Bottone EJ, Nagarsheth N, Chiu K (1998) Evidence of self-inhibition by filamentous fungi accounts for unidirectional hyphal growth in colonies. Can J Microbiol 44:390–393

Brand A, Gow NAR (2009) Mechanisms of hypha orientation of fungi. Curr Opin Microbiol 12(4):350–357. doi:10.1016/j.mib.2009.05.007

Breewer P, de Reu JC, Dracourt J-L, Rambouts FM, Abee T (1997) Nonanoic acid, a fungal self-inhibitor, prevents germination of *Rhizopus*

oligosporus sporangiospores by dissipation of the pH gradient. Appl Environ Microbiol 63:178–185
Brodhun F, Feussner I (2011) Oxylipins in fungi. FEBS J 278:1047–1063. doi:10.1111/j.1742-4658.2011.08027.x
Buller AHR (1933) Researches on fungi, vol 5. Longman, London
Butnick NZ, Yager LN, Kurtz MB, Champe SP (1984a) Genetic analysis of mutants of *Aspergillus nidulans* blocked at an early stage of sporulation. J Bacteriol 160:541–545
Butnick NZ, Yager LN, Hermann TE, Kurtz MB, Champe SP (1984b) Mutants of *Aspergillus nidulans* blocked at an early stage of sporulation secrete an unusual metabolite. J Bacteriol 160:533–540
Calvo AM, Hinze MII, Gardner HW, Keller NP (1999) Sporogenic effect of polyunsaturated fatty acids on development of *Aspergillus* spp. Appl Environ Microbiol 65:3668–3673
Cao YY, Cao YB, Xu Z, Ying K, Li Y, Xie Y, Zhu ZY, Chen WS, Jiang YY (2005) cDNA microarray analysis of differential gene expression in Candida albicans biofilm exposed to farnesol. Antimicrob Agents Chemother 49:584–589
Champe SP, El-Zayat AAE (1989) Isolation of a sexual sporulation hormone from *Aspergillus nidulans*. J Bacteriol 171:3982–3988
Champe SP, Rao P, Chang A (1987) An endogenous inducer of sexual development in *Aspergillus nidulans*. J Gen Microbiol 133:1383–1387
Chen H, Fink GR (2006) Feedback control of morphogenesis in fungi by aromatic alcohols. Genes Dev 20:1150–1161
Chen H, Fujita M, Feng Q, Clardy J, Fink GR (2004) Tyrosol is a quorum-sensing molecule in *Candida albicans*. Proc Natl Acad Sci USA 101:5048–5052
Chitarra GS, Abee T, Rombouts FM, Posthumus MA, Dijksterhuis J (2004) Germination of *Penicillium paneum* conidia is regulated by octen-3-ol, a volatile self-inhibitor. Appl Environ Microbiol 70:2823–2829
Collins TJ, Moerschbacher BM, Read ND (2001) Synergistic induction of wheat stem rust appressoria by chemical and topological signals. Physiol Mol Plant Pathol 58:259–266
Cordobés S (2006) Study of conidiation in filamentous fungi caused by signaling molecules. PhD thesis, University of Basque Country (UPV-EHU), San Sebastian
Dyer PS, O'Gorman CM (2012) Sexual development and cryptic sexuality in fungi: insights from *Aspergillus* species. FEMS Microbiol Rev 36:165–192. doi:10.1111/j.1574-6976.2011.00308.x
Dyer PS, Inderbitzin P, Debuchy R (2016) Mating-type structure, function, regulation and evolution in the pezizomycotina. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 351–385
Etxebeste O, Garzia A, Espeso EA, Ugalde U (2010) *Aspergillus nidulans* asexual development: making the most of cellular modules. Trends Microbiol 18 (12):569–576. doi:10.1016/j.tim.2010.09.007
Fillinger S, Chaveroche M-K, Shimizu K, Keller NP, d'Enfert C (2002) cAMP and ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 63:3995–4000
Fleißner A, Serrano A (2016) The Art of networking: vegetative hyphal fusion in filamentous ascomycete fungi. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 133–153
Fricker MD, Boddy L, Bebber DP (2007) Network organisation of mycelial fungi. In: Howard RJ, Gow NAR (eds) The mycota VIII. Springer, Berlin, pp 309–330
Garret MK, Robinson PM (1969) A stable inhibitor of germination produced by fungi. Arch Microbiol 67:370–377
Geiser DM, Timberlake WE, Arnold ML (1996) Loss of meiosis in *Aspergillus nidulans*. Mol Biol Evol 13:809–817
Ghosh S, Kebaara BW, Atkin AL, Nickerson KW (2008) Regulation of aromatic alcohol production in *Candida albicans*. Appl Environ Microbiol 74:7211–7218. doi:10.1128/AEM.01614-08
Gooday GW (1975) Chemotaxis and chemotropism in fungi and algae. In: Carlile MJ (ed) Primitive sensory and communication systems. Academic, London, pp 155–204
Gooday GW (1994) Hormones in mycelial fungi. In: Wessels JGH, Meihardt F (eds) The mycota I. Growth, differentiation and sexuality. Springer, Berlin, pp 401–411
Gooday GW (1999) Hyphal interactions. In: England R, Hobbs G, Bainton N, McL RD (eds) Microbial signalling and communication, Symposium 57. Society for general microbiology. Cambridge University Press, Cambridge, pp 307–334
Hadley G, Harrold CE (1958) The sporulation of *Penicillium notatum* Westling in submerged liquid culture II. J Exp Bot 9:418–425
Hall RA, De Sordi L, Maccallum DM, Topal H, Eaton R, Bloor JW, Robinson GK, Levin LR, Buck J, Wang Y, Gow NA, Steegborn C, Mühlschlegel FA (2010) CO_2 acts as a signalling molecule in populations of the fungal pathogen *Candida albicans*. PLoS Pathog 6, e1001193. doi:10.1371/journal.ppat.1001193
Hayer K, Stratford M, Archer DB (2013) Structural features of sugars that trigger or support conidial germination in the filamentous fungus *Aspergillus niger*. Appl Environ Microbiol 79(22):6924–6931. doi:10.1128/AEM.02061-13
Hayer K, Stratford M, Archer DB (2014) Germination of *Aspergillus niger* conidia is triggered by nitrogen compounds related to L-amino acids. Appl

Environ Microbiol 80(19):6046–6053. doi:10.1128/AEM.01078-14

Herrero-García E, Garzia A, Cordobes S, Espeso EA, Ugalde U (2011) 8-carbon oxylipins inhibit germination and growth, and stimulate aerial conidiation in *Aspergillus nidulans*. Fungal Biol 115:393–400. doi:10.1016/j.funbio.2011.02.005

Hickey PC, Jacobson DJ, Read ND, Glass NL (2002) Live-cell imaging of vegetative hyphal fusion in *Neurospora crassa*. Fungal Genet Biol 37:109–119

Hobot JE, Gull K (1980) The identification of a self-inhibitor from *Syncephalastrum racemosum* and its effects upon sporiongospore germination. Antonie Leeuwenhoek 46:435–441

Horinouchi S, Beppu T (1992) Autoregulatory factors and communication in actinomycetes. Annu Rev Microbiol 31:377–398

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl Environ Microbiol 67:2982–2992

Indermitte C, Liebling TH, Clemencon H (1994) Culture analysis and external interaction models of mycelial growth. Bull Math Biol 56:633–644

Inoue M, Takenaka H, Tsurushima T, Miyagawa H, Ueno T (1996) Colletofragarones A1 and A2, novel germination self-inhibitors from the fungus *Colletotrichum fragariae*. Tetrahedron Lett 37:5731–5734

Katayama M, Yanagi M, Marumo S (1989) Isolation of sporogen-PF 1, a blue light-induced sporogenic substance from *Penicillium funiculosum*. Agric Biol Chem 53:3379–3380

Kües U, Navarro-González M (2009) Communication of fungi on individual, species, kingdom, and above kingdom levels. In: Anke T, Weber D (eds) The mycota: physiology and genetics: selected basic and applied aspects, vol 15. Springer, Berlin, pp 79–106. doi:10.1007/978-3-642-00286-1_5

Lara-Ortiz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol Microbiol 50:1241–1255

Lax AR, Templeton GE, Meyer WL (1985) Isolation, purification, and biological activity of a self-inhibitor from conidia of Colletotrichum gloeosporioides. Phytopathology 75:386–390

Lee BN, Adams TH (1994) The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev 8:641–651

Lee H, Chang YC, Nardone G, Kwon-Chung KJ (2007) TUP1 disruption in *Cryptococcus neoformans* uncovers a peptide-mediated density-dependent growth phenomenon that mimics quorum sensing. Mol Microbiol 64:591–601

Leeder AC, Jonkers W, Li J, Glass NL (2013) Early colony establishment in *Neurospora crassa* requires a MAP kinase regulatory network. Genetics 195:883–898. doi:10.1534/genetics.113.156984

Leite B, Nicholson RL (1992) Mycosporine-alanine: a self-inhibitor of germination from the conidial mucilage of *Colletotrichum graminicola*. Exp Mycol 16:76–86

Leppik RA, Hollomon DW, Bottomley W (1972) Quiesone: an inhibitor of the germination of *Peronospora tabacina* conidia. Phytochemistry 11:2055–2063

Lichius A, Goryachev AB, Fricker MD, Obara B, Castro-Longoria E, Read ND (2014) CDC-42 and RAC-1 regulate opposite chemotropisms in *Neurospora crassa*. J Cell Sci 127:1953–1965. doi:10.1242/jcs.141630

Liu ZM, Kalattukudy PE (1999) Early expression of the calmodulin gene, which precedes appressorium formation in *Magnaporthe grisea*, is inhibited by self-inhibitors and requires surface attachment. J Bacteriol 181:3571–3577

Lösel DM (1967) The stimulation of spore germination in *Agaricus bisporus* by organic acids. Ann Bot 31 (2):417–425

Macko V, Staples RC (1973) Regulation of uredospore germination and germ tube development. Bull Torrey Bot Club 100:223–229

Macko V, Staples RC, Gershon H, Renwick JAA (1970) Self-inhibitor of bean rust uredospores: methyl 3,4-dimethoxycinnamate. Science 170:539–540

Macko V, Staples RC, Allen PJ, Renwick JAA (1971a) Identification of the germination self-inhibitor from wheat stem rust uredospores. Science 173:835–836

Macko V, Staples RC, Renwick JAA (1971b) Germination self-inhibitor of sunflower and snapdragon rust uredospores. Phytopathology 61:902

Malagnac P, Lalucque H, Lepere G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserine*. Fungal Genet Biol 41:982–997

Marcos AT, Schinko T, Strauss J, Cánovas D (2011) Nitric oxide (NO) is a morphogenetic signal in fungi. Fungal Genet Rep 58(suppl):171

Mazur P, Meyers HV, Nakanishi K, El-Kayat AAE, Champe SP (1990) Structural elucidation of sporogenic fatty acid metabolites from *Aspergillus nidulans*. Tetrahedron Lett 31:3837–3840

Mazur P, Nakanishi K, El-Zayat AEE, Champe SP (1991) Structure and synthesis of sporogenic psi factors from *Aspergillus nidulans*. J Chem Soc Chem Commun 20:1486–1487

McCormick JR, Flärdh K (2012) Signals and regulators that govern *Streptomyces* development. FEMS Microbiol Rev 36(1):206–231. doi:10.1111/j.1574-6976.2011.00317.x

Miyagawa H, Inoue M, Yamanaka H, Tsurushima T, Ueno T (2001) Chemistry of spore germination

self-inhibitors from the plant pathogenic fungus *Colletotrichum fragariae*. In: Baker DR, Umetsu NK (eds) Agrochemical discovery: insect, weed, and fungal control, American chemical society symposium series. Oxford University Press, Oxford, pp 62–71

Müller D, Jaffe L (1965) A quantitative study of cellular rheotropism. Biophys J 3:317–335

Nemčovič M, Jakubíková L, Víden I, Vladimír F (2008) Induction of conidiation by endogenous volatile compounds in *Trichoderma spp*. FEMS Microbiol Lett 284:231–236. doi:10.1111/j.1574-6968.2008.01202.x

Oh KB, Miyazawa H, Naito T, Matsuoka H (2001) Purification and characterisation of an autoregulatory substance capable of regulating the morphological transition in *Candida albicans*. Proc Natl Acad Sci USA 98:4664–4668

Park HS, Yu JH (2012) Genetic control of asexual sporulation in filamentous fungi. Curr Opin Microbiol 15:669–677. doi:10.1016/j.mib.2012.09.006

Pelkmans JF, Lugones LG, Wösten HAB (2016) Fruiting body formation in basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 387–405

Peraza-Reyes L, Malagnac F (2016) Sexual development in fungi. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 407–455

Podila GK, Rogers LM, Kolattukudy PE (1993) Chemical signals from avocado surface wax trigger germination and appressorium formation in *Colletotrichum gloeosporioides*. Plant Physiol 103:267–272

Polizzi V, Fazzini L, Adams A, Picco AM, De Saeger S, Van Peteghem C, De Kimpe N (2011) Autoregulatory properties of (+)-thujopsene and influence of environmental conditions on its production by *Penicillium decumbens*. Microb Ecol 62:838–852. doi:10.1007/s00248-011-9905-9

Raina S, De Vizio D, Palonen EK, Odell M, Brandt AM, Soini JT, Keshavarza T (2012) Is quorum sensing involved in lovastatin production in the filamentous fungus *Aspergillus terreus*? Process Biochem 47:843–852. doi:10.1016/j.procbio.2012.02.021

Ramage G, Saville SP, Wickes BL, Lopez-Ribot JL (2002) Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl Environ Microbiol 68:5459–5463

Rast D, Stäuble EJ (1970) On the mode of action of isovaleric acid in stimulating the germination of *Agaricus bisporus* spores. New Phytol 69:557–566

Read ND, Roca MG (2006) Vegetative hyphal fusion in filamentous fungi. In: Baluska F, Volkmann D, Barlow PW (eds) Cell-cell channels. Springer, New York, pp 87–98

Read ND, Lichius A, Shoji GAB (2009) Self-signalling and self-fusion in filamentous fungi. Curr Opin Microbiol 12:608–615. doi:10.1016/j.mib.2009.09.008

Read ND, Fleißner A, Roca M, Glass NL (2010) Hyphal fusion. In: Borkovich KA, Ebbole D (eds) Cellular and molecular biology of filamentous fungi. American Society of Microbiology, Washington, DC, pp 260–273

Read ND, Goryachev AB, Lichius A (2012) The mechanistic basis of self-fusion between conidial anastomosis tubes during fungal colony initiation. Fungal Biol Rev 26:1–11. doi:10.1016/j.fbr.2012.02.003

Ringelmann A, Riedel M, Riederer M, Hildebrandt U (2009) Two sides of a leaf blade: *Blumeria graminis* needs chemical cues in cuticular waxes of Lolium perenne for germination and differentiation. Planta 230(1):95–105. doi:10.1007/s00425-009-0924-4

Robinson PM (1973a) Autotropism in fungal spores and hyphae. Bot Rev 39:361–384

Robinson PM (1973b) Oxygen-positive chemotropic factor for fungi? New Phytol 72:1349–1356

Robinson PM (1973c) Chemotropism in fungi. Trans Br Mycol Soc 61:303–313

Robinson PM, Park D (1966) Citrinin-a fungistatic antibiotic and narrowing factor. Nature 211:883–884

Robson GD, Wiebe MG, Trinci APJ (1991) Exogenous cAMP and cGMP modulate branching in *Fusarium graminearum*. J Gen Microbiol 137:963–969

Roca MG, Read ND, Wheals AE (2005) Conidial anastomosis tubes in filamentous fungi. FEMS Microbiol Lett 249:191–198

Rodríguez-Urra AB (2012) Autoregulatory signals and its role in fungal mycelium conformation. PhD thesis, University of Basque Country (UPV-EHU), San Sebastian

Rodríguez-Urra AB, Jimenez C, Dueñas M, Ugalde U (2009) Bicarbonate gradients modulate growth and colony morphology in Aspergillus nidulans. FEMS Microbiol Lett 300:216–221. doi:10.1111/j.1574-6968.2009.01780.x

Rodríguez-Urra AB, Jiménez C, Nieto MI, Rodríguez J, Hayashi H, Ugalde U (2012) Signaling the induction of sporulation involves the interaction of two secondary metabolites in *Aspergillus nidulans*. ACS Chem Biol 7:599–606. doi:10.1021/cb200455u

Roncal T, Cordobés S, Sterner O, Ugalde U (2002) Conidiation in *Penicillium cyclopium* is induced by conidiogenone, an endogenous diterpene. Eukaryot Cell 1:823–829

Schimmel TG, Coffman AD, Parsons SJ (1998) Effect of butyrolactone I on the producing fungus, *Aspergillus terreus*. Appl Environ Microbiol 64:3707–3712

Shirtliff ME, Krom BP, Meijering RA, Peters BM, Zhu J, Scheper MA, Harris ML, Jabra-Rizk MA (2009) Farnesol-induced apoptosis in *Candida albicans*. Antimicrob Agents Chemother 53:2392–2401. doi:10.1128/AAC.01551-08

Simonin AR, Palma-Guerrero J, Fricker M, Glass NL (2012) Physiological significance of network organization in fungi. Eukaryot Cell 11:1345–1352. doi:10.1128/EC.00213-12

Spohr A, Dam-Mikkelsen C, Carlsen M, Nielsen J, Villadsen J (1998) On-line study of fungal morphology during submerged growth in a small flow-through cell. Biotechnol Bioeng 58:541–553

Tanaka S, Wada K, Matayama M, Marumo S (1984) Isolation of sporogen-AO1, a sporogenic substance, from *Aspergillus oryzae*. Agric Biol Chem 48:3189–3191

Tsitsigiannis DI, Zarnowski R, Keller N (2004a) The lipid body protein PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller N (2004b) Endogenous lipogenic regulators of spore balance in *Aspergillus nidulans*. Euk Cell 3:1398–1411

Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller NP (2005) Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*. Microbiology 151:1809–1821

Tsurushima T, Ueno T, Fukami H, Irie H, Inoue M (1995) Germination self-inhibitors from *Colletotrichum gloeosporioides* f. sp. *jussiaea*. Mol Plant-Microbe Interact 8:652–657

Wang C, Xing J, Chin C, Peters JS (2002) Fatty acids with certain structural characteristics are potent inhibitors of germination and inducers of cell death of powdery mildews. Physiol Mol Plant Pathol 61:151–161

Wang M, Hashimoto M, Hashidoko Y (2013) Carot-4-en-9,10-diol, a conidiation-inducing sesquiterpene diol produced by *Trichoderma virens* PS1-7 upon exposure to chemical stress from highly active iron chelators. Appl Environ Microbiol 79:1906–1914. doi:10.1128/AEM.03531-12

Webster J, Weber R (2007) Introduction to fungi. Cambridge University Press, Cambrigde

Wieser J, Lee BN, Fondon JW, Adams TH (1994) Genetic requirements for initiating asexual development in *Aspergillus nidulans*. Curr Genet 27:62–69

Wöstemeyer J, Schimek C, Wetzel J, Burmester A, Voigt J, Schulz E, Ellenberger S, Siegmund L (2016) Pheromone action in the fungal groups chytridiomycetes, and zygomycetes, and in the oophytes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 203–234

Yager LN (1992) Early developmental events during asexual and sexual sporulation in *Aspergillus nidulans*. In: Bennet JW, Klich MA (eds) *Aspergillus*: biology and industrial applications. Butterworth-Heinemann, Boston, pp 19–41

10 Pheromone Action in the Fungal Groups Chytridiomycetes and Zygomycetes and in the Oophytes

J. Wöstemeyer[1], C. Schimek[2], J. Wetzel[1], A. Burmester[1], J. Voigt[1], E. Schulz[1], S. Ellenberger[1], L. Siegmund[1]

CONTENTS

I. Introduction

Sexual differentiation and development depend on the exchange of communication molecules between complementary cells, hyphae and structures. In the phylogenetically basic organismic groups, dealt with in this chapter, soluble and often volatile molecules are involved in partner recognition. These primary recognition systems contribute substantially to distinction between the same and foreign species and must reliably provide recognition of complementary mating types. These signals need to be synthesised and sent out at appropriate stages of development and ideally allow distinction from potentially interfering environmental signals. They need to be perceived by the partner, interpreted and finally used for initiating adequate developmental programmes. Apart from these basic attributes of communication via pheromones, there is essentially very little similarity between the molecules involved in different fungal groups, and even within these groups, different communication signals are often revealed, if the immediate phylogenetic range of traditional model organisms is exceeded.

II. Communication and Development

Although there is no experimental evidence for a common material basis of fungal communication, some chemical principles are ascribed as typical to major fungal groups. The phylogenetically highly diverse group of flagellated basal fungi, traditionally subsumed as chytridiomycetes, is characterised by utilising sesquiterpenes as attractants between sexually complementary mobile gametes, sirenin and parisin (Fig. 10.1), at least in the traditional model organism *Allomyces arbusculus* that has recently been taken out of the chytridiomycetes sensu stricto and is now classified together with its relatives in the separate phylum Blastocladiomycota (James et al. 2006a; Hibbett et al. 2007).

The zygomycetes that have been analysed with respect to recognition at the level of diffusible pheromones seem to rely predomi-

[1]Lehrstuhl für Allgemeine Mikrobiologie und Mikrobengenetik, Friedrich-Schiller-Universität Jena, Neugasse 24, 07743 Jena, Germany; e-mail: b5wojo@rz.uni-jena.de
[2]Fernhochschule Riedlingen, Lange Straße 19, 88499 Riedlingen, Germany

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

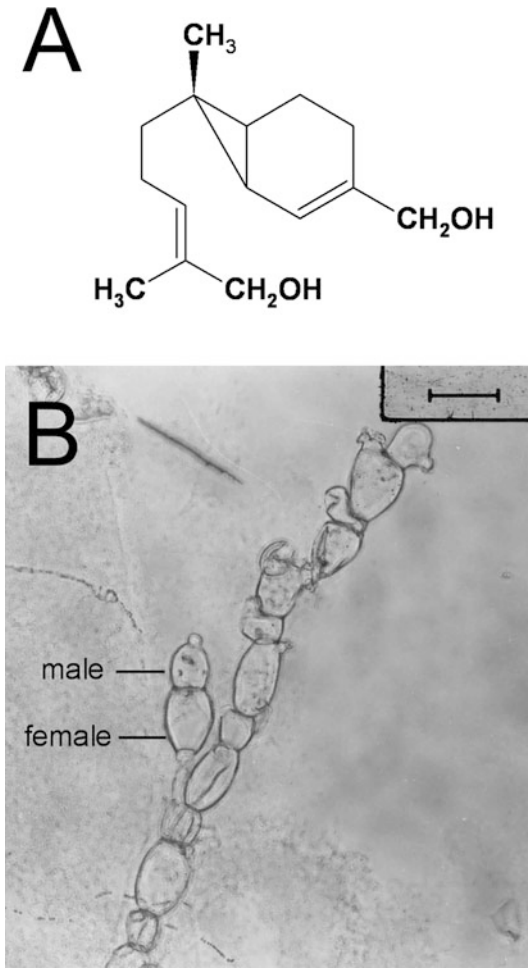


Fig. 10.1 (**a**) Structure of sirenin, the sesquiterpenoid gamete attractant produced by female gametangia of *Allomyces* sp. (**b**) Sexual differentiation in *A. macrogynus*: paired female and male gametangia develop on the same hypha. Bar = 50 μm (Photograph by S. Münch)

nantly on oxidative degradation products of carotene that, by tradition, are described as trisporic acid derivatives or trisporoids (Figs. 10.2 and 10.3; Schimek et al. 2003). A chemically very similar concept of chemical communication has been implemented during evolution by making use of retinoids for inter- and intracellular communication in animals. Here too, the active signal molecules are synthesised by oxidative degradation of carotene (Kedishvili 2013). Plants also have developed an apocarotenoid, abscisic acid, to act as a hormone that mediates embryogenesis, seed maturation and several stress responses (Nambara and Marion-Poll 2005). Structural relationships between enzymes and receptors that bind these trisporoid-related molecules have recently been analysed at the level of protein modelling. Structural relationships between these proteins are small, indicating that evolution has found several independent gateways towards apocarotenoid processing (Ellenberger et al. 2013).

In contrast to these fungal groups that often are addressed as lower fungi, the Dikaryomycota (Ascomycotina, Basidiomycotina) seem to rely predominantly on communication schemes based on small peptides (see Dyer et al. 2016; Pelkmans et al. 2016; Peraza-Reyes and Malagnac 2016; Schmoll and Wang 2016).

The non-mycotan group Oomycota makes use of steroid compounds, e.g. the chemically well-characterised compounds oogoniol and antheridiol (Fig. 10.5) in *Achlya* spp. This steroid system seems to be less widespread within oomycetes than trisporoids that are within zygomycetes. Plant pathogens of the genus *Phytophthora* communicate via different, nonsteroid substances (Yajima et al. 2008).

The regulatory substances involved in recognition and control of sexual processes of fungi have been variously termed 'hormones', indicating the activity of the substance beyond its producing cell or tissue, 'pheromones' or 'gamones' to describe substances involved in attraction between motile gametes. Other terms, e.g. 'attractant', 'morphogen' and 'sex factor', are also found in the relevant literature. Throughout this text, the term 'pheromone' will be used to indicate chemical acting at a distance on members of the same and sometimes of other species. Where the importance of a substance as mediator of specifically a sexual process is to be stressed, the term 'sex pheromone' is used.

A. Chytridiomycota

1. Background and Models

The former phylum Chytridiomycota, recently recognised as polyphyletic, included all zoosporic fungi with a single posteriorly located whiplash flagellum. Analysis of rDNA sequence data and ultrastructure (James et al. 2006a, b)

has led to establishing a novel taxon at the phylum level, Blastocladiomycota, to which the well-known model organisms from the genera *Allomyces*, for studying sexual communication, and *Blastocladiella*, for studying chytrid physiology, belong (Porter et al. 2011). No sexual or other communication systems have been studied for species belonging to the remaining, much smaller group Chytridiomycota and also not to Neocallimastigomycota (Hibbett et al. 2007), formerly also included in Chytridiomycota. Nevertheless, the termini chytridiomycetes or chytrids are still generally in use, not meant to refer to a monophyletic taxon but to address the lifestyle of uniflagellated fungi, and will, for reasons of compatibility with relevant literature, still be used in this chapter.

Sexual reproduction has been documented in detail only for surprisingly few chytrids, mostly from the *Allomyces* kinship (Blastocladiomycota). *Coelomomyces* sp. can also be crossed by isogamous fusion (Whisler et al. 1975) of electron microscopically well-characterised zoospores (Lucarotti and Federici 1984), a process that has been employed for breeding of more effective mosquito pathogens (Federici 1979). The genus *Polyphagus* (core Chytridiomycota) follows a completely different fusion mechanism, where a nonmotile 'male' hypha contacts larger 'female' structures, a process best described as somatogamy (Powell 1979). *Monoblepharis* sp., also among core Chytridiomycota, releases mobile sperm cells that fertilise oogonia (Sparrow 1965). This latter system has been analysed with respect to control of sexual morphogenesis to light (Marek 1984), but unfortunately no efforts have been taken to characterise diffusible or volatile communication substances that mediate sexual differentiation or gamete attraction in organisms other than *Allomyces* sp. The physiology of mating-type recognition in these phylogenetically ancient fungi can certainly be a rewarding field of inventive research. With respect to describing fusion structures at the morphological level, electron-microscopical evidence for nuclear fusion has been provided for the diatom parasite *Zygorhizidium planktonicum* (core Chytridiomycota; Doggett and Porter 1996).

A very interesting feature is the lack of sexual fusion in *Blastocladiella emersonii*, although two different swarmer types are formed that, similar to the situation in *Allomyces arbusculus*, differ with respect to size and formation of orange colour, the latter being restricted to the smaller cell type. This interesting difference between swarmer cells on a single organism, putatively representing mating types, was described in detail already together with the original species description of *B. emersonii* (Cantino and Hyatt 1953) that later on was studied in many different physiological and genetic respects (Viera and Gomes 2010; Viera and Camilo 2011) .

Nevertheless, although even the formation of sexually differentiated structures does not necessarily lead to fusion events or sexual reproduction, sexual systems in chytrids definitely have been evolved early during evolution. Mineralised specimens from the early Devonian, 400 million years ago, clearly contained sexual structures that are essentially indistinguishable from those found in modern chytrids of the *Blastocladiella* type (Remy et al. 1994).

Nonparasitic chytridiomycetes live mainly in freshwater habitats or in moist soil. Within this group, sexual reactions follow several different mechanisms, typically but not exclusively involving interactions between motile gametes. In the genus *Allomyces*, the two uniflagellate gamete types develop in 'male' and 'female' gametangia, which are usually located on the same haploid hypha. In *A. macrogynus*, the gametangia are paired, the male being terminal and the female subterminal (Fig. 10.1b). The gametes express a mutual attraction system. The larger and colourless gametes, regarded as female, attract the faster swimming, smaller male gametes (Pommerville 1978, 1981), which are coloured in intense orange due to their high content of γ-carotene. Fusion results in biflagellate zygotes which, in turn, give rise to the diploid vegetative stage of the life cycle.

2. Communication Molecules

Gamete attraction in *Allomyces* is predominantly mediated by a sesquiterpene, the female pheromone sirenin (Fig. 10.1a; Machlis 1958). Male gametes release a different compound to the surrounding water, usually termed parisin that exerts an attractive function on female

gametes. Parisin has properties similar to sirenin. It is an essentially thermostable, hydrophobic and ether-soluble substance that is inactivated only by hydrolytic treatment with strong acidic or alkaline solutions (Pommerville and Olson 1987), but its chemical structure has not been elucidated. Unfortunately, the term parisin has also been introduced for a polyketide-derived acetogenin of plant origin (Queiroz et al. 2003); this compound must not be confounded with the *Allomyces* pheromone. Female gametes never react to sirenin or any of its artificial derivatives (Carlile and Machlis 1965a; Pommerville et al. 1988), and male gametes never react to parisin (Pommerville and Olson 1987).

As sirenin and several derivatives have early been made available by chemical synthesis (Plattner and Rapoport 1971), and today can even be produced directly as pure enantiomers (Gant et al. 1995), the structural requirements for biological activity were elucidated. There are mandatory requirements for the primary hydroxyl group in the aliphatic side chain (Machlis 1973) and for the general geometry of the bicyclic ring system (Pommerville et al. 1988). The hydroxymethyl group at the bicyclic ring system can be omitted without impeding activity in the picomolar concentration range. It cannot be substituted, however, by large hydrophobic groups. Introducing a benzyl ether ($-CH_2-C_6H_5$) at this position drops activity 10^6-fold, from 10 pM to 10 μm. A comparable loss of activity is observed when the ring system is removed (Pommerville et al. 1988).

3. Mode of Action

The sirenin receptor is still not identified at the molecular level. Hints towards the existence and binding properties of the sirenin receptor are based on the observation that the two enantiomers, *d*- and *l*-sirenin (Fig. 10.1), are clearly distinguished in the biological system. Exclusively, the *l*-form is recognised; the *d*-form is completely inactive and does not even compete for the active form in mixing experiments. Pommerville et al. (1990) hypothesise that the different orientation towards a binding partner, the putative receptor, prevents the formation of hydrogen bonds for one of the two hydroxymethyl groups due to the difference in stereochemistry of the two enantiomers.

At least under experimental conditions, the mutual and specific attraction system of the gametes ensures that essentially all female gametes are fertilised (Pommerville 1978). Sirenin is active in concentrations as low as 10 pM. However, natural concentrations seem to be considerably higher, the concentration around female gametes amounting to 1 μM, which corresponds to the peak response of male gametes (Carlile and Machlis 1965b).

Sirenin modifies the chemotactic behaviour of the male gametes. Along a sirenin gradient, the duration of swimming phases increases, whereas the frequency of directional changes is reduced, thus enhancing accuracy of motion. This effect is mediated by a male-specific regulatory action of sirenin on Ca^{2+} influx and intracellular concentration (Pommerville 1981). The arguments for sirenin as modulator of Ca^{2+} ion concentration regulator are based on inhibitor experiments with the Ca^{2+}-specific chelator EGTA (ethylene glycol-bis-(aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) and with the membrane poisons procaine and tetracaine that prevent Ca^{2+} ions from binding to the cell membrane. This latter treatment causes the male gametes to swim in circles without being able to react to a sirenin gradient. In addition, active Ca^{2+} ion uptake was measured directly by incubating the gametes in the presence of radioactive $^{45}CaCl_2$. Addition of sirenin increases Ca^{2+} ion uptake at least 2.5-fold (Pommerville et al. 1990).

B. Zygomycota

1. Background and Models

Recently, the traditional group Zygomycota has been questioned with respect to phylogenetic consistency, leading to a certain uneasiness to address that taxon as previously. Instead, the well-known model organisms belonging to the *Mucor* kinship have been reclassified first as Mucormycotina (James et al. 2006a) and are presently addressed as Mucoromycotina

(Hibbett et al. 2007). Indeed, all organisms that have been analysed at the level of communication systems belong to the Mucoromycotina subphylum. *Blakeslea trispora* (Choanephoraceae) has found interest due to the unusually large amounts of carotene and trisporic acid, *Phycomyces blakesleeanus* (Phycomycetaceae) is recognised as the traditional model for basic biological phenomena between blue-light perception and development of sporangia, *Absidia glauca* (Absidiaceae) and *Parasitella parasitica* form a good system for studying communication between a mucoralean host and its fusion parasite, and *Mucor mucedo* is essentially the only zygomycete, where the action of trisporoid communication substance can be studied directly at the level of visible developmental steps during the differentiation of sexual structures. Thus, *Mucor mucedo* opens a convenient and presently indeed the only reasonable experimental system for studying correlations between morphological development and physiological parameters.

In zygomycetes, the two complementary mating types cannot be discriminated based on their morphological features or behaviour. In some species, different shapes of the sexual organs occur, which have been described in terms of 'macrogametangia' and 'microgametangia', but a defined assignment of these characteristics to one of the two mating types is not possible. Since the study of Blakeslee (1904), the mating types are classified as (+) and (−), following his primarily arbitrary designation of a strain pair of *Rhizopus nigricans*. The mating types of newly examined isolates are consistently determined by their behaviour in mating reactions with already classified strains of the same or of different species. Interspecific reactions that lead to induction of the first step of sexual development, the induction of zygophores, occur abundantly between members of zygomycetes. Partner recognition and regulation of the sexual differentiation programme is mediated by the β-carotene derivative trisporic acid and its biochemical precursors. Biosynthesis and physiology of trisporoids have been reviewed, among others, by Bu'Lock et al. (1976), van den Ende (1976), Jones et al. (1981), Gooday (1983), Sutter (1987) and Gooday and Adams (1993).

2. Communication Molecules

The chemistry of trisporoids has been studied in members of three families of the Mucorales: *Blakeslea trispora* (Choanephoraceae), *Mucor mucedo* (Mucoraceae) and *Phycomyces blakesleeanus* (Phycomycetaceae). All active trisporoids are variously oxygenated C18 or C19 isoprenoid molecules and modifications of a common C14-backbone structure (Fig. 10.2). Although isomerisation is possible at the C_7 and the C_9 atom of the isoprenoid side chain, only the C_9 isomers have been found in culture extracts. The conformation at this position influences the activity of the compound, with the 9-*cis* (*9Z*) isomer being approximately twice as efficient as the molecule in *trans* (*9E*) conformation (Bu'Lock et al. 1972, 1976). The influence of the stereochemistry at position C_1 has never been resolved, but, in all natural isolates, the functional carboxyl, methoxy or methoxycarbonyl groups were found in *S* position. The configuration at the other chiral centre, C_4, is of critical importance: Only the 4-*R*-hydroxy compounds are physiologically and metabolically active (Bu'Lock et al. 1976).

Another source of variance among trisporoids is the occurrence of a number of derivatives. These are characterised by the functional groups at C_2, C_3 and, mainly, C_{13} (Fig. 10.2). Hydroxyl groups at C_2 and C_3 have been determined in both *R* and *S* configuration in *B. trispora* (Sutter et al. 1989). As a general rule, the substituents at C_2 and C_4 define the molecule species, those at C_2, C_3 and C_{13} define the derivate, and the configuration at the ring carbon atoms determines the biological activity as well as the metabolic specificity. Ring configuration and conformation seem therefore to affect the ability to bind to biosynthetic enzymes or to receptors and possibly to other binding proteins.

Trisporoids belonging to the A series occur only at low levels (Austin et al. 1969; van den Ende et al. 1970; Sutter et al. 1989), and presumably for this reason, their physiological activities have not been investigated in detail. In the case of trisporic acid A, the data are ambiguous. Bu'Lock et al. (1972) reported indeed activity, but clearly lower than that of the B and C derivatives. By contrast, a fully

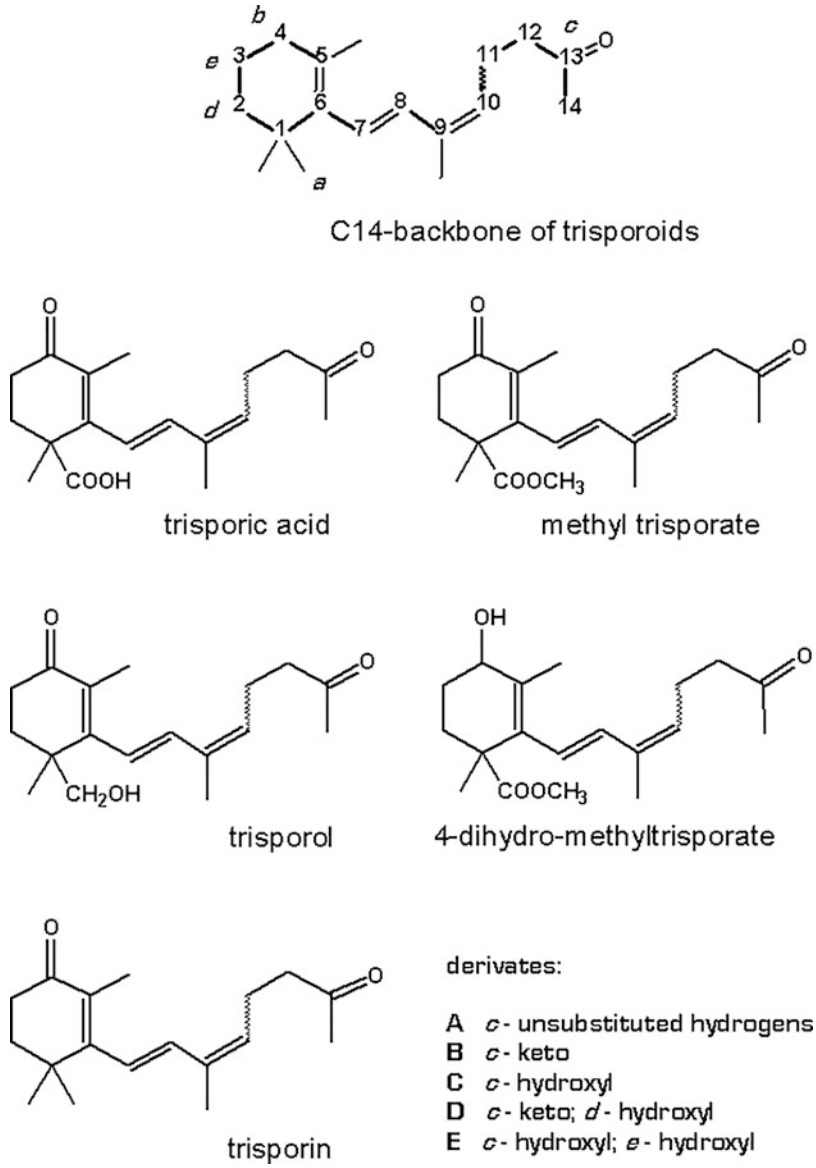


Fig. 10.2 Structure of trisporic acid and its biosynthetic precursors, sex pheromones, in zygomycetes. Only major metabolites are shown. All structures represent the B derivative with a keto function at C_{13}, the different substitutions and substitution sites of the other derivatives being indicated in the *lower right corner*. Each species produces a differently composed mixture of these compounds

synthetic trisporic acid A did not exhibit any biological activity (White et al. 1985). The precursors methyltrisporate A (Bu'Lock et al. 1972), trisporol A (White et al. 1985) and trisporin A (Schachtschabel et al. 2005) are reported to induce zygophore formation in *M. mucedo*. Trisporoids B and C have been isolated from all three species mentioned, whereas the D and E forms have been established only in *P. blakesleeanus* and *B. trispora*. This might be due to the observation that, in *M. mucedo*, the secretion of trisporoids amounts to only a very small fraction of the concentrations found for the other two species (Jones et al. 1981). The structural principles of the diverse trisporoid forms are shown in Fig. 10.2. Of all

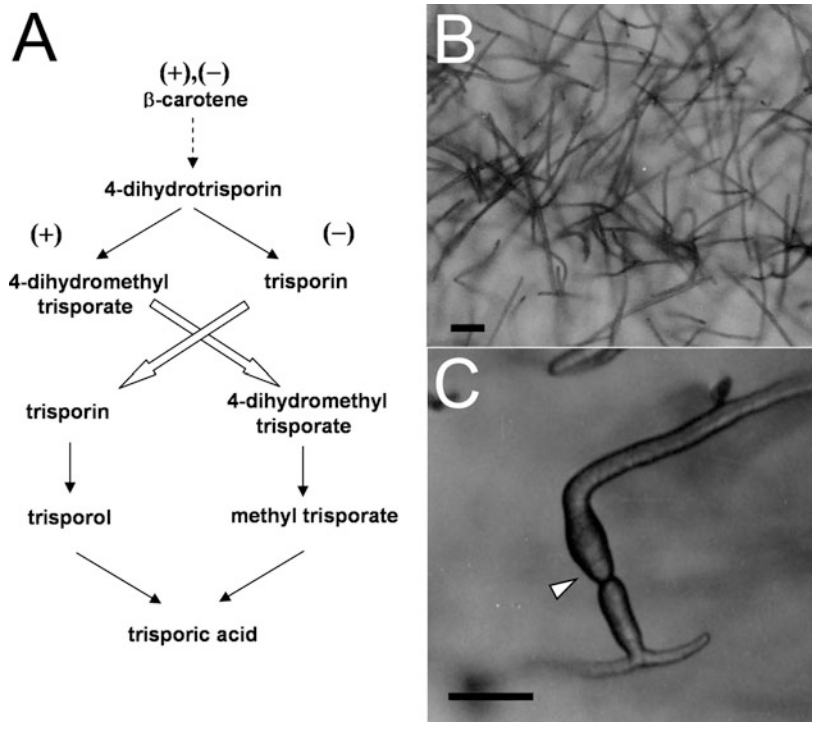


Fig. 10.3 (**a**) Simplified schematic representation of the cooperative biosynthesis of trisporic acid. The *open arrows* indicate exchange of metabolites between the mating partners. Only the compatible partner is able to convert the mating-type-specific precursors into trisporic acid. The two mating types are indicated by (+) or (−). (**b, c**) Sexual differentiation stages in *Mucor mucedo*. (**b**) Zygophores produced by *M. mucedo* (−) as reaction to stimulation by either a compatible mating partner or purified trisporoids. Bar = 120 μm. (**c**) After physical contact between the mating types, progametangia have developed from the original zygospores. The *faint line* in the upper progametangium (*arrowhead*) indicates the formation of a septum, delimiting the future gametangium. Bar = 40 μm

these compounds, the B derivatives are the most active when tested for zygophore induction in *M. mucedo* and *P. blakesleeanus*. Derivatives with anhydro cyclisation at the isoprenoid side chain or a shortened backbone (apotrisporoids) are inactive, indicating that the full-length backbone is one of the mandatory prerequisites for function. Based on analyses of various trisporoid analogues, the importance of the overall dimensions of the trisporoid molecules for bioactivity was confirmed: Exclusively compounds with side chains similar to those occurring in nature revealed activity in the *Mucor* bioassay (Schachtschabel et al. 2005), which tests the induction of zygophores (Fig. 10.3b; Schimek et al. 2003). Early precursors and chemically similar compounds such as retinyl acetate, retinol, retinal, retinoic acid, β-ionone and abscisic acid are completely inactive, too (Bu'Lock et al. 1972). A major requirement for activity is the polarity of the functional groups at the longer side chain, whereas oxygen substituents at the ring carbons are not essential. Only the early precursor 4-dihydrotrisporin, with a hydroxyl group at C_4, does not exhibit activity in this biotest (Schachtschabel et al. 2005).

More information on the specific action of individual trisporoids, ideally with defined chemical modifications, will be necessary to fully understand the biological reasons for the plethora of trisporoids that are found in mucoralean fungi. As the options for chemical synthesis of such substances have recently considerably been improved, relationships between structure and function of trisporoids

are developing towards a highly promising field of research (Gonzalez-Delgado et al. 2014; Schachtschabel and Boland 2007).

3. Biosynthesis

Trisporic acid, the major sex pheromone, is synthesised by a rather elaborate pathway. Neither of the mating types is able to complete the synthesis on its own, at least not in amounts that are necessary for inducing sexual reactions. Communication and exchange of metabolites between two sexually compatible mating partners is indispensable. This concept of cooperative biosynthesis of active pheromones by exchange of mating-type-specific precursors has recently been modelled for an idealised mucoralean fungus by a mathematical approach based on differential equations (Werner et al. 2012). The major result is that exchange of precursors between the mating types leads to bistable behaviour of the system, with clear transitions between low- and high-level synthesis of the pheromones, which must physiologically be expected. In this respect, the complementary synthesis is clearly superior to other models, either assuming exchange of only the end product or just providing the pheromone by one of the mating types.

The biosynthesis starts from β-carotene, as has been ascertained by feeding experiments with radioactively labelled β-carotene (Austin et al. 1970). Whereas the late steps along the pathway to trisporic acid have attracted interest early, the first steps, especially the primary cleavage of β-carotene, have only recently gained the interest of researchers. However, even before cleavage had been measured at the enzymatic level, there was a general consent that β-carotene in zygomycetes is oxidatively cleaved by an enzyme similar to those carotene dioxygenases or monooxygenases that lead to abscisic acid (Schwartz et al. 1997; Tan et al. 1997), retinoids (Ebnet et al. 1999; Blomhoff and Blomhoff 2006), strigolactones (Matusova et al. 2005) or mycorradicin, a substance that is specifically formed in the interaction between arbuscular mycorrhizal fungi and their host plant (Strack and Fester 2006). Alternatively or additionally, but experimentally not proven, an oxidation product of carotene, 4-hydroxy-isocryptoxanthin, has been proposed as substrate for the initial cleavage reaction (Gessler et al. 2002). Following a screening procedure for relatives of the conserved RPE65 protein domain, identified in a retinal pigment epithelial membrane protein and in carotene oxygenases in mammalia and plants (Kloer et al. 2005), two putative carotene oxygenase genes, *TSP3* and *TSP4*, have been identified primarily in the genome of a clinical isolate of *Rhizopus oryzae* (Burmester et al. 2007). The corresponding *TSP3* gene has been cloned and sequenced from *Blakeslea trispora* (Burmester et al. 2007). The gene product has been identified at the level of enzymatic activity by expressing the protein in an *Escherichia coli* strain that had been genetically modified for synthesis of carotene (von Lintig and Vogt 2000). Expressing the *TSP3* gene leads to loss of carotene colour in that strain (Burmester et al. 2007). Whether the molecule is cleaved symmetrically or asymmetrical was an issue of intense debate. A good experimental indication for asymmetric attack of oxygenases on β-carotene was obtained by chemical analysis of cleavage products in culture supernatants of *Phycomyces blakesleeanus*. In addition to the expected C18-trisporoid compounds and the previously described C15-apotrisporoids, the missing link between these two products, two isomers of a C7-compound, (2*E*,4*E*)-6-hydroxy-5-methyl-hexa-2,4-dienoic acid as well as the corresponding 2-methyl isomer, were identified (Polaino et al. 2010). A more detailed chemical analysis of apocarotenoids extracted from supernatants of mixed, sexually compatible cultures led to the identification of as many as seven C18 trisporoids; four C15 compounds, best described as cyclofarnesoids; and the two isomeric methylhexanoid C7 structures already mentioned (Polaino et al. 2012). Screening the genome sequence of *Phycomyces blakesleeanus* revealed five genes coding for putative carotene oxygenases, all of which are transcribed (Medina et al. 2011). The previously recognised *Phycomyces* gene *carS* codes for an enzyme that, after expression in a carotene producing strain of *Escherichia coli*, cleaves β-carotene to the

C25 compound β-apo-12′-carotenal (Tagua et al. 2012). This compound is further processed by the product of the gene *AcaA* to the C18 structure β-apo-13-carotenone. These results, obtained by heterologous expression of *Phycomyces* gene products in *E. coli*, corroborate the assumption, already inferred from chemical analysis, that the first biochemical steps from carotene to trisporoids consist of cleaving the β-carotene backbone at the double bonds between C_{11}' and C_{12}' by CarS followed by action of AcaA between C_{13} and C_{14} (Medina et al. 2011). These results are not consistent with the older hypothesis of retinal, the cleavage product of a symmetrically acting 15-15′-β-carotene dioxygenase, being the precursor of trisporic acid. Retinal has been spectroscopically detected in a *P. blakesleeanus* single wild-type strain culture in concentrations of 5–50 μg per gram mycelial dry weight (Meissner and Delbrück 1968). However, the putative retinal was never verified by chemical analysis. Other results, pointing towards symmetrical cleavage of β-carotene, were obtained with *Blakeslea trispora* and are essentially based on conversion of radioactively labelled retinyl acetate or retinol into trisporoids, which must not necessarily be interpreted as indication for a C20 compound as primary cleavage product (Austin et al. 1970; Bu'Lock et al. 1974). Precautions need to be considered with respect to the conjugated C18 ketone d'orenone, probably corresponding to the C18 ketone compound introduced by Bu'Lock et al. (1974, 1976), that was hypothesised to constitute the first cleavage product of β-carotene in *B. trispora* (Schachtschabel et al. 2008). On the other hand, this deviating experimental observation could also be due to differences in biochemical processes between *P. blakesleeanus* and *B. trispora*. A more recent chemical analysis of the situation in culture supernatants of *B. trispora* reveals two C18 trisporoids, three C15 cyclofarnesyl compounds, one C13 structure and five 2-methylhexane derivatives (Barrero et al. 2011). These results strongly resemble those in *P. blakesleeanus*, clearly rule out retinal or any other symmetric cleavage product as precursor of trisporoids, but do not yet stringently answer the question, if, as assumed for *P. blakesleeanus*, the primary cleavage event produces a C25 and an additional C15 product, although this interpretation seems highly likely regarding the nearly congruent results of the chemical analysis for these two fungi.

Other authors too (Gessler et al. 2002) clearly favoured asymmetric cleavage of β-carotene as predominant biosynthetic pathway towards trisporoids. Interestingly, these authors stressed the possibility of nonenzymatic cleavage at the C_{13-14} double bond, an alternative pathway leading to the C18 ketone. Taking together the results of chemical analysis from *P. blakesleeanus* (Polaino et al. 2012) and *B. trispora* (Barrero et al. 2011), as well as the studies on expression of dioxygenase genes in *E. coli* (Medina et al. 2011), this mechanism provides an alternative that may have some importance in cultures that are not sexually induced. The significance of this reaction may be linked to coping with oxidative stress (Gessler et al. 2002).

After processing the β-carotene skeleton to the first trisporoid, the C14 compound 4-dihydrotrisporin, the pathway towards trisporic acid differs clearly between the mating types. Whereas the oxidation of 4-dihydrotrisporin to trisporic acid is performed via a series of dehydrogenase reactions in (−) mating types, (+) types follow a pathway that depends most probably on oxidases. Specific for the (−) type is the ability to oxidise the hydroxyl group at C_4 to form the ketone. 4-Dihydrotrisporin is thus converted to trisporin, the major (−)-specific pheromone (Fig. 10.3). Depending on the species, the hydroxylation of the C_1 methyl group may occur in the (−) and in the (+) mating type, or solely in the (−) mating type. The resulting alcohols are designated 4-dihydrotrisporol and trisporol, respectively (Bu'Lock et al. 1973; Gooday 1983). Oxidation of the alcohol at C1 to the carboxyl function (Fig. 10.2) is strictly specific for the (+) mating type (Fig. 10.3). Consequently, trisporin and trisporol need to be transferred from the producing (−) type into the (+) mating type to complete trisporic acid synthesis.

In the (+) mating type, 4-dihydrotrisporin is converted, probably via 4-dihydrotrisporol, into 4-dihydromethyltrisporate (Fig. 10.3). This step involves two reactions, oxidation of the C_1 hydroxymethyl group, followed by methylation of the resulting carboxylate to the methylcarbox-

ylate ester. 4-Dihydromethyltrisporate and methyltrisporate are converted to trisporic acid exclusively or at least highly predominantly by the (−) mating partner. The analogous result was obtained for the esterase reaction, necessary for demethylation. In the heterothallic species *Mucor mucedo*, the dehydrogenase reaction was located by histochemical staining solely to zygophores of the (−) partner. Interestingly, in the homothallic species *Zygorhynchus moelleri*, the copulating branches can be differentiated, with the main branch behaving clearly as (−) partner expressing the dehydrogenase activity (Werkman 1976). The naturally occurring overproducer of trisporoids, *B. trispora*, has evolved an interesting reaction for preventing the accumulation of larger concentrations of trisporic acid by unmated (+) strains which would otherwise be possible (Sutter and Whitaker 1981a, 1981b). *B trispora* degrades trisporin to trisporone (apotrisporin; Sutter and Zawodny 1984).

4. Mode of Action

Trisporoids participate in the regulation of sexual processes at different levels. In mated cultures, the amount of terpenoids is generally increased (Thomas and Goodwin 1967; Bu'Lock and Osagie 1973), but only the ergosterol content was demonstrated to be directly influenced by externally added trisporic acid (Gooday 1978; van den Ende 1978). The most conspicuous effect of trisporoids is the increase of β-carotene biosynthesis in certain species, most prominently in *B. trispora* and *P. blakesleeanus* (e.g. Caglioti et al. 1966; Thomas and Goodwin 1967; Vail et al. 1967; Govind and Cerdá-Olmedo 1986). This is generally inferred from the observation that carotene production is increased in mated cultures, compared to single mating types. The trisporoid content of these cultures was not determined. Feofilova et al. (1976) described an increase of carotene production after adding trisporic acid in a single growing *B. trispora* strain and, based on the effects of cycloheximide and actinomycin D, concluded trisporic acid regulation to take place at the translational level. On the other hand, Schmidt et al. (2005) have shown that the transcription levels of two genes involved in carotene synthesis, the genes for phytoene dehydrogenase (*carB*) and lycopene cyclase/phytoene synthase (*carRA*) of *B. trispora*, are strongly induced in mated cultures. Neither the effects of specific trisporoids on the regulation of genes for carotene biosynthesis nor the influence of carotene or its precursors on trisporoid synthesis has been analysed. Purified trisporic acid B and C enhanced carotene synthesis in *B. trispora* (van den Ende 1968), as well as synthetic racemic methyltrisporate B and C, but not methyltrisporate E in *P. blakesleeanus* (Govind and Cerdá-Olmedo 1986). A series of synthetic C13 and C15 trisporoid intermediates had no effect on carotenogenesis (Yakovleva et al. 1980).

Carotene synthesis and its regulation in mucoralean fungi have been studied intensively in the past decades, due to the inherent biotechnical implications. Besides sexual interactions, carotene production is also regulated by light, especially by blue light, as well as by various chemicals (e.g. Cerdá-Olmedo and Hüttermann 1986; Ruiz-Hidalgo et al. 1997; Velayos et al. 2003; Quiles-Rosillo et al. 2005; Vereshchagina et al. 2012). In addition, genetic regulation of carotenogenesis has been reviewed (Wöstemeyer et al. 2005; Corrochano and Garre 2010).

A positive feedback regulatory cycle has been postulated, according to which trisporoids enhance their own production in mated cultures (Werkman and van den Ende 1973), probably via inducing carotene synthesis. This theory implies that, similar to abscisic acid formation in plants (Taylor et al. 2000), the rate-limiting step of trisporoid synthesis is the cleavage of carotene and that only a certain amount of total carotene can act as substrate for conversion to trisporoids. Sexual reactions and trisporoids are only two of the several factors controlling carotene synthesis, whereas the presence of β-carotene is supposed to be an absolute prerequisite for trisporoid formation. Mutants of *P. blakesleeanus* deficient in carotene synthesis show both largely reduced sexual reactions and trisporoid production (Sutter 1975; Sutter et al. 1996). The assumption of general applicability of this hypothesis, although reasonable, has not been experimentally validated for those species

with naturally low carotene content. Conspicuously, mutants with exceptionally high carotene content are deficient in sexual morphogenesis, too (Salgado et al. 1989; Ootaki et al. 1996). However, their ability for trisporoid synthesis has not been analysed. In *P. blakesleeanus*, mutant analyses revealed that complete sexual reactions occur over a wide range of β-carotene levels, ranging between 5 (Salgado et al. 1991; Ootaki et al. 1996) and 3000 μg/g mycelial dry weight (Salgado and Cerdá-Olmedo 1992; Mehta et al. 1997).

M. mucedo is the usual and indeed the only reasonable organism among mucoralean fungi for observing the effect of externally added trisporoids on early steps of sexual differentiation. The trisporic acid precursors trisporin, trisporol, 4-dihydromethyltrisporate and methyltrisporate induce the development of sexually committed hyphae, the zygophores, in *M. mucedo* (Fig. 10.3b) and *P. blakesleeanus*. This reaction can be observed in actually mated cultures, as a response to externally added trisporoids. Trisporic acid also induces zygophores in *M. mucedo*, but not in *P. blakesleeanus* (Sutter et al. 1996). Several derivatives exhibit strictly mating-type-specific effects by triggering reactions only in the complementary mating type. The most active compound in bioassays with *M. mucedo* (−) is trisporic acid B, followed by 4-dihydromethyltrisporate B, where 27 and 107 pmole (10 and 30 ng) of substance are sufficient to induce a response (Sutter and Whitaker 1981b). A comprehensive model of the actual events in trisporoid action is still lacking.

As zygophore induction can be observed when the mating partners are separated by a gap or a mechanical barrier, permeable for gases, volatile precursors have been postulated early (Burgeff 1924) and later experimentally shown to be responsible for mediating zygotropism (Plempel 1962). The term zygotropism describes the directed growth of zygophores leading first to contact and adhesion, followed by fusion of zygophores with complementary mating type Mesland et al. 1974; Fig. 10.3c). 4-Dihydromethyltrisporate, a (+) type-specific substance, and trisporin, a (−) type-specific substance, are the promising candidates for effects exerted by volatiles. Trisporol and definitely trisporic acid, on the other hand, are certainly not or not sufficiently volatile. Their action depends on transport by diffusion through the substrate or via immediate hyphal contact between the partners.

As, at least in *M. mucedo*, the model for microscopic observation of early sexual stages, all trisporoid effects are triggered by trisporic acid as well as by its precursors at comparable concentrations, and with similar efficiency, the true function of trisporic acid is not yet fully understood. The main reason for the biochemical pathway towards trisporic acid may reside in higher stability against oxidative processes of the compound compared to the precursors. Sutter (1975) interpreted the precursors as interhyphal chemical messengers, the true mating-type-specific pheromones, in contrast to the trisporic acids, which may act as intrahyphal chemical regulators. Some experimental observations indicate that all precursors are internally converted to trisporic acid, which would then be the main regulatory molecule. A different chain of thought leads to the idea that trisporic acid is more a transport form of all trisporoids and, after having been taken up by the mating partner, is reduced again to the active and mating-type-specific pheromones. Most probably, all trisporoids are involved in more general regulatory networks, as is the case for the structurally similar compounds retinoic acid in animals and abscisic acid in plants. Both are involved in regulation of gene expression on a large scale. Directly and by interaction with a number of other signal molecules and binding proteins, these compounds ultimately act at the transcriptional level (e.g. Bastien and Rochette-Egly 2004; Chung et al. 2005; Al-Tanoury et al. 2013). A correlation with intracellular cAMP levels was observed in *M. mucedo* (Bu'Lock et al. 1976). Retinol (vitamin A) was found to act as structural analogue to trisporoids in enhancing carotene synthesis (Eslava et al. 1974), but is completely inactive in zygophore induction (Bu'Lock et al. 1976; C. Schimek, personal observation). Trisporoid receptors have not been isolated or recognised, but the binding sites for two enzymes involved in trisporic acid biosynthesis, 4-dihydromethyltrisporate dehydrogenase and

4-dihydrotrisporin dehydrogenase, have been identified and described by protein modelling in silico (Ellenberger et al. 2013).

Trisporoid actions might differ between diverse species, according to their inherent differences in structuring mating reactions. In *Absidia glauca* and many other species, distinct zygophores are not formed. Therefore, zygotropism cannot occur in this organism. Instead, progametangia apparently develop between aerial hyphae of the mating partners, which either get in close contact or even touch each other accidentally during vegetative growth (peg-to-peg interaction). This procedure for initiating zygospore development is highly efficient, especially as *A. glauca* does not show any form of contact avoidance between neighbouring colonies. In this situation, the secretion of trisporic acid precursors, especially of those that are volatile, does not seem necessary and indeed has never been studied. In other species, e.g. *P. blakesleeanus* and *B. trispora*, zygophores are formed in the upper substrate layer or at the interphase between substrate and air. Under such circumstances, signal molecules spreading by diffusion through the substrate are more plausible and probably more efficient than volatiles. The domain of volatiles is the *M. mucedo* situation, encountered indeed in many different mucoralean genera, where specialised zygophores grow away from the substrate mycelium into the air and need to come into contact via direct growth along a gradient of zygotropic substances. From early studies of zygomycete sexuality, and based on numerous observations of partial interspecific reactions, it has repeatedly been argued that the trisporoid signal system is active throughout a large group of organisms. In addition to the well-known heterothallic species already mentioned, trisporoid action was also established for the sexual reactions of the homothallic species. Trisporoids with activity in the *M. mucedo* zygophore induction assay were found in *Zygorhynchus moelleri* and *Z. heterogamus*, whereas no zygophore inducing substances were found in cultures of *Syzygites megalocarpus* and *Mucor genevensis*. However, also these latter species were able to convert precursors from *M. mucedo* to trisporic acid (Werkman and van den Ende 1974). Thus, it is highly plausible that also homothallic species rely on the trisporoid system for controlling early steps in sexual communication and development. Later, trisporoid activity has also been described in a different order of zygomycetes, the Mortierellales. A substance with chromatographic behaviour and spectroscopic characteristics of 4-dihydromethyltrisporate was isolated from *Mortierella indohii* and from other *Mortierella* species. This substance was shown to induce sexual reactions in *M. mucedo* and carotene synthesis in *P. blakesleeanus*. The enzyme 4-dihydromethyltrisporate dehydrogenase and the corresponding gene were also found in mycelia of several mortierellalian fungi (Schimek et al. 2003). Surprisingly, the gene for this enzyme, *Tsp1*, was recently also found in the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (syn. *Glomus irregulare*, formerly *G. intraradices*; Halary et al. 2013). There is, however, no experimental incidence for the corresponding substrate or a biological function of trisporoids in Glomeromycota (formerly: Glomales, Zygomycota).

Moreover, the biotrophic fusion parasite *Parasitella parasitica* (Mucorales, Mucoraceae) depends on trisporoid pheromones for identifying suitable hosts. In some host-parasite interactions, especially in confrontations with *A. glauca*, this dependency leads to strictly mating-type-specific infestations (Wöstemeyer et al. 1995). Studies on the enzyme 4-dihydromethyltrisporate dehydrogenase and its gene in *P. parasitica* revealed common regulatory pathways between sexual and parasitic communication (Schultze et al. 2005). Overall, sexuality and parasitism in *P. parasitica* seem to share much more features than just the striking morphological similarity between sexual zygospores and the result of interspecific communication and development, the sikyospores (Fig. 10.4; Kellner et al. 1991). More clues elucidating the relations between sexuality and parasitism in zygomycetes can be expected from the analysis of the *P. parasitica* genome that has recently been made available (Ellenberger et al. 2014; ENA-PRJEB7124).

Apart from trisporoids that mediate communication between the complementary mating types and definitely induce the formation of

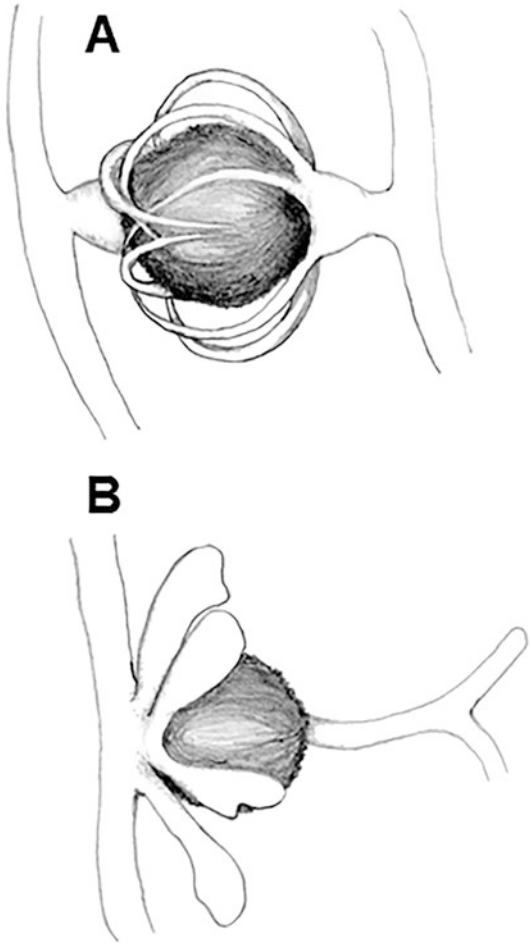


Fig. 10.4 (**a**) Drawing of a mature zygospore formed between two complementary, sexually committed hyphae of *Absidia glauca*. (**b**) A sikyospore developed by the biotrophic fusion parasite *P. parasitica* at the end of parasitic growth on a sexually complementary hypha of its host, *A. glauca*. Host and parasite need to belong to complementary mating types in interactions between these two species. The drawings are slightly simplified but drawn to scale

early differentiation structures, the zygophores, other signals are necessary to complete the pathway from zygophores via hyphal fusion to gametangia and finally to zygosporangia and zygospores. In any case, direct contact between sexually committed structures is strictly required, at least in *M. mucedo*, for continuing the developmental programme from zygophores to progametangia that only subsequently are able to fuse. The contact between surface-bound components apparently bears signal character, and indeed mating-type-specific surface components have been identified in *A. glauca*. One genetic difference between mating types, reflected at the protein level, relates to a mating-type-specific plasmid, encoding a secreted, non-glycosylated surface protein (Teepe et al. 1988; Hänfler et al. 1992). Homothallic derivatives of *A. glauca*, obtained by protoplast fusion of complementary mating types, do not express the protein, indicating that at least in this homothallic situation, the protein is not necessary for zygospore formation (Wöstemeyer et al. 1990).

Recently, also links between sexual differentiation and polyamine metabolism have been seen in the homothallic species *Zygorhynchus moelleri* and the heterothallic *A. glauca*. Zygospore development is prevented by inhibiting polyamine biosynthesis or transglutaminase activity, indicating that conjugated polyamines may be necessary for morphogenesis of later sexual structures. Both conjugated polyamines and transglutaminase activity were detected at hyphal surfaces of progametangia and gametangia and are found to be asymmetrically distributed between the mating types (J. Voigt, E. Schulz, unpublished).

5. Genetic Control

Although sexuality and the trisporoid communication system of *Mucor*-related fungi have been in the focus of research for more than six decades, studying the underlying genetic system has always lagged behind. This is mainly due to the inherent obstacles that render all zygomycete systems difficult to work with. Traditional Mendelian genetics was always severely hampered by the high number of nuclei in the fusing gametangia and by the inability to be sure about the number of nuclear fusions and of meioses that take place in this situation. Coping with germination rates of zygospores is another severe issue. In many species, germination does not occur at all, at least not under laboratory conditions, and the best germination rates possible, in *P. blakesleeanus*, range around a few per cent.

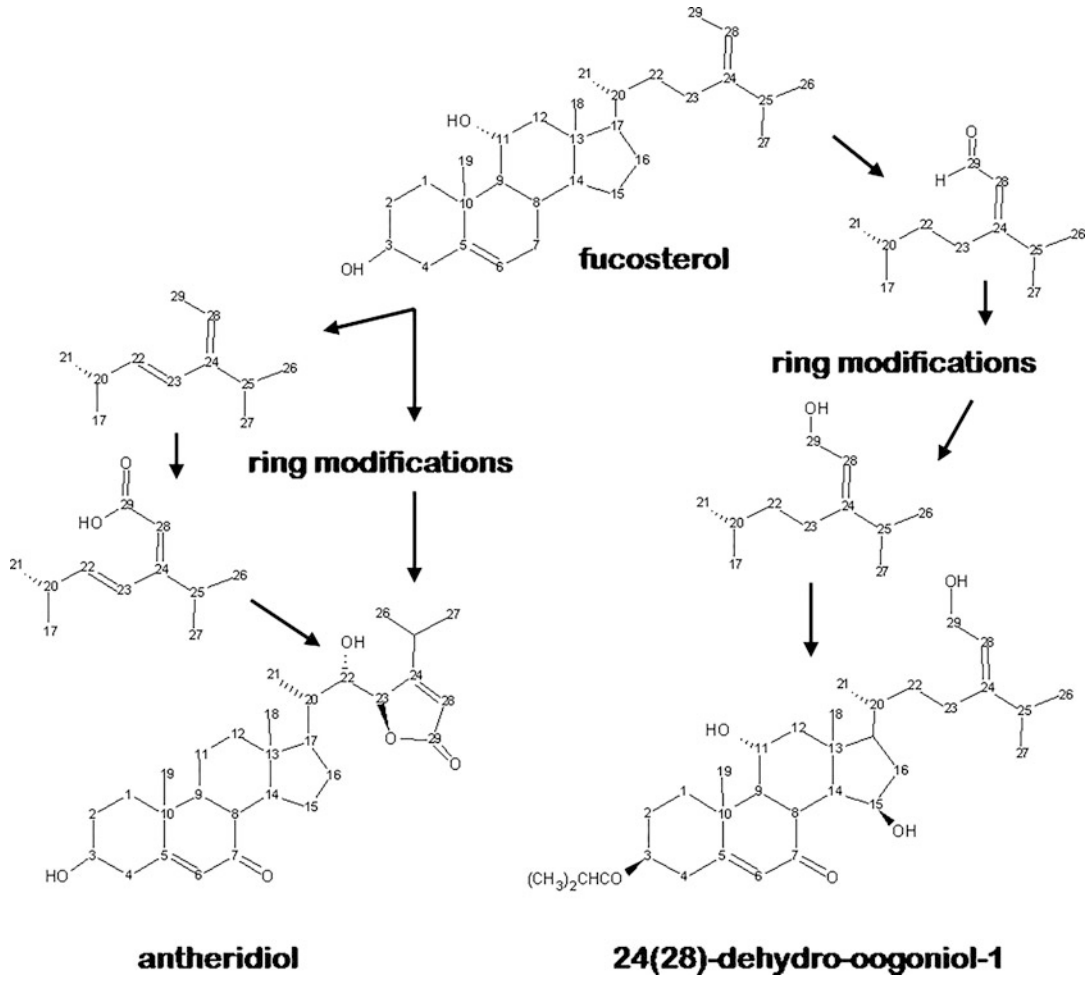


Fig. 10.5 Biosynthesis pathway of the steroid sex pheromones of *Achlya* spp., derived from the common precursor fucosterol. Antheridiol is produced by females via independently occurring modifications at the ring system and the side chain. 24(28)-Dehydro-oogoniol-1 represents a number of active oogoniols produced by male strains. The unesterified oogoniol and its 24(28)-dehydro-derivative are modified by three different substituents at C_3: *oogoniol-1* $(CH_3)_2CHCO$, *oogoniol-2* CH_3CH_2CO and *oogoniol-3* CH_3CO. In males, biosynthesis takes place in a more strictly observed sequence of conversion steps. Ring modifications must be finished before the final modifications at the side chain may take place

Deduced from the general principle of trisporic acid synthesis, the cooperatively shared pathway between the complementary mating types, two hypotheses concerning the genetic situation have been proposed. First, a single mating-type locus would exist, with two alleles or maybe idiomorphs, designated (+) or (−). The mating-type locus would then determine which of the two possible enzyme sets for trisporic acid synthesis are expressed, either the (−) specific dehydrogenases or the (+) specific oxidases. In principle, this hypothesis goes back to Bu'Lock et al. (1976). The second basic hypothesis states that all necessary genes would exist in both mating types, but the synthesis of one set of mating-type-specific enzymes is repressed and blocked, depending on the mating type (Nieuwenhuis and van den Ende 1975). Accordingly, the sex-specific enzymatic steps were proposed to be predominantly suppressed in single growing cultures, but become derepressed in the mating situation

(Bu'Lock et al. 1973, 1976). Many observations along the line of these hypotheses, based essentially on the concept of cooperative biosynthesis of trisporic acid, suggest that a basic regulator or master transcription factor as, e.g. in the mating-type system of bakers' yeast, *Saccharomyces cerevisiae* (Lee et al. 2010) is not mandatory for zygomycetes.

Intriguingly, a single mating-type locus has been identified and mapped in *P. blakesleeanus* by conventional genetics (Álvarez et al. 1980; Orejas et al. 1987; Eslava and Álvarez 1996) and, later, with higher resolution and accuracy by including PCR-RFLP markers (Chaudhary et al. 2013). This locus has been designated *sexM* for the (−) and *sexP* for the (+) mating type. Both mating-type alleles encode different HMG-domain transcription factors and are transcribed in opposite directions relative to the adjacent genes (Idnurm et al. 2008). Until today, no target genes for these transcription factors have been identified. Homologues of *sexM* and *sexP* are the rule in other mucoralean fungi *Rhizopus oryzae* (Gryganskyi et al. 2010), the homothallic species *Syzygites megalocarpus* (Idnurm 2011), *Mucor mucedo* (Wetzel et al. 2012) and *Mucor circinelloides* (Li et al. 2011). In this latter organism, a Δ*sexM* mutant was obtained that behaved completely sexually sterile in crossing experiments (Li et al. 2011). This important experiment unequivocally proves the mandatory contribution of the *sex* locus to sexual development. The effect of *sexM* or *sexP* expression on pheromone synthesis has unfortunately not been studied, but in *M. mucedo*, trisporoids themselves regulate transcription of the *sex* locus (Wetzel et al. 2012). Transcription of *sexM* is considerably stimulated by adding trisporoids, whereas *sexP* is only marginally affected. This experimental result provides a link between trisporoids and the regulatory properties of the *sex* locus, although stimulation of pheromone synthesis by action of the *sex* loci would appear more illuminative. The corresponding experiment can presently best be done in *M. circinelloides* by analysing the Δ*sexM* mutants for activity of the enzymes involved in trisporoid biosynthesis. Generally, *sexM* is transcribed nearly exclusively during mating, whereas *sexP* is additionally transcribed during vegetative growth (Lee and Heitman 2014). The transcription factor protein, encoded by *sexM* but not *sexP*, contains a nuclear localisation sequence. By following the behaviour of a translational fusion chimaera of sexM with a fluorescent protein in *Saccharomyces cerevisiae*, transport to the nucleus could directly be shown in the microscope (Wetzel et al. 2012).

In addition to the already mentioned gene with intriguing similarity to the mucoralean *Tsp1* gene in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*, genes for HMG proteins, strongly resembling the genes encoded by *sexM* and *sexP*, have been identified by several authors (Halary et al. 2013; Lee et al. 2014; Riley and Corradi 2013), which, together with other results from genome analysis of *Glomus*-like fungi, opens prospects for interpreting arbuscular mycorrhizal fungi as basically sexual organisms (Riley et al. 2014), perhaps via mechanisms that presently escape our vision, but putatively resemble the genetic sexual system of zygomycetes.

Whereas no information is available on the enzymes or the corresponding genes contributing to trisporic acid synthesis in the (+) mating type of mucoralean fungi, two reactions, specific for the (−) mating type, have been analysed in some detail. The first reaction in (−) mating types leads from the common precursor 4-dihydrotrisporin to trisporin, the substance that is passed on to (+) mating types and that is, in addition, supposed to be involved in mediating zygotropism between zygophores. This oxidation step, converting the hydroxyl group at the C4-position of the ionone ring to a keto group, is catalysed by the NADP-dependent 4-dihydrotrisporin dehydrogenase, the product of the *TSP2* gene. The enzyme has been purified from *M. mucedo* (Wetzel et al. 2009). The gene is found in both mating types; transcription, although dependent on developmental stage, can also be seen in both partners, but expression of active enzyme is specific for the (−) mating type. However, an inactive form of the 4-dihydrotrisporin dehydrogenase was also found in the (+) mating type. Activity could be restored by renaturing SDS-treated protein. Thus, the (−) mating-type specificity of active TSP2 protein is accomplished at the post-translational level

presumably by a binding protein abolishing activity in the (+) type (Wetzel et al. 2009). At the molecular level, 4-dihydrotrisporin dehydrogenase belongs into the short-chain dehydrogenase superfamily, characterised by a Rossmann fold. The three-dimensional structure of the protein has been calculated and modelled (Ellenberger et al. 2013).

The second (−) type-specific dehydrogenase acts on the (+)-specific substrate 4-dihydromethyltrisporate dehydrogenase that is taken up by the (−) mating type and converted by a dehydrogenase, the product of the gene *TSP1*, to methyltrisporate. This gene has been isolated from *M. mucedo* (Czempinski et al. 1996) and from the biotrophic fusion parasite of several mucoralean fungi, *P. parasitica* (Schultze et al. 2005). The dehydrogenase activity of *TSP1* is strictly (−) type specific in these organisms (Fig. 10.3). Enzyme activity was first detected in (−) zygophores of *M. mucedo* and in the (−) equivalents of the homothallic *Zygorhynchus moelleri* (Werkman 1976). Enzyme activity was also documented in several species of *Mortierella*, thus establishing the use of trisporoids also in the only distantly related order Mortierellales (Schimek et al. 2003). In addition, this study revealed enzyme activity throughout sexually stimulated (−) type mycelium. Enzyme activity in mycelia beyond the sexually committed hyphae was also observed in *M. mucedo* and *A. glauca* (C. Schimek, unpublished data). As already suggested by Mesland et al. (1974), trisporoid biosynthesis is evidently not restricted to hyphae undergoing sexual morphogenesis. Trisporoids are usually extracted from mycelia growing in submerged cultures. The biosynthesis pathway is therefore fully active under conditions, where no zygophores and other aerial structures are formed.

Like for *TSP2*, also the *TSP1* gene is present in both mating types of *M. mucedo*, *A. glauca*, *P. parasitica* and *B. trispora*, on the whole in approximately 35 species from more than 10 families of 3 orders of the zygomycetes including *P. blakesleeanus* and *Thamnidium elegans*. Wherever both mating types were analysed, the gene was found in each of these (this institute, unpublished).

Two studies address the regulation of *TSP1* in *P. parasitica* (Schultze et al. 2005) and *M. mucedo* (Schimek et al. 2005). The regulation of trisporoid biosynthesis varies between the species. The *TSP1* gene is part of a complex gene cluster in both species, but not all of the ORFs, found in *P. parasitica*, are present in the *M. mucedo* cluster (Schimek et al. 2005; Schultze et al. 2005). *TSP1* itself is transcribed in both species, showing clearly that the major level of regulation is accomplished post-transcriptionally or post-translationally. In *P. parasitica*, gene expression and regulation follow the same pattern in intraspecific sexual interactions as well as in the parasitic interaction with the host *A. glauca*. Post-transcriptional regulation of mRNA levels for the enzyme is presumably mediated by an antisense mechanism via the interaction with an RNA transcribed in opposite direction from the partly overlapping gene for an acyl-CoA thioesterase in that itself is antisense regulated by transcripts belonging to an ORF, completely overlapping with the thioesterase gene (Schultze et al. 2005). Due to lacking any overlap between the adjacent genes for the TSP1 enzyme and the thioesterase, antisense regulation does not apply to *M. mucedo*. Indeed, analyses at the protein level show that even the TSP1 protein is formed in both mating types. Similar to the situation for the TSP2-protein in *M. mucedo*, TSP1 activity is regulated post-translationally too (Schimek et al. 2005; Wetzel et al. 2009).

Regarding the control of the pheromone pathway from β-carotene to trisporic acid in zygomycetes, as far as it is known today, reveals that essentially early steps, maybe only carotene cleavage itself by the *TSP3*-gene product, are regulated directly at the level of transcription (Burmester et al. 2007; Schimek and Wöstemeyer 2009; Sahadevan et al. 2013). Later steps, the oxidation reactions catalysed by the *TSP1*- and *TSP2*-products, are predominantly controlled at later stages during gene expression, either post-transcriptionally in *P. parasitica* or post-translationally in *M. mucedo*.

C. Oomycota

1. Background and Models

The non-mycotan phylum Oomycota, probably better addressed as oophytes, is characterised by biflagellate swarmer cells, carrying an ante-

rior flagellum with mastigonemes and an additional posterior smooth flagellum. Another characteristic is the different biochemical pathway towards the amino acid lysine. Whereas fungi use the intermediate α-aminoadipic acid, oophytes produce lysine via diaminopimelic acid, like green algae and plants. The cell wall architecture is based on cellulose and other glucans, and cell wall proteins contain the amino acid hydroxyproline. Phylogenetically, oophytes are placed in the kingdom Chromista (Beakes et al. 2012), most of which grow photoautotrophically. Taken together, oophytes resemble fungi in many morphological and ecological respects, but have a different phylogenetic history.

In oophytes, the mating types cannot be distinguished easily because clear definition features do not exist. Moreover, the terms 'sex' and 'mating type', commonly understood to establish a distinct and fixed set of features and actions, do not seem to apply to all members of the group. Rather, at least within the order Saprolegniales, a system of relative sexuality has been described besides a number of solely 'male' and solely 'female' strains, allowing the strain, producing the highest amount of the 'female' sex hormone antheridiol, to define the mating behaviour of the partner strain (Barksdale 1967; Barksdale and Lasure 1973). Traditionally, the mating types are termed 'female' and 'male'. Many strains are homothallic, or self-fertile, the two types of sexual organs, oogonia and antheridia, being formed on proximal hyphae of the same individual (Raper 1952).

Research on the sexual signal system has been performed almost exclusively on several heterothallic strains of *Achlya ambisexualis* or *A. bisexualis* and the homothallic *A. heterosexualis* (Saprolegniales). The underlying principles of sexuality are reviewed by Raper (1952), Barksdale (1969), McMorris (1978), Gooday (1983, 1994), Gooday and Adams (1993) and Mullins (1994). Since the early 1990s, little supplementary information on the biochemistry of the signal system in the traditional model organisms was added. Another source of information, especially for structural considerations, addresses the sexual processes of the plant pathogens *Phytophthora* sp. (Peronosporales; Elliott 1983) and *Pythium* sp. (Peronosporales; Knights and Elliott 1976). Recently, the chemical nature of mating hormones has been revealed for *Phytophthora nicotianae* (Qi et al. 2005; Ojika et al. 2011), and genomic sequences of various organisms belonging to this genus will certainly be helpful for elucidating the genetics of the sex system in these important plant pathogens (Judelson 2007; www.ncbi.nlm.nih.gov/gquery/?term=Phytophthora). Overall, *Phytophthora* has been reported to have higher gene numbers—around 15,000—than plant pathogens in Eumycota. Unfortunately, there are presently no reports on the sequences of mating-type genes or other genes that might be directly involved in communication between sexual partners. For the moment, the two complementary mating types are characterised at the genetic level by their non-Mendelian segregation behaviour (Fabritius and Judelson 1997) and by genetic mapping based on RAPD-, RFLP- and SSCP markers, narrowing the MAT loci down to approximately 100 kb (Judelson et al. 1995).

2. Communication Molecules

The substance named hormone A by Raper in his detailed studies of signal exchange during sexual processes in *Achlya* in the 1930s and 1940s (reviewed by Raper 1952) was subsequently isolated from a female strain of *A. bisexualis* by McMorris and Barksdale (1967) and termed antheridiol. A molecular formula, $C_{29}H_{42}O_5$, was established, and the existence of hydroxyl and carbonyl functions, as well as the presence of an α-β unsaturated γ-lactone and an α-β unsaturated ketone, was deduced. A structure, based on spectroscopy and chemical derivatisation, was proposed by Arsenault and coworkers in the following year (Arsenault et al. 1968), and these authors also first determined the steroid nature of the pheromone. This structure (Fig. 10.5) was confirmed by data derived from fully synthetic isomers (Edwards et al. 1969). Based on the standard tetracyclic steroid nucleus, with a side chain attached at C_{17}, the major difference between antheridiol and mammalian steroid hormones lies in the length of this

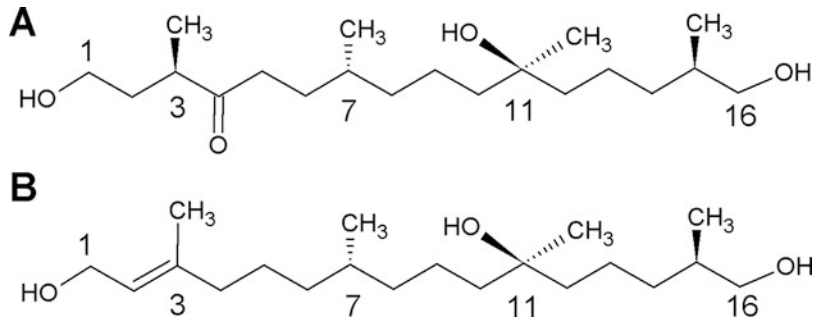


Fig. 10.6 Structure of the diffusible phytol derivatives responsible for sexual communication in the oophyte *Phytophthora infestans*. (**a**) The α1 mating pheromone, synthesised by the A1 mating type by conversion of α2, secreted by the A2 partner. (**b**) The α2 mating pheromone, synthesised by the A2 mating type

side chain. In antheridiol, the ten carbon atoms long side chain also encompasses the lactone ring. The structure contains two C=C double bonds, one at $C_{5–6}$ of the steroid nucleus and the other at $C_{24–25}$ of the side chain, as well as two carbonyl functions at C_7 and C_{26} and two hydroxyl groups at C_3 and C_{22}, respectively.

The structural feature most important for activity is the stereochemistry at C_{22} and C_{23}. From the four possible stereoisomers, only the functional antheridiol (22*S*, 23*R*) exhibits activity at 6 pg/ml, whereas the activity levels in the 22*R*, 23*S* and the 22*S*, 23*S* stereoisomers are reduced by a factor of 1000, and activity is probably even further reduced in the 22*R*, 23*R* stereoisomer (Barksdale et al. 1974). By contrast, structural changes at the ring system seem to be of minor importance. Removal of the C_7 keto group and exchange of the free hydroxyl group at C3 with its acetate ester reduce activity only by a factor of 20. By contrast, changes in the oxidation status at C_{22} or C_{23} lead to dramatic reduction in activity, whereas further structural divergence of the side chain yields completely inactive derivatives. A number of other steroids, including mammalian steroid hormones, have been shown to be equally inactive (Barksdale et al. 1974).

The male pheromone, originally termed hormone B by Raper (1952), was later renamed oogoniol (McMorris et al. 1975). The oogoniols turned out to be a mixture of active steroids. A number of these have been characterised, including the unesterified oogoniol; its isobutyrate, propionate and acetate esters, oogoniol-1, oogoniol-2 and oogoniol-3, respectively; and their 24(28)-dehydro-analogues (McMorris et al. 1975; McMorris 1978). Similarly to antheridiol, the oogoniols are 29-carbon steroids containing a $\Delta^{5–7}$-ketone chromophore (Fig. 10.5). They carry an ester substituent at C_3, hydroxyl functions at C_{11} and C_{15} and a primary hydroxyl group, too, at C_{29} at the end of the side chain. Oogoniols do not contain a lactone ring (McMorris et al. 1975), but analogously to antheridiols, the physiological activity strongly depends on the structure of the side chain. In fact, the minor compounds, 24(28)-dehydro-oogoniols, are about 100 times more effective than the saturated analogues and may represent the true physiologically active compounds (McMorris 1978; Preus and McMorris 1979). Possibly, biosynthetic intermediates towards dehydro-oogoniol exhibit regulatory functions; 7-deoxo-dehydro-oogoniol was described as competitive inhibitor of the pheromone (McMorris et al. 1993).

Whereas there are good reasons to assume that sexual communication in zygomycetes is based on a common chemical basis, the situation in oophytes seems to be more complicated. On the one hand, all oophytes, either from the Saprolegniales like *Achlya* sp., depend on secreting steroids for proper sexual development; on the other hand, the true mating pheromones in *Phytophthora* sp., correlated with either the A1 or the A2 mating-type locus, are the substances α1 or α2, chemically belonging to the diterpenoids, derivatives of phytol (Ojika et al. 2011; Fig. 10.6).

The steroid signal system seems to be at least genus specific, the functional substances being active only among closest neighbours.

Analyses of the induction of sexual processes indicate that steroids other than antheridiol are required in oomycetes beyond *Achlya* sp. (Knights and Elliott 1976; Musgrave et al. 1978; Kerwin and Washino 1983). The pronounced specificity of the reactions becomes apparent from the non-interchangeable character of oogoniol and antheridiol in *Achlya* species (Horgen 1977; Musgrave et al. 1978).

Self-sterile plant pathogenic species of the genera *Phytophthora* and *Pythium* are unable to synthesise sterols and are sexually inactive if no external sterols are available (Kerwin and Duddles 1989). Nevertheless, these are taken up from the surroundings, presumably from the host plants, and are supposed to be converted internally to the necessary sexual hormones (Langcake 1974; Elliott and Knights 1981). The actual structure of these active substances has not yet been determined. Many steroid compounds have been tested for their ability to induce the sexual developmental programme in the genera *Phytophthora* and *Pythium*, and the results of these analyses indicate that the usefulness of external steroids is also defined largely by their configuration and stereochemistry, particularly that of the side chain (Elliott 1979).

Steroid substances active in *Phytophthora* were found to fulfil the following criteria: They carry a hydroxyl function at C_3 of the sterol nucleus, have one C=C double bond in ring B and contain a hydrocarbon side chain longer than five carbon atoms. Increasing length of the side chain leads to increasing activity, the exact position of C_{29} being the most important feature. All compounds with the position of C_{29} fixed by the presence of a $C_{24–28}$ double bond were of high activity (Elliott et al. 1966; Elliott 1972). Methylation of C_{24} also enhances activity. Indeed, activity increases concomitantly with the size of the substituent at C_{24} (Elliott 1979). Compounds lacking the double bond in the steroid nucleus and exhibiting other undesirable structural features are completely inactive (Knights and Elliott 1976; Elliott and Sansome 1977). Despite several of the structural requirements indicating a structure of the sexual pheromone similar to those of *Achlya* sp., and thus raising the possibility of a general signal mechanism, antheridiol was found to be completely inactive in *P. cactorum* (Nes et al. 1980). This possibly reflects the outcome of evolutionary adaptation to the parasitic lifestyle. Typical plant steroids are generally far more effective than fungal or animal compounds in the plant parasitic species (Elliott et al. 1966; Nes et al. 1980).

A second signal system, active in sexual reproduction of *Phytophthora*, was proposed by Ko (1980, 1983, 1985, 1988), who observed intra- and interspecific sexual reactions leading to self-fertilisation in heterothallic *Phytophthora* spp. strains. The response, accordingly termed hormonal heterothallism and initially believed to constitute a compatibility system, occurs exclusively in the presence of members of both mating types, A1 and A2. It was recognised early that this communication system was mediated by small diffusible substances (Ko 1980), which are neither sterols nor glycerides (Chern et al. 1999), with α2 being more polar than α1 (Ko 1983; Chern et al. 1996), but the exact structures of the communication molecules were elucidated only recently (Qi et al. 2005; Ojika et al. 2011). The chemical structures are surprisingly simple: The structure of hormone α1, produced and secreted by the A1 mating type, was determined by mass, infrared and NMR spectroscopy and clearly revealed the acyclic diterpene structure of a modified, oxygenated phytol (Fig. 10.6; Qi et al. 2005). α2 is very similar and is distinguished from α1 only by the lack of the keto group in position C_4. The structures have been confirmed by chemical synthesis (Yajima et al. 2008; Harutyunyan et al. 2008; Wang et al. 2010; Yajima et al. 2011). The absolute configurations of the native, active hormones have been determined as 3*RS*,7*R*,11*R*,15*R* for α1 (Yajima et al. 2008) and 7*S*,11*R*,15*R* for α2 (Yajima et al. 2011).

A detailed study on the relationships between structure and activity was performed with nine derivatives of α1 and eight derivatives of α2 hormone (Molli et al. 2012). Activity was determined by measuring the induction of oospore formation. The substances were offered to the *Phytophthora* tester strains on small filter paper discs. Under these conditions, the natural hormones are active in amounts as

small as 3 ng. α1 tolerates more modifications than α2. Acetylation of both terminal hydroxyl groups in α1 reduces activity to only 52 %, whereas acetylation of the C_1-hydroxyl group in a_2 leads to only 3.3 % residual activity, and acetylation of the terminal hydroxyl group abolishes hormonal activity completely. These and other results support that in both α1 and α2, all functional groups, hydroxy and keto as well as the olefinic character, contribute to the biological activity of the hormones.

3. Biosynthesis

The analysis and comparison of steroid content in oophytes, together with feeding experiments using both natural and synthetic compounds, leads to a possible sequence of events in pheromone synthesis. Common precursor of antheridiol and the oogoniols is fucosterol, the most abundant sterol in oophytes (Popplestone and Unrau 1973, 1974; McMorris and White 1977). Fucosterol is also the sterol with the highest uptake rate and the most efficient conversion in *Phytophthora* (Elliott et al. 1966; McMorris and White 1977). Saprolegniales are able to synthesise fucosterol, probably via 7-dehydrofucosterol (Popplestone and Unrau 1973). In the subsequent synthesis steps leading to antheridiol, modifications of the steroid ring system and of the side chain occur independently. For the latter, the following sequence of events has been established: The initial step is the dehydrogenation at C_{22}/C_{23}, followed by an oxidation series at C_{29}. Beginning with a methyl function, the final carboxyl group is synthesised via an ethyl group and the carbonyl as intermediates. After these modifications, C_{22}/C_{23} are oxidised again, and finally, cyclisation occurs, yielding the unsaturated γ-lactone ring of the antheridiol side chain (Fig. 10.5; Popplestone and Unrau 1974; McMorris 1978).

The oogoniols, despite being derived from the same precursor, are synthesised by a different pathway. For a start, fucosterol is oxidised to give an aldehyde at C_{29}. In the subsequent steps, hydroxylation occurs at C_{11} and C_{15}, oxidation occurs at C_7, and the hydroxyl group at C_3 becomes esterified. Finally, after all the ring modifications have taken place, the $C_{24–28}$ double bond is reduced (McMorris and White 1977). No data whatsoever exist on the enzymatic mechanism of these conversions or on the genes for the corresponding enzymes.

The biosynthesis of the *Phytophthora* mating hormones α1 and α2 follows a very interesting pathway that strikingly resembles the trisporoid synthesis in zygomycetes, which is typically shared between the two mating partners. Already in 1929, observations on crossing behaviour in different isolates of *Phytophthora palmivora* led to the assumption that secretion of diffusible substances is required for sexual stimulation of sexual reactions by the partner (Ashby 1929). Only recently, the biosynthesis of the pheromones behind this communication system has been elucidated. Formation of α2 by the A2 mating type starts by converting phytol, which is almost certainly imported from the host plant and which has no pheromone activity by itself, by hydroxylation of the positions C_{11} and C_{16} and by introducing the $C_{2–3}$ double bond to the active A2-specific pheromone α2. Quantitative correlations between the amount of phytol added to an A2 strain and the amount of α2 formed, as well as labelling experiments with deuterated phytol clearly, show that the pheromone is indeed derived from phytol. The addition of deuterium-labelled α2 pheromone to an A1-culture leads to α1 pheromone, carrying a keto group at position C_4 and the deuterium label at exactly the same positions as in the α2 substance added. The A2-specific pheromone is thus taken up by the A1 mating type and converted to the A1-specific pheromone (Ojika et al. 2011). In a recent review, Lee et al. (2012) accentuated the similarity of the *Phytophthora* system with the trisporic acid system in *Mucor* relatives. Although considerably simpler in *Phytophthora*, both systems for partner recognition and induction of sexually differentiated structures in the complementary mating partners rely on the exchange of substances that are converted in the partner to the active principle. There is presently no information on the enzymes involved in phytol conversion neither on genes coding for these enzymes, involved in controlling the mating system.

4. Mode of Action

To fully understand the role of steroids in the regulation of sexual development in *Phytophthora* spp., it is necessary to strictly distinguish their effects from those of other steroid-mediated regulatory mechanisms. Early observations of the effects of externally added steroids promoting vegetative growth (Hendrix 1964, 1965; Elliott et al. 1966), and concomitantly with these being incorporated to a large extent into the cellular membranes (Langcake 1974), emphasised the function of steroids as necessary regulatory and structural cellular components. In the case of sexual processes, studies performed on compatible male and female strains elucidated a sequence of pheromone responses. Similar reactions were observed in crossings between homothallic and one compatible heterothallic strain. For antheridiol, the same sequence was also obtained with increasing concentration of externally added pheromone. Antheridiol itself is produced constitutively at low concentrations by the female. As the first decidedly sexual reaction in *Achlya ambisexualis*, apical growth in the male stops after exposure to a female strain or within an hour after antheridiol addition (Gow and Gooday 1987). In the next step, the characteristic antheridial initials are formed in a dose-dependent manner at the proximal ends of male vegetative hyphae (Barksdale 1967). Within 30 min after exposure, certain reactions are also induced in the mating partner. Exposure to antheridiol induces the synthesis and release of oogoniol in male-reacting strains (Barksdale and Lasure 1974; McMorris and White 1977) and the formation of oogonial initials. These release higher amounts of antheridiol and, consequently, chemotropically attract the antheridial branches (Barksdale 1963, 1967). Higher concentrations of antheridiol also affect the differentiation of antheridia by septum formation and are also thought to be involved in the onset of meiosis (Barksdale 1963, 1967). Continual exposure to antheridiol for at least 30 min also induces conversion of this compound to less active metabolites in male strains of the heterothallic *A. ambisexualis* or *A. bisexualis*, as well as in the homothallic species *A. americana* and *A. conspicua* (Musgrave and Nieuwenhuis 1975). In female strains, metabolism of antheridiol does not occur, nor is any antheridiol metabolism observed in those oomycetes species which do not respond to antheridiol in their sexual reactions (Musgrave et al. 1978). The authors suggest a regulatory function for this metabolism. Inactivation of antheridiol would steepen the antheridiol gradient and thus facilitate directed growth and promotion of gamete formation. As the antheridia also serve to direct male gametangial nuclei through specialised tubes directly to the oospheres enclosed in the oogonium (Raper 1952), a function of antheridiol in fertilisation may also be proposed. By strongly inducing the formation of antheridial hyphae in homothallic species growing adjacent to a female *Achlya* strain, thereby waste resources, antheridiol also acts as inhibitor for both sexual and asexual development of the homothallic strain (Barksdale 1967; Thomas and McMorris 1987).

A number of studies exist on molecular changes accompanying the onset of sexual development. Most of these observations are rather general in nature, and so the data obtained might not reflect pheromone action per se, but rather general changes in cellular regulation following the switch of commitment. Thus, exposure to antheridiol was found to increase activity and release cellulases in males. This increase occurs concomitantly to the formation of antheridial branches (Thomas and Mullins 1967, 1969; Mullins and Ellis 1974; Mullins 1979), implying that it is a necessary prerequisite to branching and the development of new apical growth sites. A similar increase in cellulase activity also accompanies massive vegetative branching (Hill 1996). The same applies to all observations concerning increase in transcription rates, cellular concentrations of rRNA and mRNA, protein synthesis and histone acetylation (Horowitz and Russell 1974; Silver and Horgen 1974; Groner et al. 1976; Horgen 1977; Sutherland and Horgen 1977; Michalski 1978; Horgen et al. 1983). Most of these effects are unspecific and reflect the expression of increasing cellular activities in the course of morphological and physiological changes during the sexual process.

The synthesis of a number of proteins is specifically and directly induced or enhanced

by antheridiol (Horton and Horgen 1985), one of these possibly being cellulase (Groner et al. 1976; cf. above). This specific pheromone-mediated response has been investigated in greater detail. Over a period of almost two decades, Brunt and Silver as well as Riehl and Toft and their co-workers have elucidated the induction and participation of certain proteins in the antheridiol-mediated sexual response of *Achlya*. They found that antheridiol-regulated proteins occur at different cellular localisation and are always to be grouped among the minor polypeptides (Brunt and Silver 1986a, b, 1987). Besides its influence on protein synthesis, treatment with antheridiol may also affect protein processing. A number of glycoproteins become deglycosylated at the onset of the sexual reaction in the male *A. ambisexualis* E87 (Brunt and Silver 1986a), implicating regulation of cellular recognition events by the pheromone. An 85-kDa protein band detected both in the nuclear and the cytoplasmic fraction (Brunt and Silver 1986b) was later found to consist partially of a previously identified (Silver et al. 1983) 85-kDa heat-shock protein (Brunt et al. 1990; Brunt and Silver 1991). Based on antibody cross-reactions and sequence similarity, this protein is classified as a member of the hsp-90 protein family (Brunt et al. 1990).

A specific antheridiol receptor was identified in the cytoplasm of male cells only (Riehl et al. 1984). As the binding activity could be recovered in fractions displaying different sedimentation coefficients, the existence of a multiprotein complex was deduced and later confirmed. At least the hsp-90 protein is an integral part of that complex (Brunt et al. 1990).

Several other heat-shock proteins, e.g. three hsp-70 proteins, a 23-kDa and a 56-kDa protein (Silver et al. 1993; Brunt et al. 1998a, b) as well as other hormone-binding and non-binding polypeptides (Riehl et al. 1985; Brunt et al. 1998b), are associated with the steroid receptor-HSP complex. They constitute a multiprotein heterocomplex, where some of the participating components are not necessarily present all the time or in all existing complexes.

At the transcriptional level, two similar but distinguishable transcriptional populations exist for *hsp70* and *hsp90*, and these are differently regulated (Silver et al. 1993; Brunt and Silver 2004). In each case, both mRNA sets are regulated by antheridiol but also react to unrelated stimuli, *hsp70* to decrease glucose concentrations (Silver et al. 1993) and *hsp90* to increase temperature (Brunt and Silver 2004). Transcript divergence for a single cDNA clone was already documented by Horton and Horgen (1989), an observation probably based on similar regulation processes.

Consistent with earlier assumptions, all authors today agree on the oophyte steroid receptor organisation and its regulation, strongly resembling animal steroid hormone systems (Riehl and Toft 1984; Riehl et al. 1985; Brunt et al. 1998b; Brunt and Silver 2004). This viewpoint is further supported by the identification of a diversity of putative transcription factor response elements in the 5′-region of the *hsp90* genes. Among these are motifs already known from animal steroid hormone response elements (Brunt et al. 1998a).

The *Phytophthora parasitica* and *P. infestans* mating-type loci have been analysed at the genetic level by the group around Judelson. Using RAPD markers to identify loci associated with the A1 and A2 phenotypes, genetic and physical mapping of these loci was accomplished. Both species are characterised by a bipolar mating system. Mating types are determined by heterozygosity at A1 (allele combination Aa) and by homozygosity at A2 (aa) at the single mating-type locus (Judelson et al. 1995; Judelson 1996a). In *P. infestans*, an unusual segregation pattern of the mating-type alleles prevails, showing a preference for two of the four possible genotypes. Despite the chromosomes bearing the A1 and A2 determinants being genetically similar, a region of structural heterozygosity, locus S1, flanks the mating-type locus in A1 isolates, whereas it is absent in A2. Although S1 contains no obvious open reading frames, a decided function of this sex-chromosome-like region in the regulation of allele segregation, DNA replication or gene expression seems plausible (Judelson 1996b; Randall et al. 2003). In *Phytophthora parasitica*, the standard Mendelian segregation pattern is realised (Fabritius and Judelson 1997). Open reading frames within the mating-type locus, providing a clue to the nature of mating-

type determination or sexual differentiation, have not been identified, although shotgun genome sequences have been established for *P. parasitica* (ENA-PRJNA181330), *P. infestans* (ENA-PRJNA17665; Haas et al. 2009), *P. capsici* (ENA-PRJNA201536; Lamour et al. 2012), *P. sojae* (ENA-PRJNA17989; Tyler et al. 2006) and *P. ramorum* (PRJNA12571; Tyler et al. 2006).

The connection between the mating-type loci and the mode of action of phytol-derived hormones in *Phytophthora* spp. is also not clear, and presently there are no hypotheses published on possible connections between the communication mediated by the α1/α2-pheromones and the reactivity to steroids.

Eight genes have been characterised which are upregulated during sexual development in *P. infestans*. As the transcription level of two of these increases during the early stages, before physical contact between partners has been established, they might be induced by the α hormones. Very low expression was also found in vegetative hyphae, which would not be uncommon in a hormone response system. Three sequences resemble that of proteins interacting with RNA—a ribonuclease activator, a RNA-binding protein of the *Drosophila melanogaster* Pumilio protein family (Puf) and RNase H—and may thus be involved in mating regulation (Fabritius et al. 2002). The Puf-like protein was later found to be transcribed not only during early sexual development but also during the early asexual development of sporangia (Cvitanich and Judelson 2003). The predicted products of two of the upregulated genes show similarities with elicitins and one to a glycoprotein cell-surface receptor (Fabritius et al. 2002). One of the elicitin-like products belongs to a *P. infestans* multigene family of putative extracellular transglutaminases, which are possibly involved in cell wall strengthening or in enhancing adhesion. The mating specifically transcribed gene is the only one of this gene family for which elicitin activity could not be predicted from the sequence data. The various putative enzymes are conceivably involved in cell wall-related processes within the different developmental processes leading to vegetative hyphae, zoospores, sexual organs or haustoria (Fabritius and Judelson 2003). Even with no evidence so far for a direct interaction of one of the upregulated genes with the α hormone system or, alternatively, with steroid signals, the character of all putative gene products suggests a possible involvement in mating-related events. Additional examples for mating-induced gene expression have been found after genomic information became available on a broad scale. Conspicuously, many of these genes seem to be species-specific in the genus *Phytophthora*. For nearly 25 % of approximately 100 genes, transcribed more than tenfold higher during mating in *P. infestans*, no homologues were found in *P. sojae*. These and other correlations between genomic architecture and development-specific gene expression have been reviewed (Judelson 2007; Prakob and Judelson 2007).

For the oomycetes sexual pheromone systems, the complexity of the hormone response and of its regulation becomes ever more apparent. The pheromone-mediated part of sexual communication and sexual development is strongly interwoven with other influences and developmental programmes, both, intracellular and external, resulting in tightly connected intracellular regulatory events. Other signal compounds, the nutritional status and the availability of phospholipids are certainly involved in the regulation of sexual reactions too.

III. Conclusions

In the three organismic groups considered, one noticeable observation concerning pheromone action recurs. The sexual interactions in large systematic units are mediated by the same or very similar substances at least at the level of genera and probably families in chytridiomycetes and oophytes and even in a whole class in zygomycetes. Nevertheless, different levels of specificity exist within each group, enabling recognition of a compatible mating partner belonging to the same species. In oophytes and zygomycetes, species specificity is, at a first level of recognition, realised by using different derivatives and isomers of a common basic compound and, subsequently and most probably more specifically, via interactions of individual surface components.

Genetic analysis of the pheromone systems has made considerable progress for zygomycetes. The other two organismic realms are

lagging behind with respect to genetic analysis of mating-type communication, but recognising the phytol derivatives in *Phytophthora* as chemical basis for communication between mating types in oophytes, it must be appreciated as major achievement in understanding recognition and sexual differentiation in this organismic group. It will be rewarding to reveal the functional connection between these substances and the mating-type locus.

The regulation of several genes involved in pheromone biosynthesis is basically known for zygomycetes, and there are good reasons for interpreting the homeodomain transcription factor genes, identified in many zygomycetes, as the functional principle in the mating-type loci, *sexM* and *sexP*. Information on the targets of these transcription factors, however, is still missing. The transcription factors must not necessarily be associated with regulating the communication system at the level of trisporoids, but could be responsible for later steps during the sexual differentiation programme, starting at the level of surface interactions.

Current knowledge on the structure of trisporoid binding proteins, together with the properties of other apocarotenoid binding proteins, will enable us to identify the intracellular events, initiated and regulated by trisporoids. Possibly, the high similarity between various apocarotenoids with regulatory potential, abscisic acid, retinoids and trisporoids will facilitate the definition of functional properties of trisporoid binding proteins and ideally trisporoid receptors. In these respects, regulation mediated by trisporoids could resemble the retinoid situation. These compounds function as intra- and intercellular communication systems in vertebrates, especially in developmental regulatory programmes. Biologically active retinoids bind to nuclear retinoid receptors belonging to the steroid/thyroid hormone nuclear receptor superfamily. Ligand-bound receptor complexes function as transcription factors by binding to specific DNA targets, the retinoid response elements. Comparable effects of intracellularly localised trisporoids, triggered by binding directly to nuclear receptor proteins and thus acting as transcription factors, are conceivable. It will be interesting to check the products of the *sexM*/*sexP* loci for interactions with trisporoids. According to signal transduction models along this line, membrane-associated receptors would not necessarily be involved in trisporoid-mediated regulation of sexual reactions in zygomycetes.

References

Al-Tanoury Z, Piskunov A, Rochette-Egly C (2013) Vitamin A and retinoid signaling: genomic and nongenomic effects. J Lipid Res 54:1761–1775

Álvarez MI, Peláez MI, Eslava AP (1980) Recombination between ten markers in *Phycomyces*. Mol Gen Genet 179:447–552

Arsenault GP, Biemann K, Barksdale AW, McMorris TC (1968) The structure of antheridiol, a sex hormone in *Achlya ambisexualis*. J Am Chem Soc 90:5635–5636

Ashby SF (1929) Strains and taxonomy of *Phytophthora palmivora* Butler (*P. faberi* Maubl.). Trans Br Mycol Soc 14:18–38

Austin DJ, Bu'Lock JD, Gooday GW (1969) Trisporic acids: sexual hormones from *Mucor mucedo* and *Blakeslea trispora*. Nature 223:1178–1179

Austin DJ, Bu'Lock JD, Drake D (1970) The biosynthesis of trisporic acids from β-carotene via retinal and trisporol. Experientia 256:348–349

Barksdale AW (1963) The role of hormone A during sexual conjugation in *Achlya ambisexualis*. Mycologia 55:627–632

Barksdale AW (1967) The sexual hormones of the fungus *Achlya*. Ann N Y Acad Sci 144:313–319

Barksdale AW (1969) Sexual hormones of *Achlya* and other fungi. Science 166:831–837

Barksdale AW, Lasure LL (1973) Induction of gametangial phenotypes in *Achlya*. Bull Torrey Bot Club 100:199–202

Barksdale AW, Lasure LL (1974) Production of hormone B by *Achlya heterosexualis*. Appl Microbiol 28:544–546

Barksdale AW, McMorris TC, Seshadri R, Arunachalam T (1974) Response of *Achlya ambisexualis* E87 to the hormone antheridiol and certain other steroids. J Gen Microbiol 82:295–299

Barrero AF, Herrador MM, Arteaga P, Gil J, González J-A, Alcalde E, Cerdá-Olmedo E (2011) New apocarotenoids and β-carotene cleavage in *Blakeslea trispora*. Org Biomol Chem 9:7190–7195

Bastien J, Rochette-Egly C (2004) Nuclear retinoid receptors and the transcription of retinoid target genes. Gene 17:1–16

Beakes GW, Glockling SL, Sekimoto S (2012) The evolutionary phylogeny of the oomycetes "fungi". Protoplasma 249:3–19

Blakeslee AF (1904) Sexual reproduction in the Mucorineae. Proc Am Acad Arts Sci 40:205–319

Blomhoff R, Blomhoff HK (2006) Overview of retinoid metabolism and function. J Neurobiol 66:606–630

Brunt SA, Silver JC (1986a) Steroid-hormone induced changes in secreted proteins in the filamentous fungus *Achlya*. Exp Cell Res 165:22–34

Brunt SA, Silver JC (1986b) Cellular localization of steroid hormone-regulated proteins during sexual development in *Achlya*. Exp Cell Res 165:306–319

Brunt SA, Silver JC (1987) Steroid hormone-regulated basic proteins in *Achlya ambisexualis*. Exp Mycol 11:65–69

Brunt SA, Silver JC (1991) Molecular cloning and characterization of two distinct hsp85 sequences from the steroid responsive fungus *Achlya ambisexualis*. Curr Genet 19:383–388

Brunt SA, Silver JC (2004) Molecular cloning and characterization of two different cDNAs encoding the molecular chaperone hsp90 in the oomycete *Achlya ambisexualis*. Fungal Genet Biol 41:239–252

Brunt SA, Riehl R, Silver JC (1990) Steroid hormone regulation of the *Achlya ambisexualis* 85-kilodalton heat shock protein, a component of the *Achlya* steroid receptor complex. Mol Cell Biol 10:273–281

Brunt SA, Borkar M, Silver JC (1998a) Regulation of hsp90 and hsp70 genes during antheridiol-induced hyphal branching in the oomycetes *Achlya ambisexualis*. Fungal Genet Biol 24:310–324

Brunt SA, Perdew GH, Toft DO, Silver JC (1998b) Hsp90-containing multiprotein complexes in the eukaryotic microbe *Achlya*. Cell Stress Chaperones 3:44–56

Bu'Lock JD, Osagie AU (1973) Prenols and ubiquinones in single-strain and mated cultures of *Blakeslea trispora*. J Gen Microbiol 76:77–83

Bu'Lock JD, Drake D, Winstanley DJ (1972) Specificity and transformations of the trisporic acid series of fungal sex hormones. Phytochemistry 11:2011–2018

Bu'Lock JD, Jones BE, Quarrie SA, Winskill N (1973) The biochemical basis of sexuality in Mucorales. Naturwissenschaften 60:550–551

Bu'Lock JD, Jones BE, Taylor D, Winskill N, Quarrie SA (1974) Sex hormones in Mucorales. The incorporation of C_{20} and C_{18} precursors into trisporic acids. J Gen Microbiol 80:301–306

Bu'Lock JD, Jones BE, Winskill N (1976) The apocarotenoid system of sex hormones and prohormones in Mucorales. Pure Appl Chem 47:191–202

Burgeff H (1924) Untersuchungen über Sexualität und Parasitismus bei Mucorineen. Bot Abhandlungen 4:1–135

Burmester A, Richter M, Schultze K, Voelz K, Schachtschabel D, Boland W, Wöstemeyer J, Schimek C (2007) Cleavage of β-carotene as the first step in sexual hormone synthesis in zygomycetes is mediated by a trisporic acid regulated β-carotene oxygenase. Fungal Genet Biol 44:1096–1108

Caglioti L, Cainelli G, Camerino B, Mondelli R, Prieto A, Quilico A, Salvatori T, Selva A (1966) The structure of trisporic-C acid. Tetrahedron Suppl 7:175–187

Cantino EC, Hyatt MT (1953) Phenotypic "sex" determination in the life history of a new species of *Blastocladiella,* B. emersonii. Ant v Leeuwenh 19:25–70

Carlile MJ, Machlis L (1965a) A comparative study of the chemotaxis of the motile phases of *Allomyces*. Am J Bot 52:484–486

Carlile MJ, Machlis L (1965b) The response of male gametes of *Allomyces* to the sexual hormone sirenin. Am J Bot 52:478–483

Cerdá-Olmedo E, Hüttermann A (1986) Förderung und Hemmung der Carotinsynthese bei *Phycomyces* durch Aromaten. Angew Bot 69:59–70

Chaudhary S, Polaino S, Shakya VPS, Idnurm A (2013) A new genetic linkage map of the zygomycete fungus *Phycomyces blakesleeanus*. PLoS ONE 8, e58931. doi:10.1371/journal.pone.0058931

Chern LL, Ko WH, Tang CS (1996) Factors affecting yields of α hormones of *Phytophthora parasitica* obtained by adsorption. Can J Microbiol 42:172–176

Chern LL, Tang CS, Ko WH (1999) Chemical characterization of alpha hormones of *Phytophthora parasitica*. Bot Bull Acad Sinica 40:79–84

Chung HJ, Fu HY, Thomas TL (2005) Abscisic acid-inducible nuclear proteins bind to bipartite promoter elements required for ABA response and embryo-regulated expression of the carrot *Dc3* gene. Planta 220:424–433

Corrochano LM, Garre V (2010) Photobiology in the Zygomycota: multiple photoreceptor genes for complex responses to light. Fungal Genet Biol 47:893–899

Cvitanich C, Judelson HS (2003) A gene expressed during sexual and asexual sporulation in *Phytophthora infestans* is a member of the Puf family of translational regulators. Eukaryot Cell 2:465–473

Czempinski K, Kruft V, Wöstemeyer J, Burmester A (1996) 4-Dihydromethyltrisporate dehydrogenase from *Mucor mucedo*, an enzyme of the sexual hormone pathway: purification, and cloning of the corresponding gene. Microbiology 142:2647–2654

den Ende V (1968) Relationship between sexuality and carotene synthesis in *Blakeslea trispora*. J Bacteriol 96:1298–1303

Doggett MS, Porter D (1996) Sexual reproduction in the fungal parasite, *Zygorhizidium planktonicum*. Mycologia 88:720–732

Dyer PS, Inderbitzin P, Debuchy R (2016) Mating-type structure, function, regulation and evolution in the pezizomycotina. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 351–385

Ebnet E, Fischer M, Deininger W, Hegemann P (1999) Volvoxrhodopsin, a light-regulated sensory photoreceptor of the spheroidal green alga *Volvox carteri*. Plant Cell 11:1473–1484

Edwards JA, Mills JS, Sundeen J, Fried JH (1969) The synthesis of the fungal sex hormone antheridiol. J Am Chem Soc 91:1248–1249

Ellenberger S, Schuster S, Wöstemeyer J (2013) Correlation between sequence, structure and function for trisporoid processing proteins in the model zygomycete *Mucor mucedo*. J Theor Biol 320:66–75

Ellenberger S, Burmester A, Wöstemeyer J (2014) Complete mitochondrial DNA sequence of the mucoralean fusion parasite *Parasitella parasitica*. Genome Announc 2:e00912–e00914. doi:10.1128/genomeA.00912-14

Elliott CG (1972) Sterols and the production of oospores by *Phytophthora cactorum*. J Gen Microbiol 72:321–327

Elliott CG (1979) Influence of the structure of the sterol molecule on sterol-induced reproduction in *Phytophthora infestans*. J Gen Microbiol 115:117–126

Elliott CG (1983) Physiology of sexual reproduction in *Phytophthora*. In: Erwin DC, Bartnicki-Garcia S, Tsao PH (eds) Phytophthora, Its biology, taxonomy, ecology and pathology. American Phytopathological Society, St Paul, pp 71–81

Elliott CG, Knights BA (1981) Uptake and interconversion of cholesterol and cholesteryl esters by *Phytophthora cactorum*. Lipids 16:1–7

Elliott CG, Sansome E (1977) The influence of sterols on meiosis in *Phytophthora cactorum*. J Gen Microbiol 98:141–145

Elliott CG, Hendrie MR, Knights BA (1966) The sterol requirement of *Phytophthora cactorum*. J Gen Microbiol 42:425–435

Eslava AP, Álvarez MI (1996) Genetics of *Phycomyces*. In: Bos CJ (ed) Fungal genetics: principles and practice. Marcel Dekker, New York, pp 385–406

Eslava AP, Álvarez MI, Cerdá-Olmedo E (1974) Regulation of carotene biosynthesis in *Phycomyces* by vitamin A and β-ionone. Eur J Biochem 48:617–623

Fabritius AL, Judelson HS (1997) Mating type loci segregate aberrantly in *Phytophthora infestans* but normally in *Phytophthora parasitica*: implications for models of mating-type determination. Curr Genet 32:60–65

Fabritius AL, Judelson HS (2003) A mating-induced protein of *Phytophthora infestans* is a member of a family of elicitors with divergent structures and stage-specific patterns of expression. Mol Plant Microbe Interact 16:926–935

Fabritius AL, Cvitanich C, Judelson HS (2002) Stage-specific gene expression during sexual development in *Phytophthora infestans*. Mol Microbiol 45:1057–1066

Federici BA (1979) Experimental hybridization of *Coelomomyces dodgei* and *Coelomomyces punctatus*. Proc Natl Acad Sci USA 76:4425–4428

Feofilova EP, Fateeva TV, Arbuzov VA (1976) Mechanism of the action of trisporic acids on carotene-synthesizing enzymes of a (−) strain of *Blakeslea trispora*. Microbiologiya 45:153–155

Gant TG, Noe MC, Corey EJ (1995) The first enantioselective synthesis of the chemotactic factor sirenin by an intramolecular [2+1] cyclization using a new chiral catalyst. Tetrahedron Lett 36:8745–8748

Gessler NN, Sokolov AV, Belozerskaya TA (2002) Initial stages of trisporic acid synthesis in *Blakeslea trispora*. Appl Biochem Microbiol 38:536–543

Gonzalez-Delgado JA, Escobar G, Arteaga JF, Barrero AF (2014) Easy access to a cyclic key intermediate for the synthesis of trisporic acids and related compounds. Molecules 19:1748–1762

Gooday GW (1978) Functions of trisporic acid. Philos Trans R Soc Lond B 284:509–520

Gooday GW (1983) Hormones and sexuality in fungi. In: Bennett JW, Ciegler A (eds) Secondary metabolism and differentiation in fungi. Dekker, New York, pp 239–266

Gooday GW (1994) Hormones in mycelial fungi. In: Wessels JGH, Meinhardt F (eds) The mycota, vol 1, Growth, differentiation and sexuality. Springer, Berlin/New York, pp 401–411

Gooday GW, Adams DJ (1993) Sex hormones in fungi. Adv Microb Physiol 34:69–145

Govind NS, Cerdá-Olmedo E (1986) Sexual activation of carotenogenesis in *Phycomyces blakesleeanus*. J Gen Microbiol 132:2775–2780

Gow NAR, Gooday GW (1987) Effects of antheridiol on growth, branching and electrical currents of hyphae of *Achlya ambisexualis*. J Gen Microbiol 133:3531–3536

Groner B, Hynes N, Sippel AE, Schütz G (1976) Induction of specific proteins in hyphae of *Achlya ambisexualis* by the steroid hormone antheridiol. Nature 261:599–601

Gryganskyi AP, Lee SC, Litvintseva AP, Smith ME, Bonito G, Porter TM, Anishchenko IM, Heitman J, Vilgalys R (2010) Structure, function, and phylogeny of the mating locus in the *Rhizopus oryzae* complex. PLoS ONE 5, e15273. doi:10.1371/journal.pone. 0015273

Haas BJ, Kamoun S, Zody MC, Jiang RHY, Handsaker RE, CanoLM GM, Kodira CD, Raffaele S, Torto-Alalibo T, Bozkurt TO, Ah-Fong AMV, Alvarado L, Anderson VL, Armstrong MR, Avrova A, Baxter L, Beynon J, Boevink PC, Bollmann SR, Bos JIB, Bulone V, Cai G, Cakir C, Carrington JC, Chawner M, Conti L, Costanzo S, Ewan R, Fahlgren N, Fischbach MA, Fugelstad J, Gilroy EM, Gnerre S, Green PJ, Grenville-Briggs LJ, Griffith J, Grünwald NJ, Horn K, Horner NR, Hu C-H, Huitema E, Jeong D-H, Jones AME, Jones JDG, Jones RW, Karlsson EK, Kunjeti SG, Lamour K, Liu Z, Ma LJ, MacLean D, Chibucos MC, McDonald H, McWalters J, Meijer HJG, Morgan W, Morris PF, Munro CA, O'Neill C, Ospina-Giraldo M, Pinzón

A, Pritchard L, Ramsahoye B, Ren Q, Restrepo S, Roy S, Sadanandom A, Savidor A, Schornack S, Schwartz DC, Schumann UD, Schwessinger B, Seyer L, Sharpe T, Silvar C, Song J, Studholme DJ, Sykes S, Thines M, van de Vondervoort PJI, Phuntumart V, Wawra S, Weide R, Win J, Young C, Zhou S, Fry W, Meyers BC, vanWest P, Ristaino J, Govers F, Birch PRJ, Whisson SC, Judelson HS, Nusbaum C (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. Nature 461:393–398

Halary S, Daubois L, Terrat Y, Ellenberger S, Wöstemeyer J, Hijri M (2013) Mating type gene homologues and putative sex pheromone-sensing pathway in arbuscular mycorrhizal fungi, a presumably asexual plant root symbiont. PLoS ONE 8, e80729. doi:10.1371/journal.pone.0080729

Hänfler J, Teepe H, Weigel C, Kruft V, Lurz R, Wöstemeyer J (1992) Circular extrachromosomal DNA codes for a surface protein in the (+) mating type of the zygomycete *Absidia glauca*. Curr Genet 22:319–325

Harutyunyan SR, Zhao Z, den Hartog T, Bouwmeester K, Minnard AJ, Feringa BL, Govers F (2008) Biologically active *Phytophthora* mating hormone prepared by catalytic asymmetric total synthesis. Proc Natl Acad Sci USA 105:8507–8512

Hendrix JW (1964) Sterol induction of reproduction and stimulation of growth of *Pythium* and *Phytophthora*. Science 144:1028–1029

Hendrix JW (1965) Influence of sterols on growth and reproduction of *Pythium* and *Phytophthora*. Phytopathology 55:790–797

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Lumbsch HT, Lutzoni F, Matheny PB, Mclaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111:509–547

Hill TW (1996) Electrophoretic characterization of endo-(1,4)-β-glucanases secreted during assimilative growth and antheridiol-induced branching in *Achlya ambisexualis*. Can J Microbiol 42:557–561

Horgen PA (1977) Cytosole-hormone stimulation of transcription in the aquatic fungus *Achlya ambisexualis*. Biochem Biophys Res Commun 75:1022–1028

Horgen PA, Iwanochko M, Bettiol MF (1983) Antheridiol, RNA polymerase II and sexual development in the aquatic fungus, *Achlya*. Arch Microbiol 134:314–319

Horowitz DK, Russell PJ (1974) Hormone-induced differentiation of antheridial branches in *Achlya ambisexualis*: dependence on ribonucleic acid synthesis. Can J Microbiol 20:977–980

Horton JS, Horgen PA (1985) Synthesis of an antheridiol-inducible polypeptide during sexual morphogenesis of *Achlya ambisexualis*. Can J Biochem 63:355–366

Horton JS, Horgen PA (1989) Molecular cloning of cDNAs regulated during steroid-induced sexual differentiation in the aquatic fungus *Achlya*. Exp Mycol 13:263–273

Idnurm A (2011) Sex determination in the first-described sexual fungus. Eukaryot Cell 10:1485–1491

Idnurm A, Walton FJ, Floyd A, Heitman J (2008) Identification of the *sex* genes in an early diversed fungus. Nature 251:193–198

James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R (2006a) A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98:860–871

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgylys R (2006b) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443:818–822

Jones BE, Williamson IP, Gooday GW (1981) Sex pheromones in *Mucor*. In: O'Day DA, Horgen PA (eds) Sexual interactions in eukaryotic microbes. Academic, New York, pp 179–198

Judelson HS (1996a) Genetic and physical variability at the mating type locus of the oomycete, *Phytophthora infestans*. Genetics 144:1005–1013

Judelson HS (1996b) Chromosomal heteromorphism linked to the mating type locus of the oomycetes *Phytophthora infestans*. Mol Gen Genet 252:155–161

Judelson HS (2007) Genomics of the plant pathogenic oomycete *Phytophthora*: insights into biology and evolution. Adv Genet 57:97–141

Judelson HS, Spielman LJ, Shattock RC (1995) Genetic mapping and non-mendelian segregation of mating type loci in the oomycete, *Phytophthora infestans*. Genetics 141:503–512

Kedishvili NY (2013) Enzymology of retinoic acid biosynthesis and degradation. J Lipid Res 54:1744–1760

Kellner M, Burmester A, Wöstemeyer A, Wöstemeyer J (1991) Sex and parasitism in mucoraceous fungi: transfer of genetic information between *Parasitella simplex* and its host *Absidia glauca*. Mycologist 5:120–122

Kerwin JL, Duddles ND (1989) Reassessment of the role of phospholipids in sexual reproduction by sterol-auxotrophic fungi. J Bacteriol 171:3831–3839

Kerwin JL, Washino RK (1983) Sterol induction of sexual reproduction in *Lagenidium giganteum*. Exp Mycol 7:109–115

Kloer DP, Ruch S, Al-Babili S, Beyer P, Schulz GE (2005) The structure of a retinal-forming carotenoid oxygenase. Science 308:267–269

Knights BA, Elliott CG (1976) Metabolism of Δ^5- and $\Delta^{5,7}$- sterols by *Phytophthora cactorum*. Biochim Biophys Acta 441:341–346

Ko WH (1980) Hormonal regulation of sexual reproduction in *Phytophthora*. J Gen Microbiol 116:459–463

Ko WH (1983) Isolation and partial characterization of α hormones produced by *Phytophthora parasitica*. J Gen Microbiol 129:1397–1401

Ko WH (1985) Stimulation of sexual reproduction of *Phytophthora* by phospholipids. J Gen Microbiol 131:2591–2594

Ko WH (1988) Hormonal heterothallism and homothallism in *Phytophthora*. Annu Rev Phytopathol 26:57–73

Lamour KH, Mudge J, Gobena D, Hurtado-Gonzales OP, Schmutz J, Kuo A, Miller NA, Rice BJ, Raffaele S, Cano LM, Bharti AK, Donahoo RS, Finley S, Huitema E, Hulvey J, Platt D, Salamov A, Savidor A, Sharma R, Stam R, Storey D, Thines M, Win J, Haas BJ, Dinwiddie DL, Jenkins J, Knight JR, Affourtit JP, Han CJ, Chertkov O, Lindquist EA, Detter C, Grigoriev IV, Kamoun S, Kingsmore SF (2012) Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. Mol Plant-Microbe Interact 25:1350–1360

Langcake P (1974) Uptake of sterols by *Phytophthora infestans*, their intracellular distribution and metabolism. Trans Br Mycol Soc 64:55–65

Lee SC, Heitman J (2014) Sex in the mucoralean fungi. Mycoses 57(suppl 3):18–24

Lee SC, Ni M, Li WJ, Heitman J (2010) The evolution of sex: a perspective from the fungal kingdom. Microbiol Mol Biol Rev 74:298–340

Lee SC, Ristaino JB, Heitman J (2012) Parallels in intercellular communication in oomycete and fungal pathogens of plants and humans. PLoS Pathog 8, e1003028. doi:10.1371/journal.ppat.1003028

Lee SC, Sun S, Heitman J (2014) Unseen sex in ancient virgin fungi. New Phytol 201:3–5

Li CH, Cervantes M, Springer DJ, Boekhout T, Ruiz-Vazquez RM, Torres-Martinez SR, Heitman J, Lee SC (2011) Sporangiospore size dimorphism is linked to virulence of *Mucor circinelloides*. PLoS Pathog 7, e1002086. doi:10.1371/journal.ppat.1002086

Lucarotti CJ, Federici BA (1984) Ultrastructure of the gametes of *Coelomomyces dodgei* couch (blastocladiales, chytridiomycetes). Protoplasma 121:77–86

Machlis L (1958) Evidence for a sexual hormone in *Allomyces*. Physiol Plant 11:181–192

Machlis L (1973) Factors affecting the stability of various sirenins and analogues and the uptake of sirenin by the sperm of *Allomyces*. Plant Physiol 52:527–530

Marek LE (1984) Light affects *in vitro* development of gametangia and sporangia of *Monoblepharis macrandra* (chytridiomycetes, monoblepharidales). Mycologia 76:420–425

Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiol 139:920–934

McMorris TC (1978) Sex hormones of the aquatic fungus *Achlya*. Lipids 13:716–722

McMorris TC, Barksdale AW (1967) Isolation of a sex hormone from the water mold *Achlya ambisexualis*. Nature 215:820–821

McMorris TC, White RH (1977) Biosynthesis of the oogoniols, steroidal sex hormones of *Achlya*: the role of fucosterol. Phytochemistry 16:359–362

McMorris TC, Seshadri R, Weihe GR, Arsenault GP, Barksdale AW (1975) Structures of oogoniol-1, −2, and −3, steroidal sex hormones of the water mould, *Achlya*. J Am Chem Soc 97:2544–2545

McMorris TC, Toft DO, Moon S, Wang W (1993) Biological responses of the female strain *Achlya ambisexualis* 734 to dehydro-oogoniol and analogues. Phytochemistry 32:833–837

Medina HR, Cerdá-Olmedo E, Al-Babili S (2011) Cleavage oxygenases for the biosynthesis of trisporoids and other apocarotenoids in *Phycomyces*. Mol Microbiol 82:199–208

Mehta BJ, Salgado LM, Bejarano ER, Cerdá-Olmedo E (1997) New mutants of *Phycomyces blakesleeanus* for β-carotene production. Appl Environ Microbiol 63:3657–3661

Meissner G, Delbrück M (1968) Carotenes and retinal in *Phycomyces* mutants. Plant Physiol 43:1279–1283

Mesland DAM, Huisman JG, van den Ende H (1974) Volatile sexual hormones in *Mucor mucedo*. J Gen Microbiol 80:111–117

Michalski CJ (1978) Protein synthesis during hormone stimulation in the aquatic fungus, *Achlya ambisexualis*. Biochem Biophys Res Commun 84:417–427

Molli SD, Qi J, Yajima A, Shikai K, Imaoka T, Nukada T, Yabuta G, Ojika M (2012) Structure-activity relationship of α hormones, the mating factors of phytopathogen *Phytophthora*. Bioorg Med Chem 20:681–686

Mullins JT (1979) A freeze-fracture study of hormone-induced branching in the fungus *Achlya*. Tissue Cell 11:585–595

Mullins JT (1994) Hormonal control of sexual dimorphism. In: Wessels JGH, Meinhardt F (eds) The mycota, vol 1, 1st edn, Growth, differentiation and sexuality. Springer, Berlin/New York, pp 413–421

Mullins JT, Ellis EA (1974) Sexual morphogenesis in *Achlya*: ultrastructural basis for the hormonal induction of antheridial hyphae. Proc Natl Acad Sci USA 71:1347–1350

Musgrave A, Nieuwenhuis D (1975) Metabolism of radioactive antheridiol by *Achlya* species. Arch Microbiol 105:313–317

Musgrave A, Ero L, Scheffer R (1978) The self-induced metabolism of antheridiol in water moulds. Acta Bot Neerl 27:397–404

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 56:165–185

Nes WD, Patterson GW, Bean GA (1980) Effect of steric and nuclear changes in steroids and triterpenoids on sexual reproduction in *Phytophthora infestans*. Plant Physiol 66:1008–1011

Nieuwenhuis M, van den Ende H (1975) Sex specificity of hormone synthesis in *Mucor mucedo*. Arch Microbiol 102:167–169

Ojika M, Molli SD, Kanazawa H, Yajima A, Toda K, Nukada T, Mao H, Murata R, Asano T, Qi J, Sakagami Y (2011) The second *Phytophthora* mating hormone defines interspecies biosynthetic cross-talk. Nat Chem Biol 7:591–593

Ootaki T, Yamazaki Y, Noshita T, Takahashi S (1996) Excess carotenoids disturb prospective cell-to-cell recognition system in mating responses of *Phycomyces blakesleeanus*. Mycoscience 37:427–435

Orejas M, Peláez MI, Álvarez MI, Eslava AP (1987) A genetic map of *Phycomyces blakesleeanus*. Mol Gen Genet 210:69–76

Pelkmans JF, Lugones LG, Wösten HAB (2016) Fruiting body formation in basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 387–405

Peraza-Reyes L, Malagnac F (2016) Sexual development in fungi. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 407–455

Plattner JJ, Rapoport H (1971) The synthesis of *d*- and *l*- sirenin and their absolute configurations. J Am Chem Soc 93:1758–1761

Plempel M (1962) Die zygotropische Reaktion der Mucorineen. Planta 58:509–520

Polaino S, Herrador MM, Cerdá-Olmedo E, Barrero AF (2010) Splitting of β-carotene in the sexual interaction of *Phycomyces*. Org Biomol Chem 8:4229–4231

Polaino S, Gonzales-Delgado JA, Arteaga P, Herrador MM, Barrero AF, Cerdá-Olmedo E (2012) Apocarotenoids in the sexual interaction of *Phycomyces blakesleeanus*. Org Biomol Chem 10:3002–3009

Pommerville JC (1978) Analysis of gamete and zygote motility in *Allomyces*. Exp Cell Res 113:161–172

Pommerville JC (1981) The role of sex pheromones in *Allomyces*. In: O'Day DH, Horgen PH (eds) Sexual interactions in eukaryotic microbes. Academic, New York, pp 52–73

Pommerville J, Olson LW (1987) Evidence for a male-produced pheromone in *Allomyces macrogynus*. Exp Mycol 11:145–248

Pommerville JC, Strickland B, Romo D, Harding KE (1988) Effects of analogs of the fungal sexual pheromone sirenin on male gamete motility in *Allomyces macrogynus*. Plant Physiol 88:139–142

Pommerville JC, Strickland JB, Harding KE (1990) Pheromone interactions and ionic communication in gametes of aquatic fungus *Allomyces macrogynus*. J Chem Ecol 16:121–131

Popplestone CR, Unrau AM (1973) Major sterols of *Achlya bisexualis*. Phytochemistry 12:1131–1133

Popplestone CR, Unrau AM (1974) Studies on the biosynthesis of antheridiol. Can J Chem 52:462–468

Porter TM, Martin W, James TY, Longcore JE, Gleason FH, Adler PH, Letcher PM, Vilgalys R (2011) Molecular phylogeny of the Blastocladiomycota (fungi) based on nuclear ribosomal DNA. Fungal Biol 115:381–392

Powell MJ (1979) What are the chromidia of *Polyphagus euglenae*? Am J Bot 66:1173–1180

Prakob W, Judelson HS (2007) Gene expression during oosporogenesis in heterothallic and homothallic *Phytophthora*. Fungal Genet Biol 44:726–739

Preus MW, McMorris TC (1979) The configuration at C-24 in oogoniol (24*R*-3β,11α,15β,29-tetrahydroxystigmast-5-en-7-one) and identification of 24 (28)-dehydroogoniols as hormones in *Achlya*. J Am Chem Soc 101:3066–3071

Qi JH, Asano T, Jinno M, Matsui K, Atsumi K, Sakagami Y, Ojika M (2005) Characterization of a *Phytophthora* mating hormone. Science 309:1828

Queiroz EF, Roblot F, Laprévote O, Paulo M d Q, Hocquemiller R (2003) Two unusual acetogenins from the roots of Annona salzmannii. J Nat Prod 66:755–758

Quiles-Rosillo MD, Ruiz-Vásquez RM, Torres-Martínez S, Garre V (2005) Light induction of the carotenoid biosynthesis pathway in *Blakeslea trispora*. Fungal Genet Biol 42:141–153

Randall TA, Fong AA, Judelson HS (2003) Chromosomal heteromorphism and an apparent translocation detected using a BAC contig spanning the mating type locus of *Phytophthora infestans*. Fungal Genet Biol 38:75–84

Raper JR (1952) Chemical regulation of sexual processes in the thallophytes. Bot Rev 18:447–545

Remy W, Taylor TN, Hass H (1994) Early devonian fungi: a blastocladalean fungus with sexual reproduction. Am J Bot 81:690–702

Riehl RM, Toft DO (1984) Analysis of the steroid receptor of *Achlya ambisexualis*. J Biol Chem 259:15324–15330

Riehl RM, Toft DO, Meyer MD, Carlson GL, McMorris TC (1984) Detection of a pheromone-binding protein in the aquatic fungus *Achlya ambisexualis*. Exp Cell Res 153:544–549

Riehl RM, Sullivan WP, Vroman BT, Bauer VJ, Pearson GR, Toft DO (1985) Immunological evidence that the non-hormone binding component of avian steroid receptor exists in a wide range of tissues and species. Biochemistry 24:6586–6591

Riley R, Corradi N (2013) Searching for clues of sexual reproduction in the genomes of arbuscular mycorrhizal fungi. Fungal Ecol 6:44–49

Riley R, Charron P, Idnurm A, Farinelli L, Dalpe Y, Martin F, Corradi N (2014) Extreme diversification of the mating type-high-mobility group (MAT-HMG) gene family in a plant-associated arbuscular mycorrhizal fungus. New Phytol 201:254–268

Ruiz-Hidalgo MJ, Benito EP, Sandmann G, Eslava AP (1997) The phytoene dehydrogenase gene of *Phycomyces*: regulation of its expression by blue light and vitamin A. Mol Gen Genet 253:734–744

Sahadevan Y, Richter-Fecken M, Kaerger K, Voigt K, Boland W (2013) Early and late trisporoids differentially regulate β-carotene production and gene transcript levels in the mucoralean fungi *Blakeslea trispora* and *Mucor mucedo*. Appl Environ Microbiol 79:7466–7475

Salgado LM, Cerdá-Olmedo E (1992) Genetic interactions in the regulation of carotenogenesis in *Phycomyces*. Curr Genet 21:67–71

Salgado LM, Bejarano ER, Cerdá-Olmedo E (1989) Carotene-superproducing mutants of *Phycomyces blakesleeanus*. Exp Mycol 13:332–336

Salgado LM, Avalos J, Bejarano ER, Cerdá-Olmedo E (1991) Correlation between *in vivo* and *in vitro* carotenogenesis in *Phycomyces*. Phytochemistry 30:2587–2591

Schachtschabel D, Boland W (2007) Efficient generation of a trisporoid library by combination of synthesis and biotransformation. J Org Chem 72:1366–1372

Schachtschabel D, Schimek C, Wöstemeyer J, Boland W (2005) Biological activity of trisporoids and trisporoid analogues in *Mucor mucedo* (−). Phytochemistry 66:1358–1365

Schachtschabel D, David A, Menzel K-D, Schimek C, Wöstemeyer J, Boland W (2008) Cooperative biosynthesis of trisporoids by the (+) and (−) mating types of the zygomycete *Blakeslea trispora*. ChemBioChem 9:3004–3012

Schimek C, Wöstemeyer J (2009) Carotene derivatives in sexual communication of zygomycete fungi. Phytochemistry 79:1867–1875

Schimek C, Kleppe K, Saleem A-R, Voigt K, Burmester A, Wöstemeyer J (2003) Sexual reactions in Mortierellales are mediated by the trisporic acid system. Mycol Res 107:736–747

Schimek C, Petzold A, Schultze K, Wetzel J, Wolschendorf F, Burmester A, Wöstemeyer J (2005) 4-Dihydromethyltrisporate dehydrogenase, an enzyme of the sex-hormone pathway in *Mucor mucedo*, is constitutively transcribed but its activity is differently regulated in (+) and (−) mating types. Fungal Genet Biol 42:804–812

Schmidt AD, Heinekamp T, Matuschek M, Liebmann B, Bollschweiler C, Brakhage AA (2005) Analysis of mating-dependent transcription of *Blakeslea trispora* carotenoid biosynthesis genes *carB* and *carRA* by quantitative real-time PCR. Appl Microbiol Biotechnol 67:549–555

Schmoll M, Wang T-F (2016) Sexual development in trichoderma. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 457–474

Schultze K, Schimek C, Wöstemeyer J, Burmester A (2005) Sexuality and parasitism share common regulatory pathways in the fungus *Parasitella parasitica*. Gene 348:33–44

Schwartz SH, Tan BC, Gage DA, Zeevaart JAD, McCarty DR (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science 276:1872–1874

Silver JC, Horgen PA (1974) Hormonal regulation of presumptive mRNA in the fungus *Achlya ambisexualis*. Nature 249:252–254

Silver JC, Andrews DR, Pekkala D (1983) Effect of heat-shock on synthesis and phosphorylation of nuclear and cytoplasmic proteins in the fungus *Achlya*. Can J Biochem Cell Biol 61:447–455

Silver JC, Brunt SA, Kyriakopoulou G, Borkar M, Nazarian-Armavil V (1993) Regulation of two different *hsp70* transcript populations in steroid hormone-induced fungal development. Dev Genet 14:6–14

Sparrow FK (1965) A preliminary note on a population of *Monoblepharis*. Trans Br Mycol Soc 48:55–58

Strack D, Fester T (2006) Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. New Phytol 172:22–34

Sutherland RB, Horgen PA (1977) Effects of the steroid sex hormone, antheridiol, on the initiation of RNA synthesis in the simple eukaryote, *Achlya ambisexualis*. J Biol Chem 252:8812–8820

Sutter RP (1975) Mutations affecting sexual development in *Phycomyces blakesleeanus*. Proc Natl Acad Sci USA 72:127–130

Sutter RP (1987) Sexual development. In: Lipson E, Cerdá-Olmedo E (eds) *Phycomyces*. Cold Spring Harbor Press, Cold Spring Harbor, pp 317–336

Sutter RP, Whitaker JP (1981a) Sex pheromone metabolism in *Blakeslea trispora*. Naturwissenschaften 68:147–148

Sutter RP, Whitaker JP (1981b) Zygophore-stimulating precursors (pheromones) of trisporic acids active in (−)-*Phycomyces blakesleeanus*. J Biol Chem 256:2334–2341

Sutter RP, Zawodny PD (1984) Apotrisporin: a major metabolite of *Blakeslea trispora*. Exp Mycol 8:89–92

Sutter RP, Dadok J, Bothner-By AA, Smith RR, Mishra PK (1989) Cultures of separated mating types of *Blakeslea trispora* make D and E forms of trisporic acids. Biochemistry 28:4060–4066

Sutter RP, Grandin AB, Dye BD, Moore WR (1996) (−) Mating type-specific mutants of *Phycomyces* defective in sex pheromone biosynthesis. Fungal Genet Biol 20:268–279

Tagua VG, Medina HR, Martín-Domínguez R, Eslava AP, Corrochano LM, Cerdá-Olmedo E, Idnurm A (2012) A gene for carotene cleavage required for pheromone biosynthesis and carotene regulation in the fungus *Phycomyces blakesleeanus*. Fungal Genet Biol 49:398–404

Tan BC, Schwartz SH, Zeevaart JA, McCarty DR (1997) Genetic control of abscisic acid biosynthesis in maize. Proc Natl Acad Sci USA 94:12235–12240

Taylor IB, Burbidge A, Thompson AJ (2000) Control of abscisic acid synthesis. J Exp Bot 51:1563–1574

Teepe H, Böttge J-A, Wöstemeyer J (1988) Isolation and electrophoretic analysis of surface proteins of the zygomycete *Absidia glauca*. FEBS Lett 234:100–106

Thomas DM, Goodwin TW (1967) Studies on carotenogenesis in *Blakeslea trispora*. I. General observations on synthesis in mated and unmated strains. Phytochemistry 6:355–360

Thomas DDS, McMorris TC (1987) Allomonal functions of steroid hormone, antheridiol, in water mold *Achlya*. J Chem Ecol 13:1131–1137

Thomas DDS, Mullins JT (1967) Role of enzymatic wall-softening in plant morphogenesis: hormonal induction in *Achlya*. Science 156:84–85

Thomas DS, Mullins JT (1969) Cellulase induction and wall extension in the water mold *Achlya ambisexualis*. Physiol Plant 22:347–353

Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RHY, Aerts A, Arredondo FD, Baxter L, Bensasson D, Beynon JL, Chapman J, Damasceno CMB, Dorrance AE, Dou D, Dickerman AW, Dubchak IL, Garbelotto M, Gijzen M, Gordon SG, Govers F, Grunwald NJ, Huang W, Ivors KL, Jones RW, Kamoun S, Krampis K, Lamour KH, Lee M-K, McDonald WH, Medina M, Meijer HJG, Nordberg EK, Maclean DJ, Ospina-Giraldo MD, Morris PF, Phuntumart V, Putnam NH, Rash S, Rose JKC, Sakihama Y, Salamov AA, Savidor A, Scheuring CF, Smith BM, Sobral BWS, Terry A, Torto-Alalibo TA, Win J, Xu Z, Zhang H, Grigoriev IV, Rokhsar DS, Boore JL (2006) *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. Science 313:1261–1266

Vail WJ, Morris C, Lilly VG (1967) Hormone-like substances which increase carotenogenesis in + and – sexes of *Choanephora cucurbitarum*. Mycologia 59:1069–1074

Van den Ende H (1976) Sexual interactions in plants. Academic, London

Van den Ende H (1978) Sexual morphogenesis in the Phycomycetes. In: Smith JE, Berry DR (eds) The filamentous fungi. Arnold, London, pp 257–274

Van den Ende H, Wiechmann AH, Reyngoud DJ, Hendriks T (1970) Hormonal interactions in *Mucor mucedo* and *Blakeslea trispora*. J Bacteriol 101:423–428

Velayos A, Papp T, Aguilar-Elena R, Fuentes-Vicente M, Eslava AP, Iturriaga EA, Álvarez MI (2003) Expression of the *carG* gene, encoding geranylgeranyl pyrophosphate synthase, is up-regulated by blue-light in *Mucor circinelloides*. Curr Genet 43:112–120

Vereshchagina OA, Memorskaya AS, Tereshina VM (2012) Trisporoids under the stimulation of carotenogenesis in *Blakeslea trispora*. Microbiology 81:570–577

Viera ALG, Camilo CM (2011) *Agrobacterium tumefaciens*-mediated transformation of the aquatic fungus *Blastocladiella emersonii*. Fungal Genet Biol 48:806–811

Viera ALG, Gomes SL (2010) Global gene expression analysis during sporulation of the aquatic fungus *Blastocladiella emersonii*. Eukaryot Cell 9:415–423

Von Lintig J, Vogt K (2000) Filling the gap in vitamin A research - molecular identification of an enzyme cleaving beta-carotene to retinal. J Biol Chem 275:11915–11920

Wang S-Y, Song P, Chan L-Y, Loh T-P (2010) Total synthesis of *Phytophthora* mating hormone α1. Org Lett 12:5166–5169

Werkman BA (1976) Localization and partial characterization of a sex-specific enzyme in homothallic and heterothallic Mucorales. Arch Microbiol 109:209–213

Werkman BA, van den Ende H (1973) Trisporic acid synthesis in *Blakeslea trispora*. Interaction between plus and minus mating types. Arch Microbiol 90:365–374

Werkman BA, van den Ende H (1974) Trisporic acid synthesis in homothallic and heterothallic Mucorales. J Gen Microbiol 82:273–278

Werner S, Schroeter A, Schimek C, Vlaic S, Wöstemeyer J, Schuster S (2012) Model of the synthesis of trisporic acid in Mucorales showing bistability. IET Syst Biol 6:207–214

Wetzel J, Scheibner O, Burmester A, Schimek C, Wöstemeyer J (2009) 4-Dihydrotrisporin-dehydrogenase, an enzyme of the sex hormone pathway of *Mucor mucedo*: purification, cloning of the

corresponding gene, and developmental expression. Eukaryot Cell 8:88–95

Wetzel J, Burmester A, Kolbe M, Wöstemeyer J (2012) The mating-related loci *sexM* and *sexP* of the zygomycetous fungus *Mucor mucedo* and their transcriptional regulation by trisporoid pheromones. Microbiology 158:1016–1023

Whisler HC, Zebold SL, Shemanchuk JA (1975) Life history of *Coelomomyces psorophorae*. Proc Natl Acad Sci USA 72:693–696

White JD, Takabe K, Prisbylla MP (1985) Stereoselective synthesis of trisporic acids A and B, their methyl esters, and trisporol A and B, hormones and prohormones of mucoraceous fungi. J Org Chem 50:5233–5244

Wöstemeyer A, Teepe H, Wöstemeyer J (1990) Genetic interactions in somatic inter-mating type hybrids of the zygomycete *Absidia glauca*. Curr Genet 17:163–168

Wöstemeyer J, Wöstemeyer A, Burmester A, Czempinski K (1995) Relationships between sexual processes and parasitic interactions in the host-pathogen system *Absidia glauca–Parasitella parasitica*. Can J Bot 73:S243–S250

Wöstemeyer J, Grünler A, Schimek C, Voigt K (2005) Genetic regulation of carotenoid biosynthesis in fungi. In: Arora DK (ed) Applied mycology and biotechnology, vol 5, Genes, genomics and bioinformatics. Elsevier, Dordrecht, pp 257–274

Yajima A, Qin Y, Zhou X, Kawanishi N, Xiao X, Wang J, Zhang D, Wu Y, Nukada T, Yabuta G, Qi J, Asano T, Sakagami Y (2008) Synthesis and absolute configuration of hormone α1. Nat Chem Biol 4:235–237

Yajima A, Toda K, Molli SD, Ojika M, Nukada T (2011) Syntheses of the four stereoisomers of *Phytophthora* mating hormone α2 and a concise synthesis of mating hormone α1. Tetrahedron 67:8887–8894

Yakovleva IM, Vakulova LA, Feofilova EP, Bekhtereva MN, Samakhvalov GI (1980) Influence of synthetic compounds structurally close to trisporic acids on carotenogenesis and lipogenesis of the *Mucor* fungus *Blakeslea trispora*. Microbiologiya 49:274–278

11 Photomorphogenesis and Gravitropism in Fungi

Luis M. Corrochano[1], Paul Galland[2]

CONTENTS

[1]Departamento de Genética, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain; e-mail: corrochano@us.es
[2]Fachbereich Biologie, Philipps-Universität Marburg, Karl-von-Frisch Str. 8, 35032 Marburg, Germany; e-mail: galland@staff.uni-marburg.de

I. Introduction

Fungi use signals from the environment to regulate growth and development. Fungal responses to light and gravity have been investigated in most detail, and we have a great understanding of the mechanisms of fungal photoreception in several model fungi. Light regulates fungal metabolism and gene expression, resets the circadian clock, and regulates fungal morphogenesis. Light and gravity direct the growth of reproductive structures in many fungi through a coordinated mode of action. Understanding the molecular mechanisms that allow the perception of light and gravity by fungi will help to understand similar phenomena in more complex organisms like plants. The increased number of fungal genomes available, the availability of fungal collections of gene knockouts, and the popularization of techniques to tag proteins for visualization and biochemical characterization have increased enormously our understanding of fungal photoreception during the last 10 years. The photoreceptor repertoire of fungi has been analyzed in detail from the fungal genomes available, and the mechanisms of photoreceptor activity and their role in the fungal cell are being investigated by gene inactivation and detailed biochemical characterizations. At the same time, the cytoskeleton and the fungal organelles that may participate in gravity sensing are being characterized with advanced visualization techniques that should yield new

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

insights into the mechanisms of gravity sensing in the fungal cell. Readers interested in fungal photomorphogenesis and gravitropism should consult our chapter in the previous edition of this book (Corrochano and Galland 2006). Here, we update and review our current knowledge in fungal photomorphogenesis and gravitropism.

II. Photomorphogenesis

Light regulates several aspects of fungal morphogenesis that include spore germination, hyphal growth, and the initiation and development of structures for sexual or asexual reproduction. In addition, light regulates the direction and speed of growth of reproductive structures, and many fungal fruiting bodies show a phototropic response similar to that observed in plants. We summarize our current knowledge on fungal photomorphogenesis with an emphasis in the reception of the signal by photoreceptors, the transduction of the signal inside the cell, and the effects of light on fungal morphogenesis.

A. Fungal Photoreceptors and Their Mode of Action

Photoreceptor proteins receive the light signal through specialized light-absorbing molecules, chromophores, and transduce the energy of each photon into the cell to promote a response. Photoreceptor genes and proteins have been described and characterized in most organisms, including fungi (Briggs and Spudich 2005). Most fungi see blue light using homologs of the WC complex first identified in the fungus *Neurospora crassa*, but fungal genomes have genes for other blue-light photoreceptors (cryptochromes), red-light photoreceptors (phytochromes), and opsins which are similar to the ones used for animal vision (Corrochano 2007; Herrera-Estrella and Horwitz 2007; Purschwitz et al. 2006; Corrochano and Avalos 2010; Idnurm et al. 2010; Corrochano and Garre 2010). The variety of photoreceptor genes in all fungal lineages suggests that the last common ancestor of the fungi was capable of perceiving light in a wide range of colors.

1. The WC Complex: A Photoreceptor that Regulates Transcription

The white collar complex (WCC) from *N. crassa* is a protein complex composed of proteins white collar-1 (WC-1) and white collar-2 (WC-2) (Ballario et al. 1996; Linden and Macino 1997). Both proteins contain a zinc finger at their C-terminal end for DNA binding, and WC-1 contains a LOV domain that binds a flavin chromophore. These domains allow the WCC to act as a light-dependent transcription factor complex (Froehlich et al. 2002; He et al. 2002). Another LOV domain-containing protein is VVD (Heintzen et al. 2001), and it plays a key role in the regulation of the activity of the WCC. The role of the WCC and VVD in the photoreception of *N. crassa* has been reviewed recently (Chen et al. 2010b; Olmedo et al. 2013; Idnurm et al. 2010; Schafmeier and Diernfellner 2011), and our current view of the mechanism of activation of gene transcription by the WCC is summarized below. The mechanism for the WCC-mediated activation of transcription involves several steps. In the dark, the WCC binds to the promoters of light-regulated genes (Smith et al. 2010; Wu et al. 2014) and interacts with the histone acetyltransferase NGF-1 (Brenna et al. 2012). The activation of WC-1 by light provokes a conformational change in the protein that leads to the formation of WCC homodimers through the LOV domains of WC-1 (Malzahn et al. 2010). The conformational change should lead to the activation of the HAT (histone acetyltransferase) activity of NGF-1 and the transient light-dependent acetylation of lysine 14 in histones H3 that are located in the promoters of the light-regulated genes, like *al-3* (Brenna et al. 2012; Grimaldi et al. 2006). The histone deacetylation would change the chromatin in the promoter allowing better accessibility for the RNA polymerase. This model is supported by the observation that strains with a replacement of lysine 14 by glutamine in histone H3 or with mutations in the gene *ngf-1* for a histone acetylase show a blind phenotype (Grimaldi et al.

2006). Light promotes the transient transcription of *wc-1* (Ballario et al. 1996; Káldi et al. 2006) and phosphorylation of WC-1 that leads to WC-1 degradation and its replacement in the WCC by newly synthesized but inactive WC-1 protein (He and Liu 2005; Schwerdtfeger and Linden 2000, 2001; Talora et al. 1999). WC-2 is also phosphorylated after light exposure, but the modification is more subtle (Schwerdtfeger and Linden 2000).

Abundance, phosphorylation, and activity of the WCC are controlled by FRQ, a master regulator of the *Neurospora* circadian clock. The oscillation in the amount of FRQ during a circadian cycle results in changes in the abundance, phosphorylation, and activity of the WCC during growth in the dark (Baker et al. 2012; Brunner and Káldi 2008). FRQ is required for full *wc-1* photoactivation and for the translation of the *wc-1* mRNA, resulting in cycles of WC-1 protein in dark-grown mycelia (Cheng et al. 2001, 2002; Lee et al. 2000; Merrow et al. 2001; Schafmeier et al. 2006). In addition, the RNA helicase FRH is associated with the WCC and mediates the interaction between FRQ and the WCC (Cheng et al. 2005). FRQ plays a relevant role in the regulation of the phosphorylation status of the WCC and its activity. The lack of FRQ results in hypophosphorylated and transcriptionally active WCC, while the WCC is phosphorylated and inactive in the presence of FRQ. The oscillation in the amount of FRQ during a circadian cycle results in changes in the phosphorylation status of the WCC and its potential activity during the circadian cycle in the dark (Brunner and Schafmeier 2006).

Other proteins interact with the WCC to regulate its activity. The protein phosphatases PP2A and PP4 interact with the WCC and participate in the dephosphorylation and activation of the WCC (Schafmeier et al. 2005; Cha et al. 2007). On the other hand, the kinases CK-1a and CKII interact with FRQ for the phosphorylation and inactivation of the WCC (He et al. 2006). The observation that CK-1a phosphorylates WC-2 in vitro suggests that this enzyme may play a role in the regulation of the activity of the WCC (Querfurth et al. 2007). Another kinase that regulates the abundance of the WCC on a light-independent manner is GSK, the glycogen synthase kinase. GSK binds WC-1 and WC-2, presumably promoting its degradation via phosphorylation (Tataroglu et al. 2012). The protein kinase A, PKA, stabilizes the WC proteins, inhibits WCC binding to the *frq* promoter in the dark, and inhibits the light function of the WCC as shown by the enhanced light-dependent accumulation of *al-3* mRNA in a strain lacking PKA (Huang et al. 2007). These observations suggest that PKA may have a leading role in the light-dependent phosphorylation of the WCC that occurs during the release of the WCC from light-regulated promoters. Indeed, PKA inhibits the nuclear localization of the WCC (Cha et al. 2008). Protein kinase C (PKC) interacts with WC-1 in vivo and phosphorylates the Zn finger domain in vitro in dark-grown mycelia or after 2 h of illumination, when light-induced gene expression has ceased. A strain with reduced amounts of PKC has increased levels of WC-1 confirming that PKC is a negative regulator of WC-1 (Franchi et al. 2005). In contrast, a strain lacking PKA has reduced levels of WC-1 and WC-2 (Huang et al. 2007). Regardless of the effect of these kinases on the amount of WC proteins, reduced activities of either PKA or PKC promote light-dependent gene transcription (Franchi et al. 2005; Huang et al. 2007), suggesting that the role of these two kinases is to inactivate the WCC. An interaction between the WCC and the Ccr4–Not complex has been detected and is required for maintaining the levels of the WCC suggesting a regulatory role for this complex (Huang et al. 2013).

2. Regulation of Transcription by the WCC: Adaptation to Light

When dark-grown mycelia are exposed to light the amount of WCC-dependent mRNAs increases rapidly and then decreases after extended illumination, a process known as photoadaptation (Arpaia et al. 1999; Lauter and Yanofsky 1993; Schwerdtfeger and Linden 2001, 2003). As expected, the WCC only binds transiently to the promoter of light-regulated genes (He and Liu 2005; Olmedo et al. 2010b).

A key element in the regulation of photoadaptation is the LOV domain photoreceptor VVD. The gene *vvd* is activated by light and strains with mutations in *vivid* exhibits sus-

tained WC-1 light-dependent phosphorylation, a reduction in the amount of WC-1 in the cell, and sustained gene photoactivation (Heintzen et al. 2001; Malzahn et al. 2010; Schwerdtfeger and Linden 2001, 2003; Shrode et al. 2001). Light-activated VVD forms dimers through interactions between each LOV domain (Zoltowski and Crane 2008; Peter et al. 2012), and newly synthesized VVD disrupts interactions between two WCCs resulting in a reduction of the transcriptional response and photoadaptation (Malzahn et al. 2010; Hunt et al. 2010; Chen et al. 2010a). Although the transcription of *wc-1* is activated in response to light, this newly synthesized WC-1 would not be enough to compensate for WC-1 degradation. Instead, the binding to VVD prevents degradation of WC-1 creating a pool of light-activated WC-1 that is transcriptionally inactive. As expected, overexpression of VVD results in desensitization of the WCC (Malzahn et al. 2010).

Other elements participate in the regulation of photoadaptation. The light-dependent phosphorylation of the WCC reduces its capacity to bind light-inducible promoters, suggesting that phosphorylation plays a prominent role in the transient activity of the WCC and photoadaptation (He and Liu 2005; Schafmeier et al. 2005). Photoadaptation is modified by inhibitors or mutations of the protein kinase C (Arpaia et al. 1999; Franchi et al. 2005) and requires protein synthesis (Schwerdtfeger and Linden 2001).

RCO-1 and RCM-1, the *Neurospora* homologs of the corepressor complex Tup1-Ssn6 in yeast, play a role in photoadaptation. RCO-1 and RCM-1 accumulate in *Neurospora* nuclei (Olmedo et al. 2010a), and both proteins interact to form a repressor complex similar to that observed in yeast (Sancar et al. 2011). When exposed to 5 h of light, the *rco-1* and *rcm-1* mutants show a sustained expression of light-induced genes (Olmedo et al. 2010a) suggesting that the *Neurospora* RCO-1/RCM-1 complex is involved in the repression of light-dependent gene transcription that leads to photoadaptation. The absence of the RCO-1/RCM-1 repressor complex leads to a reduction in the amount of VVD that is available for the regulation of the WCC. The reduction in the amount of VVD results in increased WCC binding to the promoters of light-regulated genes in the dark and after long exposures to light, leading to the modification of photoadaptation that has been observed in *rco-1* and *rcm-1* mutants. These results indicate that the photoadaptation phenotype of mutants in the RCO-1/RCM-1 repressor complex is, at least in part, an indirect consequence of the reduction of *vvd* transcription and the resulting modification in the regulation of transcription by the WCC (Ruger-Herreros et al. 2014).

Three additional mutant strains that displayed defects in photoadaptation have been identified using a genetic selection system. These mutations altered photoadaptation of only a specific group of genes (*con-10* and *con-6*), suggesting that regulation of photoadaptation is relatively gene specific. Unfortunately, none of the relevant genes have yet been identified (Navarro-Sampedro et al. 2008).

In addition, epigenetic modifications play a role in the transcriptional response to light such as the trimethylation of lysine 9 of histone H3 (H3K9me3) by DIM-5. The levels of H3K9me3 in the promoter of *frq* increased during exposure to light with a maximum after 30 min of light. Strains lacking DIM-5 show an increased in light-dependent mRNA accumulation for *frq* and increased accumulation of WC-2 in the *frq* promoter. The results suggest that the modification of histone H3 after light exposure should play a role in the release of the WCC from the *frq* promoter after light-dependent activation (Ruesch et al. 2015).

The events leading to gene photoactivation can be summarized as follows. Light reception by the FAD chromophore of WC-1 should trigger the formation of a flavin–cysteinyl adduct, causing a conformational change that leads to WCC dimerization and promoter binding, chromatin remodeling, and the activation of gene transcription. Light exposure of dark-grown mycelia stimulates the transcription of *vvd*, *frq*, and other light-induced genes and promotes the phosphorylation of WC-1. Gene photoactivation is transient. Newly synthesized VVD competes with the light-activated WC-1 and disrupts the formation of WCC dimers reducing WCC binding to the promoter. The

WCC bound to VVD is not transcriptionally active and results in the attenuation of the response to light. Different fractions of the light-activated WCC are stabilized by FRQ and dephosphorylated and partially degraded, probably through an interaction with the protein kinase C (PKC). After gene transcription, epigenetic modification of histones helps to release the WCC from the promoters.

3. Other *N. crassa* Photoreceptors

The *N. crassa* genome contains genes for additional photoreceptors, but they should operate as secondary photoreceptors to modulate the activity of the WCC (Olmedo et al. 2010c). Cryptochromes are plant blue-light photoreceptors very similar to photolyases, enzymes required for blue-light-dependent DNA repair. They bind noncovalently the flavin chromophore FAD and other, probably secondary, chromophores (pterin or deazaflavin) and do not show photolyase activity (Fortunato et al. 2015; Chaves et al. 2011; Liu et al. 2011). *Neurospora* CRY belongs to the cryptochrome-DASH subfamily, and the gene is induced by light in a WC-1-dependent manner and by the circadian clock (Froehlich et al. 2010). CRY binds the chromophores FAD and MTHF (methenyltetrahydrofolate) when expressed in *E. coli* and binds DNA (Froehlich et al. 2010). A role for CRY modulating the activity of the WCC for the activation of some light-induced genes such as *con-10* has been proposed (Olmedo et al. 2010c). However, genome-wide activation of transcription by light is not significantly altered in the *Neurospora cry* mutant (Froehlich et al. 2010). CRY plays a secondary role in the regulation of the circadian clock as an element of a secondary oscillator that is only detected in a specific genetic background (Nsa et al. 2015).

Phytochromes are red and far-red photoreceptors present in plants, fungi, and bacteria that bind a bilin chromophore (Burgie and Vierstra 2014; Chen and Chory 2011; Rockwell et al. 2006; Karniol et al. 2005). The two *N. crassa* phytochromes, PHY-1 and PHY-2, contain an amino-terminal sensory domain that forms the pocket necessary for chromophore binding and a carboxy-terminal output domain that is likely involved in relaying the light signal to other proteins (Froehlich et al. 2005). The *phy* genes are not regulated by light, but *phy-1* is regulated by the circadian clock. Phosphorylated and unphosphorylated versions of PHY-1 have been observed in the *Neurospora* cytoplasm (Froehlich et al. 2005). The *phy-2* mutant shows an increased light-dependent accumulation of *con-10* mRNA, suggesting that its activation by light is negatively regulated by PHY-2 (Olmedo et al. 2010c). However, the results of genome-wide microarray hybridization experiments showed that only a few genes had an altered expression in response to light in *phy-1* and *phy-2* mutants (Chen et al. 2009), suggesting that phytochromes may modify light responses in a gene-specific manner.

Rhodopsins are membrane-embedded seven-transmembrane helix photoreceptors composed of a retinal chromophore bound to an opsin apoprotein (Ernst et al. 2014; Sharma et al. 2006; Spudich 2006). The *Neurospora* opsin, NOP-1, shows a slow photocycle and long-lived intermediates, consistent with a role as a sensory photoreceptor (Bergo et al. 2002; Bieszke et al. 1999a, b; Brown et al. 2001; Furutani et al. 2004). However, the inactivation of *nop-1* did not result in a blind phenotype (Bieszke et al. 1999a). The mRNA for the *nop-1* gene accumulates during asexual or sexual development, with a large amount of the mRNA observed late during conidiation, but not during early vegetative growth (Bieszke et al. 1999a). A regulatory role for the NOP-1 protein has been suggested based on changes in mRNA accumulation of light- and conidiation-regulated genes (Bieszke et al. 2007; Olmedo et al. 2010c).

4. Photoreceptors in Other Fungi

The surge in fungal genome sequencing projects has provided enormous information regarding the presence of photoreceptor genes among representatives of the fungal kingdom (Idnurm et al. 2010; Rodriguez-Romero et al. 2010; Herrera-Estrella and Horwitz 2007; Corrochano 2007).

Proteins similar to WC-1 and WC-2 have been identified and characterized in most fungal groups, and presumably their mode of action should be similar to that of the WCC as shown by the blind phenotype of strains with mutations in their *wc* homologs (Idnurm et al. 2010). In fact, interactions between MadA and MadB, the *Phycomyces blakesleeanus* homologs of WC-1 and WC-2, suggested the presence of a photosensitive Mad complex in *Phycomyces* that may operate with a mechanism similar to that of the WCC (Sanz et al. 2009).

However, the characterization of light responses in several fungi has shown differences with the model fungus *Neurospora crassa*. Unlike *Neurospora*, the ascomycete *Aspergillus nidulans* can perceive blue and red light to regulate morphogenesis and secondary metabolism (Bayram et al. 2010). A phytochrome gene, *fphA*, has been described and characterized in the ascomycete *A. nidulans* (Blumenstein et al. 2005; Brandt et al. 2008). The *Aspergillus* phytochrome has a chromophore binding domain and an output domain composed of a histidine kinase domain and a response regulator domain. The FphA phytochrome bounds the chromophore biliverdin when it was expressed in *E. coli* and showed the red/far-red-light photoreversibility that is typical of phytochromes. In addition, the expressed FphA phytochrome showed a reduced but detectable autophosphorylation. FphA is located in the cytoplasm of germinating spores, and this localization is not changed after red-light exposure. In vivo experiments have showed that FphA molecules interact physically (Blumenstein et al. 2005; Brandt et al. 2008). An *Aspergillus* strain with a deletion of the *fphA* gene showed a reduction in the red-light-dependent inhibition of sexual development and in the red-light-dependent stimulation of asexual development (conidiation). However, lack of red-light sensitivity in the strain without a functional *fphA* gene was not complete, since this strain produced only about 10 % of the sexual structures under red light that developed in the dark. This observation suggested that other red-light-absorbing protein was responsible for most of the red-light repression of sexual development in *Aspergillus* (Blumenstein et al. 2005). The *Aspergillus* phytochrome forms a complex with WC proteins and interacts with the WC-2 homolog LreB in the nucleus, despite the preferred localization of the phytochrome in the cytoplasm (Purschwitz et al. 2008). The interaction between FphA and LreB occurs through the histidine kinase domain and the response regulator domain of FphA (Purschwitz et al. 2009). These results suggest the active transport of the phytochrome to the *Aspergillus* nuclei, perhaps to modulate the transcriptional activity of the WC complex. A key protein in the photobiology of *A. nidulans* is VeA. A mutation in *veA* allows *Aspergillus* to conidiate in the dark, suggesting a role for VeA as a negative regulator of red-light-induced conidiation (Mooney and Yager 1990). The cellular location of VeA depends on the presence of light. VeA is preferentially located in the nucleus in cells grown in the dark. Light exposure results in a more even distribution of VeA between the nucleus and the cytoplasm, consistent with a role for VeA as a repressor of light-dependent processes. As expected, the mutant VeA1 protein was always found in the cytoplasm regardless of light exposure (Stinnett et al. 2007). FphA forms a complex with LreA (WC-1) and LreB (WC-2) and the protein VeA in the nucleus suggesting the formation of a photoreceptor complex that regulates gene transcription after light reception (Purschwitz et al. 2008). The role of FphA and VeA seems to be central in the responses to light in *Aspergillus*, but a role of VeA in the response to light has not been observed in *Neurospora* (Bayram et al. 2008c). The *A. nidulans* cryptochrome, CryA, shows photolyase activity and functions as a repressor of sexual reproduction under ultraviolet and blue light, unlike its *Neurospora* homolog (Bayram et al. 2008a; Froehlich et al. 2010). These observations suggest differences in the mechanisms that regulate transcription by light in *N. crassa* and *A. nidulans*.

In many ascomycete and basidiomycete fungi, proteins homologous to WC-1 act as the main photoreceptor for blue light. Light regulates conidiation and enzyme production in several species of *Trichoderma* (Schmoll et al. 2010; Carreras-Villaseñor et al. 2012). In *Tri-*

choderma atroviride, the *wc* genes are named *blr-1* and *blr-2* and are required for the photoinduction of conidiation and gene expression but not for the light-dependent inhibition of hyphal growth (Casas-Flores et al. 2004; Rosales-Saavedra et al. 2006; Castellanos et al. 2010). A homolog of the *wc-1* gene has been identified in the related species *Trichoderma pleuroticola* (Steyaert et al. 2010). An increase in sensitivity to light is observed when *blr-2* is overexpressed suggesting that BLR-2 is limiting the formation of the photoresponsive complex (Esquivel-Naranjo and Herrera-Estrella 2007). A gene similar to *vvd*, *envoy*, has been isolated in *Hypocrea jecorina* (*Trichoderma reesei*). The gene is induced by light and by the presence of cellulose, suggesting a connection between light reception and carbon utilization in this fungus (Friedl et al. 2008; Schmoll et al. 2005). Light regulates gene transcription in *Hypocrea* through the activity of the BLR1/BLR2 and ENVOY (Castellanos et al. 2010), but BRL1, BRL2, and ENVOY have additional roles in the dark, as shown by the identification of genes and proteins regulated by the photoreceptors in the absence of light (Schuster et al. 2007; Sánchez-Arreguín et al. 2012; Tisch and Schmoll 2013).

Light regulates the biosynthesis of carotenoids in *Fusarium* (Avalos and Estrada 2010). Genes homologous to *wc-1* have been characterized in *Fusarium fujikuroi*, *Fusarium oxysporum*, and *Fusarium graminearum*. Mutation of the *wc-1* gene in each of these fungi reduced, but did not abolish, light-dependent accumulation of carotenoids, suggesting that the *Fusarium* WC-1 protein is required for full activation of photocarotenogenesis in coordination with other blue-light photoreceptors (Ruiz-Roldán et al. 2008; Estrada and Avalos 2008; Kim et al. 2013). The *F. fujikuroi wc-1* mutant did not show any light-dependent mRNA accumulation for the opsin genes, *carO* and *opsA*, but the mRNA levels increased in the dark, suggesting that WC-1 has a dual role as a repressor and as a light-dependent inducer (Estrada and Avalos 2008). A reduction in secondary metabolism and conidial production in the *wc-1* mutant of *F. fujikuroi* suggested that this protein plays a major regulatory role in this fungus (Estrada and Avalos 2008). In addition, the *wc-1* gene from *F. oxysporum* was required for full pathogenicity in a mice model (Ruiz-Roldán et al. 2008), a phenotype that had been observed in the *wc* mutants of *Cryptococcus neoformans* (Idnurm and Heitman 2005). Two opsin genes, *carO* and *opsA*, have been identified in the genome of *Fusarium* species. One of the opsins, CarO, is a light-driven proton pump that is abundant in conidia and retards germination under light, suggesting a role for CarO in the regulation of fungal germination (García-Martínez et al. 2015). However, the best use of an opsin as fungal photoreceptor has been described in *Blastocladiella emersonii*. The photoreceptor for phototaxis in this fungus is a fusion protein containing an opsin domain and a guanylyl cyclase catalytic domain (Avelar et al. 2014).

Genes for proteins similar to VIVID and cryptochromes are present in the genome of *F. fujikuroi*. VvdA participates in the regulation of mycelial development and is required for the accumulation of carotenoids after light (Castrillo and Avalos 2014). CryD is a member of the CRY-DASH family and participates in the regulation of secondary metabolism and the development of conidia (Castrillo et al. 2013). It is interesting to note that in the ascomycete *Sclerotinia sclerotiorum*, deletion of the CRY-DASH gene results in minor developmental defects, suggesting that this protein may have a sensory role in this fungus (Veluchamy and Rollins 2008) as in *Fusarium*.

Additional *wc* genes have been characterized in other ascomycetes and basidiomycete genomes, including the plant pathogenic fungi *Magnaporthe oryzae* (Lee et al. 2006b; Kim et al. 2011), *Bipolaris oryzae* (Moriwaki et al. 2008; Kihara et al. 2007), *Botrytis cinerea* (Canessa et al. 2013), *Alternaria alternata* (Pruss et al. 2014), the human pathogen *Aspergillus fumigatus* (Fuller et al. 2013), and the edible fungus *Cordyceps militaris* (Yang and Dong 2014). However, it is worth mentioning the discovery of a protein with a novel FAD-binding site in *Coprinopsis cinerea* that is required for the regulation by light of morphogenesis (Kuratani et al. 2010). In *C. cinerea* blue light regulates

development through a homolog of *wc-1* (Kamada et al. 2010), as in other basidiomycete fungi like *Schizophyllum commune* (Ohm et al. 2013), *Lentinula edodes* (Sano et al. 2007), or *Cryptococcus neoformans* (Idnurm and Heitman 2005; Lu et al. 2005). The discovery of Dst2 with a novel putative chromophore binding site suggests that a novel photoreceptor may play an additional role in the photobiology of *C. cinerea* (Kuratani et al. 2010).

A different evolutionary strategy in the evolution of photoreceptor genes has been observed in basal fungi. Genes for WC proteins have expanded by duplication in the genomes of fungi belonging to the Mucoromycotina (Corrochano and Garre 2010). Three genes similar to *wc-1* have been identified and characterized in *Phycomyces blakesleeanus* (Sanz et al. 2009; Idnurm et al. 2006), *Mucor circinelloides* (Silva et al. 2006), *Rhizopus delemar* (Idnurm et al. 2006), and *Pilobolus crystallinus* (Kubo 2009). In addition, four *wc-2* genes have been identified in *Phycomyces* (Sanz et al. 2009) and *Mucor* (V. Garre pers. comm.), but five *wc-2* genes have been identified in *Rhizopus* (Ma et al. 2009).

Only two of the *Phycomyces wc* genes, *madA* and *madB*, are required for photoreception since mutations in *madA* or *madB* reduce the sensitivity to light in *Phycomyces* (Cerdá-Olmedo 2001). The *madA* alleles have mutations in the flavin-binding domain or lack the Zn finger that is presumably involved in DNA binding (Idnurm et al. 2006). The only *madB* allele characterized has a splicing mutation that should produce a truncated protein without the zinc finger (Sanz et al. 2009). The blind phenotype of strains carrying *madA* or *madB* alleles suggests that the flavin-binding domain and Zn finger are both required for *Phycomyces* light responses, including gene photoactivation (Sanz et al. 2009; Idnurm et al. 2006; Rodríguez-Romero and Corrochano 2006). MadA and MadB interact to form a complex (Mad complex) in yeast two-hybrid assays and after coexpression in *Escherichia coli*, but none of these proteins interact with other *Phycomyces* WC proteins. The absence of any additional interaction between *Phycomyces* WC proteins in yeast two-hybrid assays suggests that the Mad complex must be the main photoreceptor complex in *Phycomyces* (Sanz et al. 2009).

The role of the remaining *wc* genes in *Phycomyces* photobiology is currently unknown. However, the functional domains of the WC-1 proteins suggest that they may also serve as blue-light-dependent transcription factors that may be relevant for gene photoactivation. The genes *madA* and *madB* are not induced by light, and the amount of *madA* mRNA is reduced after blue-light exposure. In contrast, *wcoA*, *wcoB*, *wctB*, and *wctD* are induced by light (Sanz et al. 2009; Idnurm et al. 2006; Rodríguez-Romero and Corrochano 2006). The differential regulation of these genes by light probably reflects different roles for the corresponding proteins in *Phycomyces* photobiology.

Unlike *Phycomyces*, the WC proteins from *Mucor* have specialized as photoreceptors for specific responses (Silva et al. 2006). The fruiting bodies of a strain with a deletion of *mcwc-1a* showed reduced phototropism, while a strain with a deletion of *mcwc-1c* showed a reduced light-dependent beta-carotene biosynthesis, suggesting that Mcwc-1a and Mcwc-1c may act as photoreceptors for phototropism and photocarotenogenesis, respectively. The activity of the third WC protein, Mcwc-1b, is modified by ubiquitylation and regulates the transcription of the genes for the biosynthesis of beta-carotene and participates in the regulation of vegetative growth (Silva et al. 2008; Navarro et al. 2013).

The transient activation of transcription by light, photoadaptation, has been described in *Neurospora* (see above), *Phycomyces* (Sanz et al. 2009; Rodríguez-Romero and Corrochano 2006), and *Aspergillus* (Ruger-Herreros et al. 2011). Photoadaptation in *Phycomyces* has been investigated using the heat-shock protein gene *hspA*. The *hspA* gene is induced by heat-shock and light (Rodríguez-Romero and Corrochano 2004; Corrochano 2002), but photoadaptation was not prevented by changes in light intensities or dark incubations, unlike photoadaptation in *Neurospora*, suggesting the operation of a different molecular mechanism (Rodríguez-Romero and Corrochano 2006). As we have discussed above, photoadaptation in

Neurospora relies on the interaction between WC-1 and VVD, but no *vvd* homolog has been identified in the genomes of *Aspergillus* or *Phycomyces* (Idnurm et al. 2010; Lombardi and Brody 2005). Photoadaptation in these fungi is likely to occur through a novel mechanism that should be different from the one described in *Neurospora*.

B. Transducing the Light Signal

The WCC is capable of receiving the light signal to regulate transcription in a very simplified signal transduction pathway. However, other proteins have been described in several fungi that play a role in the transduction of the light signal in coordination with homologs of the WCC.

In *N. crassa*, blue light induces the phosphorylation of a 15-kDa protein that was identified as a nucleoside diphosphate kinase encoded by the *ndk-1* gene (Ogura et al. 1999). Mutations in *ndk-1* reduced the NDK-1 activity, prevented the effect of light on the polarity of the perithecial beak (the upward positioning of the end of the sexual structure in this fungus) without affecting perithecial beak phototropism (the orientation of the beak toward light), and showed alterations in other developmental responses and light-dependent carotene biosynthesis. These results suggest that NDK-1 may be an element of the signal transduction pathway for some light responses in *N. crassa* but do not rule out a structural defect in the perithecial beak of *ndk-1* strains (Lee et al. 2006a; Ogura et al. 2001). The observation that NDK-1 is present in the plasma membrane in the dark but relocates to the cytoplasm after light exposure further supports a relevant role for this protein in the light transduction pathway (Yoshida and Hasunuma 2006). Other signals may also play a role in light transduction. A *sod-1* mutant altered in the enzyme Cu,Zn superoxide dismutase lost the light-induced polarity of perithecia and also showed an enhanced synthesis of carotenes. The results suggested that intracellular reactive oxygen regulated by SOD-1 should have a role in the light transduction pathway (Yoshida and Hasunuma 2004), a conclusion further supported by the observation of physical interactions between NDK-1 and the catalase CAT-1 in yeast two-hybrid assays (Yoshida et al. 2006).

Several *A. nidulans* proteins participate in the transduction of the light signal. We described earlier the presence of a photoreceptor complex composed of the WC proteins, the phytochrome and VeA. VeA has been identified as a component of another *A. nidulans* protein complex, the *velvet* complex, with proteins VeA, VelB (a protein similar to VeA), and LaeA (the major regulator of secondary metabolism), which has been proposed to participate in the regulation by light of development and secondary metabolism (Bayram et al. 2008b). To complicate things further, VelB forms a second protein complex with VosA, another member of the velvet family, and the formation of both complexes is regulated by LaeA (Sarikaya Bayram et al. 2010). LaeA is a key element in the regulation of sexual development by light, perhaps through its regulation of the two different velvet complexes (Sarikaya Bayram et al. 2010). Proteins homologous to VeA have been characterized in several fungi (Calvo 2008). Homologs of VeA participate in the transduction of the light signal for the regulation of metabolic pathways and development in *Penicillium chrysogenum* (Hoff et al. 2010), *Mycosphaerella graminicola* (Choi and Goodwin 2011), and *Botrytis cinerea* (Schumacher et al. 2012). Another protein involved in signal transduction in *A. nidulans* is the protein kinase ImeB, which has been proposed to participate in the regulation of development and secondary metabolism by light (Bayram et al. 2009).

In addition, the COP9 signalosome (CSN) plays a major role in the transduction of the light signal in *A. nidulans* (Braus et al. 2010). A *csnD* deletion strain is missing one of the components of the CSN and is blind for light regulation of development, since it produced sexual development in plates grown in light or dark. The results with the *csnD* mutant and mutants in other components suggested that in *A. nidulans*, the CSN, which is involved in targeting proteins for degradation, is essential for light-dependent signaling and sexual development (Busch et al. 2003, 2007). The CSN and DEN1/DenA are deneddylases, proteins that remove ubiquitin-like protein Nedd8 from modified

proteins. Both proteins interact in *A. nidulans* and participate in the regulation by light of the balance between sexual and asexual development (Christmann et al. 2013). The components of the CSN and its role in the regulation of development have been described in great detail (von Zeska Kress et al. 2012; Helmstaedt et al. 2011). These results indicate a prominent role for protein modifications in the light signal transduction pathway in *A. nidulans*, a suggestion further supported by the observation that another protein modification system, sumoylation, participates in the regulation of asexual development (Harting et al. 2013).

In *Trichoderma reesei*, the transduction of the light signal through the BLR photoreceptors and ENVOY interacts with nutrient sensing through the heterotrimeric G-protein signaling pathway. The role of the BLR proteins in carbon deprivation induced conidiation, and the role of the protein kinase A in the regulation of gene expression points to an additional role for the photoreceptor in environmental sensing and interactions with the cAMP signaling pathway (Casas-Flores et al. 2006). In addition, the phenotype of single and double mutants in the G subunits or a mutant in the phosducin-like protein PhLP1, a regulator of G-protein signaling, shows modified regulation by light of cellulase gene expression (Schmoll et al. 2009; Seibel et al. 2009; Tisch et al. 2011a, b). The interactions between the light-signaling pathway and nutrient sensing by G proteins was further confirmed by the observation of a role for the cAMP signaling pathway in the regulation by light of gene expression (Tisch et al. 2011a; Schuster et al. 2012) and by the transcriptomic characterization of mutants in these pathways (Tisch et al. 2014). Interactions between light and nutrient sensing might be widespread in fungi. Light and glucose abundance regulate the light-dependent localization of VeA and photoreceptor activity in *A. nidulans* (Atoui et al. 2010).

cGMP plays a major role in the light signal transduction pathway for phototaxis in *Blastocladiella emersonii*. This basal fungus perceives light using a fusion protein between a rhodopsin and a guanylyl cyclase catalytic domain, suggesting a role for the cGMP signaling pathway in vision as it has been described in vertebrates (Avelar et al. 2014). It is tempting to speculate that this is an ancient mechanism of vision that was lost in most fungi and is still observed in vertebrates.

The search for novel mutants in fungal photoresponses may help to identify new proteins involved in signal transduction. In *Cryptococcus* a screening for suppressors of light-dependent inhibition of mating allowed the identification of the *ssn8* gene that is required for transcriptional regulation in yeast (Yeh et al. 2009).

C. The Output of Photoreception: Genes Regulated by Light

Most fungi see blue light using homologs of the WC proteins, a light-dependent transcription factor complex. It is not surprising that the first consequence of photoreception is the modification of the transcriptional machinery so that genes are activated or repressed after the cell is exposed to light. The regulation of transcription by light has been characterized using several techniques (microarrays, cDNAs, RNA-seq) in several fungi, and common themes have emerged.

A detailed analysis of light-dependent transcription using genomic microarrays identified 5.6 % of transcripts (314 out of the 5600 surveyed genes) with a peak in mRNA expression after approximately 15–30 min (early light-regulated) or 60–90 min of light treatment (late light-regulated) (Chen et al. 2009). Six genes for transcription factors were identified among the light-responsive genes (*wc-1*, *sub-1*, *csp-1*, *sah-1, vad-3,* and NCU03643.2), and one of them, the GATA family transcription factor SUB-1, was required for the activation by light of late-responsive genes. The observation of WCC binding to the promoter of *sub-1* supports the idea that the WCC activates the transcription of *sub-1* so that SUB-1 can activate late-responding genes (Chen et al. 2009). A homolog of SUB-1 has been identified in *Botrytis cinerea*, and it plays a relevant role in pathogenesis and in the regulation by light of gene expression suggesting a conservation of its function at least in related fungi (Schumacher et al. 2014). A subsequent study identified WC-

2 binding sites in the *N. crassa* genome and showed that the WCC controls a transcriptional network that regulates 20 % of all *Neurospora* genes in response to light. These genes include 24 transcription factor (TF) genes, which represents 20 % of all annotated *Neurospora* TFs, including proteins associated with development, metabolism, stress response, and 11 putative TFs of unknown function (Smith et al. 2010). A more detailed characterization of the transcriptional response to light found that 31 % of *N. crassa*-expressed genes were induced by light, while genes involved in rRNA metabolism were repressed (Wu et al. 2014).

Similar results have been obtained with other fungi. Microarray hybridization experiments performed with *A. nidulans* identified about 400 genes upregulated and about 100 genes downregulated by light, including genes that participate in the regulation of conidiation. This regulation occurred at the level of transcription with the participation of the photoreceptors for red and blue light (Ruger-Herreros et al. 2011). In the related human pathogen, *Aspergillus fumigatus* light regulates 2.6 % of genes detected by microarray hybridization (Fuller et al. 2013). Similar experiments have been performed in *Trichoderma atroviride* and *Trichoderma reesei* with wild-type strains and with strains with mutations in the photoreceptor genes. About 2.8 % of genes are regulated by light in *T. atroviride*, but genes regulated by light in the absence of the BLR photoreceptors or under red light were characterized (Rosales-Saavedra et al. 2006). However, only 72 proteins were identified that showed differential accumulation by light raising questions about the biological relevance of differential gene regulation (Sánchez-Arreguín et al. 2012). A similar fraction of genes (2.8 %) were found to be regulated by light in *T. reesei* (Tisch et al. 2011b). A major consequence of exposure to light is the activation of genes that participate in cellulose degradation and the glycoside hydrolases in particular (Tisch and Schmoll 2013; Tisch et al. 2011b). In *Botrytis cinerea* microarray hybridization identified 293 light-responsive genes, many of them involved in the response to oxidative stress, and the transcription of most of them is modulated by the SUB-1 homolog BcLTF1 (Schumacher et al. 2014).

D. Regulation by Light of Fungal Morphogenesis

The main developmental transitions in the life cycle of fungi are spore germination, hyphal growth and branching, and formation of reproductive structures for spore development and dispersal. These developmental transitions are regulated by many environmental factors, including the presence or absence of light (Corrochano and Galland 2006; Corrochano and Avalos 2010). Most fungi use blue light and homologs of the WCC to regulate development but differ in their sensitivity to light suggesting additional mechanisms or interactions with other photoreceptors in each species (Table 11.1).

1. Regulation by Light on Germination and Hyphal Growth

Blue and red light inhibits conidial germination in the human pathogen *Aspergillus fumigatus* and *A. nidulans* through the activity of the phytochrome and a blue-light photoreceptor that is not the *wc-1* homolog (Fuller et al. 2013; Röhrig et al. 2013). An opsin is required for the inhibition of germination of conidia of *F. fujikuroi* (García-Martínez et al. 2015). A similar inhibition of germination and germ tube growth has been observed in several species of rust fungi including several species of *Puccinia* (Buck et al. 2010; Dong and Buck 2011). The regulation by light on hyphal branching has been described in *N. crassa*, *Tuber borchii*, and *T. atroviride* (Ambra et al. 2004; Lauter et al. 1998; Casas-Flores et al. 2004), resulting in colonies that are more compact when grown under light. Hyphal growth in *Hypocrea atroviridis* is enhanced by light when the fungus is grown on carbon sources related to cellulose and hemicelluloses. This effect of light requires an active blue-light receptor system (BRLs proteins) and additional metabolic signals provided by the growth medium (Friedl et al. 2008).

Table 11.1 Effects of light on fungal development

Organism	Phenomenon	Threshold	Reference
Phycomyces	Induction of macrophores or inhibition of microphores	10^{-8} W/m^2	Corrochano and Cerdá-Olmedo (1990)
		10^{-4} J/m^2	Corrochano and Cerdá-Olmedo (1990)
		10^{-10} mol/m^2	Corrochano et al. (1988)
	Inhibition of sexual development	3×10^{-2} W/m^2	Yamazaki et al. (1996)
Neurospora	Induction of protoperithecia	4 J/m^2	Degli-Innocenti et al. (1983)
	Circadian clock resetting	10^{-5} mol/m^2	Crosthwaite et al. (1995)
Aspergillus	Induction of conidiation	135 J/m^2	Mooney and Yager (1990)
		8×10^{-4} mol/m^2	Mooney and Yager (1990)
	Inhibition of sexual development	10^{-4} μmol/m$^2 \times$ s (4.3×10^{-5} W/m^2)	Bayram et al. (2008a)
Trichoderma	Induction of conidiation	10^{-5} mol/m^2	Horwitz et al. (1990)
Alternaria	Inhibition of photoinduced conidiation	10^{-5} mol/m^2	Kumagai (1989)
Paecilomyces	Induction of conidiation	1.8×10^{-4} mol/m^2	Sánchez-Murillo et al. (2004)

2. Regulation by Light of Asexual and Sexual Development

The regulation by light on fungal reproduction has been investigated in great detail. Prominent examples are the activation by light of conidiation and the repression by light of sexual development in *N. crassa* (Chen et al. 2010b) and *A. nidulans* (Bayram et al. 2010), the regulation by light of conidiation in species of *Trichoderma* (Carreras-Villaseñor et al. 2012; Schmoll et al. 2010) and *Fusarium* (Avalos and Estrada 2010), and the regulation by light of fruit body formation in *C. cinerea* (Kamada et al. 2010) and *P. blakesleeanus* (Corrochano and Garre 2010).

In *N. crassa*, the regulation by light of conidiation and the inhibition of perithecial development requires the WCC (Chen et al. 2010b). The regulation by light of conidiation appears to act through the direct activation of transcription of a key regulator of conidiation, the gene for the transcription factor *fluffy* (Bailey and Ebbole 1998). The transcription of *fluffy* is sufficient to induce conidiation in vegetative cells (Bailey-Shrode and Ebbole 2004). Light activates the transcription of *fluffy* in vegetative cells through the binding of the WCC to the promoter providing a simple mechanism for the regulation by light of conidiation (Olmedo et al. 2010b). A similar observation has been made in *A. nidulans* where the key regulator of conidiation, *brlA*, is regulated by light through the activity of the photoreceptors for blue and red lights (Ruger-Herreros et al. 2011). It is possible that similar regulation by light of key developmental regulators may explain the mechanisms that control conidiation in other fungi. A detailed spectral characterization of conidiation in *A. nidulans* showed that both blue light and red light were required for full conidial induction, contradicting previous observations that suggested a prominent role for red light only (Purschwitz et al. 2008). Blue light and red light for the activation of conidiation must be sensed by the WC proteins and the phytochrome (in a protein complex described earlier), since only double mutants in the *wc* and phytochrome genes were blind (Purschwitz et al. 2008).

Blue light and red light inhibit sexual development in *A. nidulans* (Purschwitz et al. 2008), an observation that has been explored fully with detailed stimulus–response experiments with light of different wavelengths (Bayram et al. 2008a). A low-intensity component (10^{-4}–10^{-2} μmol/m$^2 \times$ s) and a high-intensity component (above 10^{-4} μmol/m$^2 \times$ s) were observed in the inhibition of sexual development after exposure with near-UV, blue, and red lights, suggesting the activity of a complex photosensory system. Deletion of the cryptochrome gene reduced the sensitivity in the

near-UV/blue region of the spectrum for the inhibition of sexual development, supporting a role for the *A. nidulans* cryptochrome in the blue-light-dependent inhibition of sexual development (Bayram et al. 2008a). However, the absence of sexual inhibition by light in the phytochrome and *wc* gene double mutants suggested that, in addition to the cryptochrome, the *A. nidulans* WC complex participates in sensing blue light for this developmental response (Purschwitz et al. 2008). No results have been reported about the possible interactions between the cryptochrome and other *Aspergillus* photoreceptors (WC proteins and phytochromes), but the results summarized here and the observation of physical interactions between the phytochrome and the WC proteins (Purschwitz et al. 2008) suggest the possibility of a physical interaction between the cryptochrome and any of the other photoreceptor proteins. The results summarized here suggest that the effect of light on *A. nidulans* development is mediated by the coordinated activity of a cryptochrome (only data for sexual development are available), the WC complex, and a phytochrome, perhaps acting in a coordinated manner in a photoreceptor complex located in the nucleus.

The regulation by light of conidiation or fruit body development in several species of fungi requires homologs of the WCC suggesting the ancient origin of this photoreceptor complex for fungal vision (Idnurm et al. 2010; Corrochano 2007; Rodriguez-Romero et al. 2010). Homologs of the WCC are required the regulation by light of fruit body formation in *C. cinerea* (Kamada et al. 2010) and *P. blakesleeanus* (Corrochano and Garre 2010), but the role of other proteins or novel photoreceptors cannot be ruled out. For example, the regulation by light of fruit body formation in *C. cinerea* requires a protein with a novel flavin-binding site that may be a new photoreceptor (Kuratani et al. 2010), and in *M. circinelloides* the regulatory protein CrgA and the photoreceptor Mcwc-1b participate in the regulation of vegetative reproduction (Navarro et al. 2013). In species of *Trichoderma* light regulates conidiation through homologs of the WCC (Schmoll et al. 2010; Carreras-Villaseñor et al. 2012) in a complex interaction with ENVOY and the regulatory cascade for nutrient sensing (Tisch et al. 2011a). ENVOY and the WCC homolog participate in mating and its regulation by light in *Hypocrea jecorina* (*Trichoderma reesei*) in a complex mechanism that includes the regulation of mating type genes and the biosynthesis of pheromones (Chen et al. 2012; Seibel et al. 2012).

The effect of light on development has been investigated in other fungi. In species of *Fusarium* light regulates the biosynthesis of carotenoids and development (Avalos and Estrada 2010). Most of the characterization has been done with *F. fujikuroi* (García-Martínez et al. 2015; Castrillo and Avalos 2014; Castrillo et al. 2013; Estrada and Avalos 2008), but the regulation by light of conidiation has been characterized in *F. graminearum* (Kim et al. 2013), *F. verticillioides* (Fanelli et al. 2012b), and *F. proliferatum* (Fanelli et al. 2012a). Sexual and asexual development is controlled by light in *B. cinerea*, but the regulation by light can only be observed in certain isolates suggesting that light regulation is not a general feature of the species (Schumacher et al. 2012, 2014; Canessa et al. 2013). Conidiation is induced by light in *Penicillium chrysogenum*, and the regulation requires the velvet complex as in *A. nidulans* (Hoff et al. 2010), but conidiation was repressed in two species of *Puccinia* (Dong and Buck 2011) or *Exserohilum turcicum* (Flaherty and Dunkle 2005), an indication that light can exert opposite effects in different fungi.

The regulation by light of fruit body development in edible fungi adds a biotechnological aspect to research in photomorphogenesis. Growth conditions have been investigated that promote fruit body development for *Pleurotus ostreatus*, including the effect of light and light/dark cycle (Lee et al. 2011; Arjona et al. 2009).

III. Gravitropism

The effect of gravity on plants and fungi is usually associated with gravitropism, i.e., the

directed growth of elongating organs parallel or antiparallel to the gravity vector. While gravitropism represents no doubt the most apparent gravireaction, gravity exerts in addition a substantial influence on morphogenesis, i.e., shape and form of plants and fungi. An example is the peg formation in seedlings of several plants including *Mimosa* and *Eucalyptus* and various Cucurbitaceae. The peg is a protuberance, a special hook-like organ, at the base of the hypocotyl that serves to remove the seed coat at the time of germination. That the morphogenesis of this organ is under control of gravity becomes apparent from the observation that clinostatted seedlings of *Cucurbita* develop two instead of only one peg (Takahashi 1997). Prolonged clinostatting can also induce dramatic changes in flower morphology as zygomorphic flowers can take on a radial symmetry (Rawitscher 1932). The space-filling pattern and morphology of the plant root system critically depend on the hierarchy of primary, secondary, and tertiary roots that each posses characteristic gravitropic (liminal) set point angles (Hart 1990). The important role of gravity for regular morphogenesis has been documented even for fungi. The agaric fungus, *Polyporus brumalis*, for example, develops in weightlessness flattened fruiting bodies with twisted and irregular pedicles (Zharikova et al. 1977) and the cytological fine structure of hyphae from the stem of agaric fungi changes during gravitropic bending (see below).

Gravireception is ubiquitous in the fungal kingdom and is manifested as gravimorphogenesis and gravitropism. Fruiting bodies usually grow vertically and reorient in a few hours after displacement from the plumb line. Even though the gravitropism of different classes of fungi has been described since more than a century (Table 11.2), the cellular and molecular mechanisms that underly gravioriented growth are still elusive, and as a consequence the general understanding has remained largely on a phenomenological level. In plants, graviperception is mediated by statoliths (usually amyloplasts), heavy cell organelles that sediment upon reorientation and that generate potential energy. The search for fungal statoliths (gravisusceptors), though a century old, has only very recently received novel input (see below). As in plant research, the hunt for the hypothetical gravireceptor has, however, remained unsuccessful and will continue. Though the phytohormone auxin, a hallmark of plant photo- and gravitropism research, is also present in gravitropic fungi, its role in gravi- and phototropism has yet to be elucidated.

A. Criteria for Gravisusceptors

Cell organelles or cell inclusions that function as gravisusceptors must be able to generate potential energy that exceeds the thermal noise of the cellular environment. After reorientation of the plant or fungal organ, gravisusceptors generate a gravitropic signal. To achieve this, they must have the potential to sediment (statoliths) or else to float upwards ("buoys") so that a particle gradient can be formed. To do this, they require a density different from that of the surrounding cytoplasm and additionally, a critical size to overcome the effect of thermal motion, which counteracts the effect of sedimentation or buoyancy and thus the formation of a particle gradient. Whether or not a cell organelle qualifies as a gravisusceptor can be determined with a function that was originally introduced by Einstein who modified the Boltzmann distribution by taking into account the earth's gravitational field. The function describes the ratio of sedimenting (or floating) particles separating along a distance, h:

$$N/N_0 = \exp - \left[V\left(\rho_c - \rho_{gs}\right) g \ \mathrm{h}/k_B \mathrm{T} \right] \qquad (1)$$

where N_0 and N are the number of particles separated by the distance h before and after sedimentation (flotation), respectively, V is the particle volume, ρ_c and ρ_{gs} are the specific densities (kg m^{-3}) of the cytoplasm and of the gravisusceptor, respectively, g is the constant of gravitational acceleration (9.81 m s^{-2}), k_B is the Boltzmann constant (1.38×10^{-23} J K^{-1}), and T is the absolute temperature (K). The relation is essential for calculating the minimal size and density that are required for a putative gravi-

Table 11.2 Examples for gravitropism of fungi and the putative gravisusceptors

Organism	Organ	Gravitropism	Candidates for Gravisusceptor	Reference
Basidiomycota				
Amanita	Stem	Negative	Unknown	Hofmeister (1863)
	Gills	Positive	Unknown	Moore (1991)
Coprinus	Stem	Negative	Unknown	Knoll (1909)
	Stem	Negative	Cytoplasm/vacuole	Gooday (1985)
	Gills	Positive	Unknown	Moore (1991)
Flammulina	Stem	Negative	Actin filaments	Monzer (1995)
	Stem	Negative	Nuclei	Monzer (1996)
	Gills	Positive	Unknown	
Fomes	Stem	Negative	Unknown	Buller (1922)
	Tube	Positive	Unknown	
Psalliota	Stem	Negative	Unknown	Buller (1909)
	Gills	Positive	Unknown	
Polyporus	Gills	Positive	Unknown	Sachs (1879)
Zygomycota *Phycomyces*	Sporangiophore	Negative	Octahedral	Schimek et al. (1999)
			Crystals and lipid globules	Grolig et al. (2004)
				Grolig et al. (2014)
			Cytoplasm/vacuole	Dennison and Shropshire (1984)
Pilobolus	Sporangiophore	Negative	Unknown	Horie et al. (1998)
Glomeromycota *Gigaspora*	Germ tubes	Negative	Lipid globules	Grolig et al. (2006)
	Branching hyphae	Positive	Lipid globules	Grolig et al. (2006)
				Watrud et al. (1978)
				Hong et al. (2001)

susceptor to function as statolith. To take into account the viscosity of the cytoplasm and the mobility of the sedimenting particles, one relies on the Einstein–Smoluchowski relation:

$$D/\mu = k_B T \tag{2}$$

that allows to rewrite Eq. 1 as

$$N/N_0 = \exp - \left[\mu V\left(\rho_c - \rho_{gs}\right)g \;\; h/D\right] \tag{3}$$

where μ (i.e., particle velocity/drag force, Stoke's law) represents the particle mobility (s kg^{-1}) and D the diffusion coefficient (m^2 s^{-1}) (Einstein 1905, 1906)

When gravisusceptors sediment or float upwards they generate a force, *F*, which can be calculated as

$$F = g \times V \times n \times \left(\rho_c - \rho_{gs}\right) \tag{4}$$

where *g* is the earth's gravitational acceleration (9.81 m s^{-2}), V is the volume of a gravisusceptor, n is the number of gravisusceptors, ρ_c is the density of the cytoplasm, and ρ_{gs} is the density of the gravisusceptor. The potential energy, E, of a sedimenting or floating gravisusceptor is given by

$$E = F \times d \tag{5}$$

where *F* is the static force (Newton) and d is the distance (*m*) over which the gravisusceptors are displaced. The potential energy of a gravisusceptor needs to exceed the thermal noise ($3/2\ k_B T = 6.21 \times 10^{-21}$ J at 300 K). For example, at the gravitropic threshold of *Phycomyces*, which is near $2 \times 10^{-2} \times g$ (Galland et al. 2004), the potential energy generated by floating lipid globules (Fig. 11.1) would amount to 10^{-18} J, which is still 360 times above the thermal noise. The estimated potential energies are also sufficiently high to explain the adherence of *Phycomyces* to the so-called sine law of gravitropism

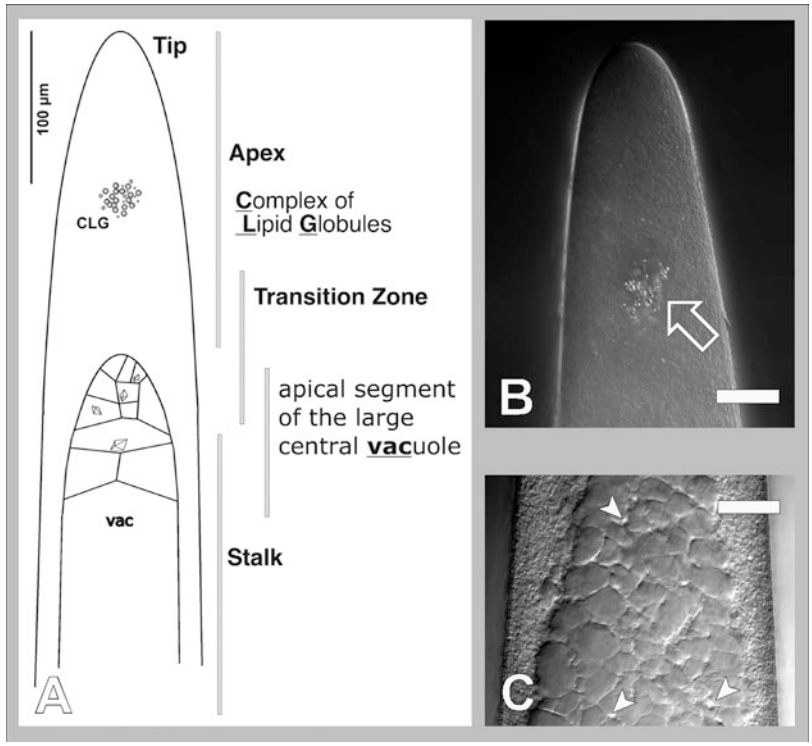


Fig. 11.1 *Phycomyces blakesleeanus*. (**a**) Schematic drawing of the tip of a stage 1 sporangiophore, i.e., without sporangium. The complex of lipid globules (CLG) and the octahedral protein crystals in the apical segment of the sporangiophore function as gravisusceptors. (**b**) Complex of lipid globules (*arrow*). (**c**) The apical segment of the central vacuole is formed by an assembly of small vacuoles; *arrowheads* indicate the octahedral protein crystals. Bars: 20 µm. Phase contrast microscopy (Modified after Grolig et al. (2014). Courtesy of Dr. Franz Grolig)

(Galland et al. 2002). For small inclination angles of the sporangiophore of 1–2°, the gravitropic stimuli are according to the sine law 1.7 to $3.4 \times 10^{-2} \times g$, which is just above the absolute gravitropic threshold and thus above the thermal noise (see above). An energy of about 10^{-16} J could be sufficient to open 10^6 mechanosensitive Ca^{2+} channels (Howard et al. 1988). Such considerations appear relevant in view of the reasonable possibility that the graviperception of fungi may involve ion transport and the requisite channels.

B. Basidiomycota

Gravitropism is ubiquitous among basidiomycota and is manifested by the lamellae as well as by the stipe. The lamellae (gills) of the pileus of agarics display positive gravitropism, i.e., they grow parallel to and in the same direction as the vector of the earth's gravitational acceleration when they are displaced from the plumb line. A displacement of vertical lamellae by as little as 5° can reduce the spore dispersal by some 50 %; an inclination angle of 30° may even completely abolish spore dispersal (Buller 1909). Stipes that are inclined bend upwards displaying thus negative gravitropism, a response that *Flammulina velutipes* completes in about 12 h (Kern and Hock 1996a, b). Basidiomycota, such as *Polyporus brumalis* or *Flammulina*, that have been cultured during microgravity in satellites or space shuttles displayed disoriented and twisted growth and sometimes even abnormal fruiting body formation (Kern and Hock 1996a, b; Zharikova et al. 1977).

The gravisensitive zone seems to be restricted to the apex of the stipe (Haindl and Monzer 1994), which comprises in *Coprinus cinereus* the upper 20–30 % (Greening et al. 1997). Gravitropic curvature of the stipe is caused by partial inhibition of the elongation growth of the upper side (Monzer et al. 1994). Gravitropic curvature is caused by differential elongation growth of the upper and lower flanks of the stipe. In *Flammulina* the outer flank grows at an increased rate, while the inner one shows a decreased growth rate. In *Coprinus* the outer flank shows a rapid increase in growth rate, while the inner flank shows a delayed increase of growth rate (Greening et al. 1997). In *Flammulina* the gravitropic bending of horizontally placed stipes is complete in about 12 h (Kern and Hock 1996a).

Inner hyphae of stipes contain large vacuoles and high turgor pressure, while outer hyphae contain smaller ones and moderate turgor. It is the high turgor of the inner hyphae that causes the elongation growth, while the outer hyphae control the differential relaxation that causes differential growth and this way gravitropic curvature. Elongation growth of hyphae of the stipe differs from that of mycelial hyphae in that cell extension occurs along the entire length of the cell; mycelial hyphae display in contrast apical growth (Kern and Hock 1996a).

Bending stress generated by the weight of the pileus does not elicit gravitropism (Greening et al. 1993). The gravistimulus is perceived even after removal of the cap; the graviperceptive and the graviresponsive zone is thus located in the apex of the stipe (Kern and Hock 1996b). However, to maintain elongation growth of the stipe for prolonged periods, the presence of the cap is necessary.

A diffusible growth promoter of unknown chemical identity plays a crucial role in the gravitropism of *Agaricus* and *Flammulina* (Gruen 1979, 1982; Hagimoto 1963). The growth promoter must be water soluble, because stipes or segments of stipes display gravitropism in air and under silicon oil, not, however, under water. Imbibition under water abolishes tropism, not, however, elongation growth (Kern and Hock 1996a, b). In order to elicit gravitropic bending, one has to postulate that the growth promoter forms a gradient in horizontal stipes that increases from the upper to the lower flank. The fact that the upper part of the stipe contains much water between the hyphae fits well this hypothesis (Kern and Hock 1996b). In *Coprinus* graviperception remains unaffected by the Ca^{2+} channel blockers verapamil or by the calmodulin inhibitor calmidazolium even though the efficiency of gravitropic bending is reduced (Novak Frazer and Moore 1993).

1. Gravisusceptors

Sedimentation of cell organelles such as observed for plant statoliths (typically amyloplasts or barium sulfate bodies in *Chara* rhizoids) has never been observed in Basidiomycota, and their statoliths have remained elusive. Upon reorientation (inversion) of *Coprinus*, no displacement of glycogen granules could be observed, and it was thus concluded that they do not represent gravisusceptors (Borriss 1934). In horizontal stipes of *Coprinus cinereus*, the cytoplasm of hyphae was displaced to the lower and vacuoles to the upper part. It is thus feasible that the cytoplasm functions as a gravisusceptor (Gooday 1985). A similar separation was reported for sporangiophores of *Phycomyces* (see below), not, however, for other basidiomycota.

In *Flammulina velutipes*, actin filaments play an important role in graviperception. Gravitropic curvature was suppressed by the actin filament-disrupting agent cytochalasin D, not, however, by the microtubule inhibitor oryzalin (Monzer 1995). The relative high density of the nuclei (1.2 g cm^{-3}) and their close association with actin filaments suggest a role for the nuclei as gravisusceptors (Monzer 1996). The density of nuclei of *Flammulina* is lower than that of plant nuclei (1.32) but higher than that of *Neurospora* (1.08). A density of 1.2 is sufficiently high for a potential gravisusceptor (Eq. 1), and actin-associated nuclei represent thus interesting contenders for gravisusceptors.

Reorientation of stipes of *Flammulina* causes the formation of numerous microvacuoles in the hyphae of the lower flank, while those of the upper flank retain a single major vacuole. It is likely that the process of vacuolization is relevant for graviperception as the process of vacuolization correlates with the differential growth rates of the hyphae of the upper and lower flanks (Kern and Hock 1996a, b).

2. Kinetics, Dose Dependence, and Threshold

When *Coprinus cinereus* is placed horizontally, gravitropic bending manifests about 25 min later (=reaction time) (Kher et al. 1992). The presentation time, i.e., the minimal stimulus time for eliciting a response, for excised and clinostatted stipe of *Coprinus cinereus* is 9.6 min (Hatton and Moore 1992). In plants the presentation time can be as short as 10–15 s or up to 4 min leading to gravitational threshold doses (expressed as the product of the earth's acceleration $g \times$ time) ranging from 10*g*s to 240*g*s (Hatton and Moore 1992; Volkmann and Sievers 1979). The corresponding value for *Coprinus* would be 576*g*s. Mechanical stress does not affect the bending reaction (Greening et al. 1993).

3. Gravimorphogenesis: Effects of Spaceflight and Simulated Microgravity

Long-term space experiments have shown that the presence of a gravitational field is of paramount importance for the morphogenesis of plants and fungi. The growth pattern and the morphogenesis of Basidiomycota are severely affected under weightlessness. *Polyporus brumalis* and *Pleurotus ostreatus* that were raised under weightlessness showed in darkness abnormal or no formation of fruiting bodies, while irradiation-induced fruiting bodies lacked, however, a hymenium. Stipes of *Flammulina* raised in weightlessness were flat instead of round (Kern and Hock 1996b). Similar results were also obtained in long-term space experiments with *Polyporus brumalis*. In weightlessness and darkness, not however in light, the formation of fruiting bodies was either arrested or twisted stems developed that lacked a cap (Kasatkina et al. 1980; Zharikova et al. 1977). When the fruiting bodies of *Pleurotus ostreatus* were clinostatted for a prolonged period, 17 genes were upregulated and 19 were downregulated; among these genes were those required for fruiting body formation (Miyazaki et al. 2010). In *Candida albicans*, a prominent fungal human pathogen, prolonged microgravity in space elicited enhanced cell aggregation and random budding and for 452 genes an altered gene expression (Crabbé et al. 2013). Simulated microgravity (clinostat) elicited in *Candida albicans* an increased filamentous growth (Altenburg et al. 2008). *Saccharomyces cerevisiae* shows several developmental changes in response to spaceflight, e.g., invasive budding and hyphal growth (Van Mulders et al. 2011).

C. Ascomycota

Even a superficial inspection of the various fructification organs of Ascomycota will provide evidence for their ubiquitous capacity of graviperception. The upright growing perithecia of *Neurospora*, the upward orientation of the apothecia of *Pezizazeae*, and the vertical fruiting bodies of *Helvellaceae*, *Helvelloidae*, and *Morchellaceae* bear ample witness for gravitropism. In spite of this fact, there exists almost no literature on the graviperception of this group of organisms.

To answer the question, whether or not single-celled organisms possess the potential to perceive gravity, yeasts were often employed in space experiments under microgravity conditions. Attempts to detect differences in the induction and the repair of DNA lesions under earth and microgravity conditions were without success for *Saccharomyces* (Pross et al. 2000; Takahashi et al. 2001). Cultures of *Saccharomyces* that were subjected to simulated weightlessness in a special apparatus providing

low-fluid shear, the "rotating wall vessel bioreactor," displayed substantially altered expression for clusters of genes that were either up- or downregulated. The genes contained promoter sequences with similarities to the Rap1p transcription factor binding site and the stress-responsive element (STRE) (Johanson et al. 2002). The experiments provide clues how physical forces acting on the cell surface could translate into differential gene expression and thus represent a model for gravimorphogenesis. A phenomenon of gravimorphogenesis was observed in bioreactor cultures of *Saccharomyces cerevisiae* that were maintained for 8 days under microgravity in a spacelab. The percentage of randomly distributed bud scars was about three times higher in the cells subjected to weightlessness (17 %) than in those maintained on earth (5 %) (Walther et al. 1996).

D. Zygomycota

Sporangiophores of *Phycomyces blakesleeanus* and *Pilobolus crystallinus* display negative gravitropism (Horie et al. 1998; Schimek et al. 1999). Mycelial hyphae, zygophores, and zygospores attached to suspensors are agravitropic. Sporangiophores of *Phycomyces* that grew in a satellite in weightlessness displayed completely random and disoriented growth (Parfyonov et al. 1979). The effectiveness of gravitropism depends to some extent on the developmental stage. Stage 1 sporangiophores of *Phycomyces*, which lack sporangia, bend gravitropically more slowly than stage 4 sporangiophores, which possess sporangia (Schimek et al. 1999; Grolig et al. 2004). In *Pilobolus* gravitropism is almost absent in stage 1, while it is well expressed in stage 4 sporangiophores (Horie et al. 1998). The latter observation might be explained by the fact that the growing zone in stage 1 sporangiophores of this fungus is merely 0.3 mm, while it is about 2–3 mm in *Phycomyces* (Horie et al. 1998).

1. Gravisusceptors

Flexure (bending stress) could potentially play a role in graviperception. Sporangiophores respond to cell wall stress generated by flexure or compression (Dennison 1961, 1964). A unilateral force of 0.5 mg elicited a bending response (Dennison and Roth 1967). Stretch elicits also a transient growth response (diminution of growth rate) when sporangiophores are stretched (elongated) by a load of 5 mg; when the load is lifted, a transient increase of growth rate is observed. Both responses are adaptive so that the growth rate returns after a few minutes to the prestimulus level (Dennison and Roth 1967). The data are in agreement with the assumption that stretch (flexure) occurring at the upper side of a horizontal sporangiophore influences gravitropism. The latencies of the respective stretch responses are about 1 min and thus much shorter than the gravitropic latencies obtained after reorientation of the sporangiophore.

The gravisusceptors of *Phycomyces* must be internal, because negative gravitropism persists in sporangiophores submerged in water or in fluids with a density exceeding that of the cytoplasm (Dennison 1961). Because cytoplasm sediments and the central vacuoles float slightly upward in horizontal sporangiophores, they probably participate in gravisusception (Dennison and Shropshire 1984). The central vacuoles contain octahedral protein crystals of high density (1.27 g cm^{-3}) (Fig. 11.1) that rapidly sediment upon reorientation of the sporangiophore and that participate in gravisusception. Mutants lacking these protein crystals are affected in gravitropism (Schimek et al. 1999; Galland et al. 2004). The crystals contain three proteins and are associated with pterin and flavin-like pigments (Eibel et al. 2000; Fries et al. 2002).

Besides the protein crystals, apical lipid globules are also involved in gravisusception. In stage-1 sporangiophores of *Phycomyces*, the lipid globules are clustered in a special organelle, a complex of lipid globules (CLG) that resides 110 μm below the apex (Fig. 11.1). Sporangiophores lacking the CLG display a greatly diminished gravitropic response (Grolig et al. 2004). The sedimentation of the octahedral crystals and the buoyancy of the lipid globules generate a potential energy that each supersedes the thermal noise by 3–4 orders of magnitude (Schimek et al. 1999; Grolig et al. 2004). The density of the lipid globules of *Phycomyces*

(0.79 g cm^{-3}) is much lower than that commonly found in oleosomes of plant material. The specific density of oleosomes (spherosomes) of peanuts, for example, is 0.92 g cm^{-3} (Jack et al. 1967) and that of oleosomes of wheat aleuron can be as high as 1.16–1.18 (Quail 1979). It is very apparent from such a comparison that the lipid globules of *Phycomyces*, which have diameters comparable to those of plant oleosomes, display traits that are indispensable for gravisusception while those of plants display ones that are suitable only for storage.

A prominent feature of the lipid globules is the fact that they are in continual non-Brownian motion that is probably powered by an actomyosin system, because the globules are localized in a cage of a compact actin mesh (Grolig et al. 2014). The lipid globules contain β-carotene and appear deep yellow. In addition, they carry pterin and flavin-like pigments that emit blue and green fluorescent light upon excitation (Grolig et al. 2004; Ogorodnikova et al. 2002). Lipid globules (droplets) are ubiquitous among oleaginous fungi (e.g., *Mortierella ramanniana*; Kamisaka et al. 1999), and it appears likely that graviperception mediated by buoyancy might represent a mechanism that is rather widespread in the fungal kingdom (Grolig et al. 2006).

2. Kinetics, Dose Dependence, and Threshold

Sporangiophores of *Phycomyces* that are placed horizontally have a rather irregular gravitropic latency of about 10–30 min or even longer when the conditions are suboptimal (Dennison 1961; Dennison and Shropshire 1984; Schimek et al. 1999). Gravitropic bending of horizontal sporangiophores is complete in 10–12 h (Schimek et al. 1999). The stimulus dependence was determined in long-term experiments on a clinostat centrifuge, and the threshold was found to be near $2 \times 10^{-2} \times g$ (Galland et al. 2004). A mutant that lacks the vacuolar protein crystals showed a slightly elevated threshold. Upon reorientation of the sporangiophore, the slowly ensuing bending response of *Phycomyces* is preceded by very fast molecular events that can be monitored spectroscopically. A gravitropic stimulus elicits so-called gravity-induced absorbance changes (GIACs) that occur almost instantaneously and that are specific for early events of the transduction chain, because they are altered in a gravitropism mutant with genotype *madJ* (Schmidt and Galland 2000, 2004; Schmidt 2006, 2007, 2011). The molecular nature of the response remains presently unidentified.

3. Cytoskeleton and Calcium

As in higher plants, the cytoskeleton appears to play a major role in the gravitropism of the *Phycomyces* sporangiophore in which actin, myosin, spectrin, and integrin were immunodetected (Doucette et al. 1994). The apical lipid globules of stage 1 sporangiophores are encased by dense mesh of actin filaments that fills the dome-like structure of the apex. Inhibitor studies show that the actin filaments cause the non-Brownian motion of the lipid globules (Grolig et al. 2014). Injection of stage 4 sporangiophores with cytochalasin D (inhibiting actin polymerization) causes a 4-h long delay of the gravitropic bending, while rhodamine phalloidin (inhibiting actin depolymerization) enhances the gravitropic bending rate and sometimes also the bending angle (Edwards et al. 1997).

Physiological studies suggest that gravitropism entails a mobilization and redistribution of Ca^{2+} and calmodulin. Gadolinium chloride, an inhibitor of plant gravitropism and stretch-activated ion channels, delays the onset of gravitropism and diminishes gravitropic curvature. An asymmetric application of gadolinium, Ca^{2+}-chelators, and compound 48/80 (inhibiting calmodulin) to the growing zone of the sporangiophore elicits curvature toward the side to which the inhibitors were applied (Edwards 1990; Stecker et al. 1990). Ca^{2+} and H^{+} fluxes at the tip of *Phycomyces* sporangiophores were directed inward. The gravitropism mutant, A909 *madJ*, displayed instead outward-directed ion fluxes (Živanović 2012). A gravistimulation of horizontally placed sporangiophores caused an influx of ions at the lower side and an efflux at the upper side. The

gravitropism of sporangiophores is moderately increasing in response to exogenous Ca^{2+} in a dynamic range from 10^{-8} to 10^{-3} M. The octahedral crystals (Fig. 11.1C) play a role in this response, because the gravitropic bending of a crystal-lacking mutant is exceeding that of the wild type about threefold (Galland et al. 2007). The result indicates that the crystals suppress the stimulatory effect of the exogenous calcium.

4. Sine Law and Exponential Law

Gravitropic bending of shoots and roots of plants obey the so-called sine rule or sine law (Sachs 1879), which states that the gravitropic stimulus can be described by the relation:

$$S = g \times \sin \gamma \qquad (6)$$

where S is the gravitropic stimulus, g the earth gravitational acceleration (9.81 m s^{-2}), and γ is the inclination angle (degrees) of the inclined plant organ.

Sporangiophores of *Phycomyces* obey this classical sine law (Galland et al. 2002). When sporangiophores are irradiated unilaterally, they bend toward the light source and a photogravitropic equilibrium is established. The photogravitropic bending angle is the result of two antagonistic responses, i.e., positive phototropism and negative gravitropism. The irradiance of unilateral light that is required to compensate the ensuing gravitropic response is well described by a novel exponential law (Grolig et al. 2000; Galland et al. 2002).

$$I = I_0 \exp(k_\lambda g \sin \gamma) \qquad (7)$$

where I is the fluence rate of the unilateral light that compensates the gravitropic response elicited by a given inclination angle γ, I_0 is the absolute threshold fluence rate (near 10^{-9} W m^{-2}, 450 nm), k_λ is a wavelength-dependent constant, g is the earth gravitational acceleration, and γ is the inclination angle of the sporangiophore (deviation from the vertical). The exponential law states that the fluence rate that compensates a gravitropic stimulus needs to be raised exponentially when the gravitropic stimulus (g sin γ) is raised linearly. Because the novel law is valid also for coleoptiles of *Avena* (Galland 2002) and also *Arabidopsis*, it describes a universal relationship for the interaction of gravi- and phototropic stimuli.

5. Gravitropism Mutants

Mutants of *Phycomyces* with a defective *madC* gene display a 10^6-fold elevated phototropic threshold (Bergman et al. 1973; Campuzano et al. 1996) and, at the same time, also a hypergravitropic phenotype (Göttig and Galland 2014). Because the *madC* gene codes for a Ras-GAP protein (Ras-GTPase-activating protein; Polaino Orts et al. 2013), this observation indicates that MadC operates as a negative regulator in gravitropism and as a positive regulator in phototropism.

Mutants of *Phycomyces* with defects in the genes *madD,E,F,G,J* are gravitropically partially defective (so-called stiff mutants). They are highly pleiotropic, because they show reduced light-growth and phototropic responses and a reduced avoidance response (Bergman et al. 1973; Campuzano et al. 1996). Mycelial responses such as photocarotenogenesis and photoinitiation of sporangiophores are, however, unaffected (see above). System analysis employing Wiener white noise or sum-of-sinusoid stimuli showed that these mutants have a lower gain than the corresponding wild-type strains or mutants that are affected only in the phototropism genes *madA-C* (Lipson 1975; Palit et al. 1989). The threshold for photogravitropic equilibrium is raised at least 6 orders of magnitude and the corresponding action spectra are greatly abnormal, a feature that indicates that these mutations directly affect the photoreceptor system (Campuzano et al. 1996). The various features indicate that (1) the photoreceptor system is affected in these gravitropism mutants, (2) that photo- and graviperception are interacting at early steps of the transduction chain, (3) and that the *madD,E,F,G* gene products interact with those of the *madA,B,C* gene products.

Another class of mutants with defects in the gene *madH* shows enhanced gravitropic and phototropic bending and also an enhanced avoidance response (Lipson et al. 1983; López-

Díaz and Lipson 1983). While the locations of the respective *mad* genes on the genetic map of *Phycomyces* have been determined (Alvarez et al. 1992), their molecular nature remains unknown.

E. Glomeromycota

Hyphae of the endomycorrhizal fungus, *Gigaspora margarita*, display either negative or positive gravitropism. Germ tubes are growing upward (negative gravitropism), while secondary, i.e., branching, hyphae, which are the sites for the formation of enichulate vesicles, grow downward (positive gravitropism) (Watrud et al. 1978; Hong et al. 2001). Calcium, an important signal element in the graviperception of plants, plays a substantial role also in the gravitropism of *Gigaspora rosea*, because lanthanum (a Ca^{2+} blocker) and EGTA (a Ca^{2+} chelator) induce hyphal branching and at the same time also inhibition of gravitropism (Berbara et al. 2002).

The gravisusceptors of these mycorrhizal fungi are presently unknown. In view of the fact that these oleaginous fungi contain numerous lipid droplets, it appears reasonable to assume that the buoyancy of these droplets mediates graviperception. We found that vertically growing hyphae of *Gigaspora margarita* possess an apical complex of lipid globules (CLG) (Grolig et al. 2006) that is similar to the one found in *Phycomyces* (Grolig et al. 2014). Even in the CLG of *Gigaspora*, the lipid globules are in continual motion. Lateral hyphae growing horizontally do not contain apical lipid globules and are agravitropic. Lipid globules occur thus only in segments that grow vertically upwards. The close correlation between gravitropic orientation with the occurrence of lipid globules clearly indicates their role as gravisusceptors (Grolig et al. 2006).

IV. Conclusions

Light and gravity are environmental stimuli responsible for the final appearance of fungi. Research on fungal photomorphogenesis and gravitropic responses has concentrated on the description and characterization of the responses to light and gravity. Genes that are required for photoreception and photomorphogenesis have been identified and, in some cases, isolated and characterized. The identification of the WCC in *N. crassa* has served as a model for other fungi, and in most cases mutants in the homologs of the WCC result in blind phenotypes. A number of WCC homologs have been identified with the increased number of fungal genome projects, and the reduction in the cost of genome sequences allows us to predict that the number of fungal photoreceptors will soar in the near future. Genome projects allowed the identification of additional photoreceptor genes in fungal genomes, many of them unexpected and most of them without a clear function in the fungal cell. Understanding the role and the coordination of fungal photoreceptors will be a major avenue of research in the future. *N. crassa* has been established as a model system for fungal photobiology, but there are indications that other fungi, even other ascomycetes, have molecular mechanisms for photoreception that are different from the one in *N. crassa*. It is becoming clear that there are limits to the use of *N. crassa* as a model for fungal photobiology.

The molecular basis of fungal gravitropism, however, is still rudimentary. Several examples of gravisusceptors and mechanisms for gravity sensing have been suggested and presented here. We hope that the combination of genetics and molecular biology with detailed cytological characterization of model systems will help to unravel the complexities of fungal gravitropism. Light and gravity interact in complex ways to direct sporangiophore growth in *Phycomyces*, possibly at the level of the photoreceptor system itself. It is thus possible that light and gravity will share some of the elements of the transduction chain in fungi. Only future research will confirm the possible relationship between light and gravity as major signals for fungal development, growth, and appearance.

References

Altenburg SD, Nielsen-Preiss SM, Hyman LE (2008) Increased filamentous growth of *Candida albicans* in simulated microgravity. Genomics Proteomics Bioinformatics 6:42–50

Alvarez MI, Benito EP, Campuzano V, Eslava AP (1992) Genetic loci of *Phycomyces blakesleeanus*. In: O'Brien SJ (ed) Genetic maps. Locus maps of complex genomes, 6th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 3120–3126

Ambra R, Grimaldi B, Zamboni S, Filetici P, Macino G, Ballario P (2004) Photomorphogenesis in the hypogeous fungus *Tuber borchii*: isolation and characterization of *Tbwc-1*, the homologue of the blue-light photoreceptor of *Neurospora crassa*. Fungal Genet Biol 41:688–697

Arjona D, Aragón C, Aguilera JA, Ramírez L, Pisabarro AG (2009) Reproducible and controllable light induction of in vitro fruiting of the white-rot basidiomycete *Pleurotus ostreatus*. Mycol Res 113:552–558

Arpaia G, Cerri F, Baima S, Macino G (1999) Involvement of protein kinase C in the response of *Neurospora crassa* to blue light. Mol Gen Genet 262:314–322

Atoui A, Kastner C, Larey CM, Thokala R, Etxebeste O, Espeso EA, Fischer R, Calvo AM (2010) Cross-talk between light and glucose regulation controls toxin production and morphogenesis in *Aspergillus nidulans*. Fungal Genet Biol 47:962–972

Avalos J, Estrada AF (2010) Regulation by light in *Fusarium*. Fungal Genet Biol 47:930–938

Avelar GM, Schumacher RI, Zaini PA, Leonard G, Richards TA, Gomes SL (2014) A rhodopsin-guanylyl cyclase gene fusion functions in visual perception in a fungus. Curr Biol 24:1234–1240

Bailey LA, Ebbole DJ (1998) The *fluffy* gene of *Neurospora crassa* encodes a Gal4p-type C6 zinc cluster protein required for conidial development. Genetics 148:1813–1820

Bailey-Shrode L, Ebbole DJ (2004) The *fluffy* gene of *Neurospora crassa* is necessary and sufficient to induce conidiophore development. Genetics 166:1741–1749

Baker CL, Loros JJ, Dunlap JC (2012) The circadian clock of *Neurospora crassa*. FEMS Microbiol Rev 36:95–110

Ballario P, Vittorioso P, Magrelli A, Talora C, Cabibbo A, Macino G (1996) White collar-1, a central regulator of blue light responses in *Neurospora*, is a zinc finger protein. EMBO J 15:1650–1657

Bayram O, Biesemann C, Krappmann S, Galland P, Braus GH (2008a) More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. Mol Biol Cell 19:3254–3262

Bayram O, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon NJ, Keller NP, Yu JH, Braus GH (2008b) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320:1504–1506

Bayram O, Krappmann S, Seiler S, Vogt N, Braus GH (2008c) *Neurospora crassa ve-1* affects asexual conidiation. Fungal Genet Biol 45:127–138

Bayram O, Sari F, Braus GH, Irniger S (2009) The protein kinase ImeB is required for light-mediated inhibition of sexual development and for mycotoxin production in *Aspergillus nidulans*. Mol Microbiol 71:1278–1295

Bayram O, Braus GH, Fischer R, Rodriguez-Romero J (2010) Spotlight on *Aspergillus nidulans* photosensory systems. Fungal Genet Biol 47:900–908

Berbara R, Fonseca H, Daft M (2002) The role of calcium in the germination and germinative hyphal growth of *Gigaspora rosea* Nicolson and Schenk. http://plantbio.berkeley.edu/~bruns/abstracts/berbara

Bergman K, Eslava AP, Cerdá-Olmedo E (1973) Mutants of *Phycomyces* with abnormal phototropism. Mol Gen Genet 123:1–16

Bergo V, Spudich EN, Spudich JL, Rothschild KJ (2002) A Fourier transform infrared study of *Neurospora* rhodopsin: similarities with archaeal rhodopsins. Photochem Photobiol 76:341–349

Bieszke JA, Braun EL, Bean LE, Kang S, Natvig DO, Borkovich KA (1999a) The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Proc Natl Acad Sci USA 96:8034–8039

Bieszke JA, Spudich EN, Scott KL, Borkovich KA, Spudich JL (1999b) A eukaryotic protein, NOP-1, binds retinal to form an archaeal rhodopsin-like photochemically reactive pigment. Biochemistry 38:14138–14145

Bieszke JA, Li L, Borkovich KA (2007) The fungal opsin gene *nop-1* is negatively-regulated by a component of the blue light sensing pathway and influences conidiation-specific gene expression in *Neurospora crassa*. Curr Genet 52:149–157

Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, Frankenberg-Dinkel N, Fischer R (2005) The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr Biol 15:1833–1838

Borriss H (1934) Beiträge zur Wachstums- und Entwicklungsphysiologie der Fruchtkörper von *Coprinus lagopus*. Planta 22:28–69

Brandt S, von Stetten D, Günther M, Hildebrandt P, Frankenberg-Dinkel N (2008) The fungal phytochrome FphA from *Aspergillus nidulans*. J Biol Chem 283:34605–34614

Braus GH, Irniger S, Bayram O (2010) Fungal development and the COP9 signalosome. Curr Opin Microbiol 13:672–676

Brenna A, Grimaldi B, Filetici P, Ballario P (2012) Physical association of the WC-1 photoreceptor and the histone acetyltransferase NGF-1 is required for blue light signal transduction in *Neurospora crassa*. Mol Biol Cell 23:3863–3872

Briggs WR, Spudich JL (eds) (2005) Handbook of photosensory receptors. Wiley-VCH, Weinheim

Brown LS, Dioumaev AK, Lanyi JK, Spudich EN, Spudich JL (2001) Photochemical reaction cycle and proton transfers in *Neurospora* rhodopsin. J Biol Chem 276:32495–32505

Brunner M, Káldi K (2008) Interlocked feedback loops of the circadian clock of *Neurospora crassa*. Mol Microbiol 68:255–262

Brunner M, Schafmeier T (2006) Transcriptional and post-transcriptional regulation of the circadian clock of cyanobacteria and *Neurospora*. Genes Dev 20:1061–1074

Buck JW, Dong W, Mueller DS (2010) Effect of light exposure on in vitro germination and germ tube growth of eight species of rust fungi. Mycologia 102:1134–1140

Buller AHR (1909) Researches on fungi, vol 1. Longman Green and Co, London

Buller AHR (1922) Researches on fungi, vol 2. Longman, Green and Co, London

Burgie ES, Vierstra RD (2014) Phytochromes: an atomic perspective on photoactivation and signaling. Plant Cell 26(12):4568–4583

Busch S, Eckert SE, Krappmann S, Braus GH (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 49:717–730

Busch S, Schwier EU, Nahlik K, Bayram O, Helmstaedt K, Draht OW, Krappmann S, Valerius O, Lipscomb WN, Braus GH (2007) An eight-subunit COP9 signalosome with an intact JAMM motif is required for fungal fruit body formation. Proc Natl Acad Sci USA 104:8089–8094

Calvo AM (2008) The VeA regulatory system and its role in morphological and chemical development in fungi. Fungal Genet Biol 45:1053–1061

Campuzano V, Galland P, Alvarez MI, Eslava AP (1996) Blue-light receptor requirement for gravitropism, autochemotropism and ethylene response in *Phycomyces*. Photochem Photobiol 63:686–694

Canessa P, Schumacher J, Hevia MA, Tudzynski P, Larrondo LF (2013) Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: characterization of the White Collar Complex. PLoS ONE 8, e84223

Carreras-Villaseñor N, Sánchez-Arreguín JA, Herrera-Estrella AH (2012) *Trichoderma*: sensing the environment for survival and dispersal. Microbiology 158:3–16

Casas-Flores S, Ríos-Momberg M, Bibbins M, Ponce-Noyola P, Herrera-Estrella A (2004) BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. Microbiology 150:3561–3569

Casas-Flores S, Rios-Momberg M, Rosales-Saavedra T, Martínez-Hernández P, Olmedo-Monfil V, Herrera-Estrella A (2006) Cross talk between a fungal blue-light perception system and the cyclic AMP signaling pathway. Eukaryot Cell 5:499–506

Castellanos F, Schmoll M, Martínez P, Tisch D, Kubicek CP, Herrera-Estrella A, Esquivel-Naranjo EU (2010) Crucial factors of the light perception machinery and their impact on growth and cellulase gene transcription in *Trichoderma reesei*. Fungal Genet Biol 47:468–476

Castrillo M, Avalos J (2014) Light-mediated participation of the VIVID-like protein of *Fusarium fujikuroi* VvdA in pigmentation and development. Fungal Genet Biol 71:9–20

Castrillo M, García-Martínez J, Avalos J (2013) Light-dependent functions of the *Fusarium fujikuroi* CryD DASH cryptochrome in development and secondary metabolism. Appl Environ Microbiol 79:2777–2788

Cerdá-Olmedo E (2001) *Phycomyces* and the biology of light and color. FEMS Microbiol Rev 25:503–512

Cha J, Huang G, Guo J, Liu Y (2007) Posttranslational control of the *Neurospora* circadian clock. Cold Spring Harb Symp Quant Biol 72:185–191

Cha J, Chang SS, Huang G, Cheng P, Liu Y (2008) Control of WHITE COLLAR localization by phosphorylation is a critical step in the circadian negative feedback process. EMBO J 27:3246–3255

Chaves I, Pokorny R, Byrdin M, Hoang N, Ritz T, Brettel K, Essen LO, van der Horst GT, Batschauer A, Ahmad M (2011) The cryptochromes: blue light photoreceptors in plants and animals. Annu Rev Plant Biol 62:335–364

Chen M, Chory J (2011) Phytochrome signaling mechanisms and the control of plant development. Trends Cell Biol 21:664–671

Chen CH, Ringelberg CS, Gross RH, Dunlap JC, Loros JJ (2009) Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. EMBO J 28:1029–1042

Chen CH, Demay BS, Gladfelter AS, Dunlap JC, Loros JJ (2010a) Physical interaction between VIVID and white collar complex regulates photoadaptation in *Neurospora*. Proc Natl Acad Sci USA 107:16715–16720

Chen CH, Dunlap JC, Loros JJ (2010b) *Neurospora* illuminates fungal photoreception. Fungal Genet Biol 47:922–929

Chen CL, Kuo HC, Tung SY, Hsu PW, Wang CL, Seibel C, Schmoll M, Chen RS, Wang TF (2012) Blue light acts as a double-edged sword in regulating sexual development of *Hypocrea jecorina* (*Trichoderma reesei*). PLoS ONE 7, e44969

Cheng P, Yang Y, Liu Y (2001) Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. Proc Natl Acad Sci USA 98:7408–7413

Cheng P, Yang Y, Gardner KH, Liu Y (2002) PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. Mol Cell Biol 22:517–524

Cheng P, He Q, He Q, Wang L, Liu Y (2005) Regulation of the *Neurospora* circadian clock by an RNA helicase. Genes Dev 19:234–241

Choi YE, Goodwin SB (2011) MVE1, encoding the velvet gene product homolog in *Mycosphaerella graminicola*, is associated with aerial mycelium formation, melanin biosynthesis, hyphal swelling, and light signaling. Appl Environ Microbiol 77:942–953

Christmann M, Schmaler T, Gordon C, Huang X, Bayram O, Schinke J, Stumpf S, Dubiel W, Braus

GH (2013) Control of multicellular development by the physically interacting deneddylases DEN1/DenA and COP9 signalosome. PLoS Genet 9, e1003275

Corrochano LM (2002) Photomorphogenesis in *Phycomyces*: differential display of gene expression by PCR with arbitrary primers. Mol Genet Genomics 267:424–428

Corrochano LM (2007) Fungal photoreceptors: sensory molecules for fungal development and behaviour. Photochem Photobiol Sci 6:725–736

Corrochano LM, Avalos J (2010) Light sensing. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Washington, DC, pp 417–441

Corrochano LM, Cerdá-Olmedo E (1990) Photomorphogenesis in *Phycomyces*: competence period and stimulus–response relationships. J Photochem Photobiol B5:255–266

Corrochano LM, Galland P (2006) Photomorphogenesis and gravitropism in fungi. In: Kües U, Fischer R (eds) The mycota I. Growth, differentiation and sexuality, vol I, 2nd edn, The mycota. Springer, Berlin, pp 233–259

Corrochano LM, Garre V (2010) Photobiology in the Zygomycota: multiple photoreceptor genes for complex responses to light. Fungal Genet Biol 47:893–899

Corrochano LM, Galland P, Lipson ED, Cerdá-Olmedo E (1988) Photomorphogenesis in *Phycomyces*: fluence-response curves and action spectra. Planta 174:315–320

Crabbé A, Nielsen-Preiss AM, Woolley CM, Barrila J, Buchanan K, McCracken J, Inglis DO, Searles SC, Nelman-Gonzales MA, Ott CM, Wilson JW, Pierson DL, Stefanyshyn-Piper HM, Hyman LE, Nickerson CA (2013) Spaceflight enhances cell aggregation and random budding in *Candida albicans*. PLoS ONE 8, e80677

Crosthwaite SK, Loros JJ, Dunlap JC (1995) Light-induced resetting of a circadian clock is mediated by a rapid increase in *frequency* transcript. Cell 81:1003–1012

Degli-Innocenti F, Pohl U, Russo VEA (1983) Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem Photobiol 37:49–51

Dennison DS (1961) Tropic responses of *Phycomyces* sporangiophores to gravitational and centrifugal stimuli. J Gen Physiol 45:23–38

Dennison DS (1964) The effect of light on the geotropic responses of *Phycomyces* sporangiophore. J Gen Physiol 47:651–665

Dennison DS, Roth CC (1967) *Phycomyces* sporangiophores: fungal stretch receptors. Science 156:1386–1388

Dennison D, Shropshire W Jr (1984) The gravireceptor of *Phycomyces*: its development following gravity exposure. J Gen Physiol 84:845–859

Dong W, Buck JW (2011) Effect of light on in vivo urediniospore germination, lesion development and sporulation of *Puccinia hemerocallidis* on daylily and *Puccinia pelargoniizonalis* on geranium. Mycologia 103:1277–1283

Doucette A, Voorthuis R, Havrilla M (1994) Cytoskeletal and associated components and calcium binding proteins of *Phycomyces* gravity sensitive sporangiophores. Am Soc Grav Space Biol 8:82

Edwards KL (1990) Gravity detection mechanism in a single cell, the *Phycomyces* sporangiophore. NASA space/gravitational biology accomplishments. NASA Tech Mem 4160:42–43

Edwards KL, Chew J, Harris S, Di Muzio E (1997) Effects of microinjected cytoskeletal inhibitors on the gravitropic response of the *Phycomyces* sporangiophore. Am Soc Gravitat Space Biol Bull 11:47

Eibel P, Schimek C, Fries V, Grolig F, Schapat T, Schmidt W, Schneckenburger H, Ootaki T, Galland P (2000) Statoliths in *Phycomyces*: characterization of octahedral protein crystals. Fungal Genet Biol 29:211–220

Einstein A (1905) Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. Ann Phys 17:549–560

Einstein A (1906) Zur Theorie der Brownschen Bewegung. Ann Phys 19:371–381

Ernst OP, Lodowski DT, Elstner M, Hegemann P, Brown LS, Kandori H (2014) Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. Chem Rev 114:126–163

Esquivel-Naranjo EU, Herrera-Estrella A (2007) Enhanced responsiveness and sensitivity to blue light by *blr-2* overexpression in *Trichoderma atroviride*. Microbiology 153:3909–3922

Estrada AF, Avalos J (2008) The White Collar protein WcoA of *Fusarium fujikuroi* is not essential for photocarotenogenesis, but is involved in the regulation of secondary metabolism and conidiation. Fungal Genet Biol 45:705–718

Fanelli F, Schmidt-Heydt M, Haidukowski M, Geisen R, Logrieco A, Mule G (2012a) Influence of light on growth, fumonisin biosynthesis and FUM1 gene expression by *Fusarium proliferatum*. Int J Food Microbiol 153:148–153

Fanelli F, Schmidt-Heydt M, Haidukowski M, Susca A, Geisen R, Logrieco A, Mule G (2012b) Influence of light on growth, conidiation and fumonisin production by *Fusarium verticillioides*. Fungal Biol 116:241–248

Flaherty JE, Dunkle LD (2005) Identification and expression analysis of regulatory genes induced during conidiation in *Exserohilum turcicum*. Fungal Genet Biol 42:471–481

Fortunato AE, Annunziata R, Jaubert M, Bouly JP, Falciatore A (2015) Dealing with light: the widespread and multitasking cryptochrome/photolyase family

in photosynthetic organisms. J Plant Physiol 172C:42–54
Franchi L, Fulci V, Macino G (2005) Protein kinase C modulates light responses in *Neurospora* by regulating the blue light photoreceptor WC-1. Mol Microbiol 56:334–345
Friedl MA, Schmoll M, Kubicek CP, Druzhinina IS (2008) Photostimulation of *Hypocrea atroviridis* growth occurs due to a cross-talk of carbon metabolism, blue light receptors and response to oxidative stress. Microbiology 154:1229–1241
Fries V, Krockert T, Grolig F, Galland P (2002) Statoliths in *Phycomyces*: spectrofluorometric characterization of octahedral protein crystals. J Plant Physiol 159:39–47
Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. Science 297:815–819
Froehlich AC, Noh B, Vierstra RD, Loros J, Dunlap JC (2005) Genetic and molecular analysis of phytochromes from the filamentous fungus *Neurospora crassa*. Eukaryot Cell 4:2140–2152
Froehlich AC, Chen CH, Belden WJ, Madeti C, Roenneberg T, Merrow M, Loros JJ, Dunlap JC (2010) Genetic and molecular characterization of a cryptochrome from the filamentous fungus *Neurospora crassa*. Eukaryot Cell 9:738–750
Fuller KK, Ringelberg CS, Loros JJ, Dunlap JC (2013) The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. MBio 4
Furutani Y, Bezerra AG Jr, Waschuk S, Sumii M, Brown LS, Kandori H (2004) FTIR spectroscopy of the K photointermediate of *Neurospora* rhodopsin: structural changes of the retinal, protein, and water molecules after photoisomerization. Biochemistry 43:9636–9646
Galland P (2002) Tropisms of *Avena* coleoptiles: sine law for gravitropism, exponential law for photogravitropic equilibrium. Planta 215:779–784
Galland P, Wallacher Y, Finger H, Hannappel M, Tröster S, Bold E, Grolig F (2002) Tropisms in *Phycomyces*: sine law for gravitropism, exponential law for photogravitropic equilibrium. Planta 214:931–938
Galland P, Finger H, Wallacher Y (2004) Gravitropism in *Phycomyces*: threshold determination on a clinostat centrifuge. J Plant Physiol 161:733–739
Galland P, Fries V, Grolig F, Schmidt W (2007) Gravireception in *Phycomyces blakesleeanus*. Adv Space Res 39:1134–1139
García-Martínez J, Brunk M, Avalos J, Terpitz U (2015) The CarO rhodopsin of the fungus *Fusarium fujikuroi* is a light-driven proton pump that retards spore germination. Sci Rep 5:7798
Gooday GW (1985) Elongation of the stipe of *Coprinus cinereus*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, Cambridge, pp 311–331
Göttig M, Galland P (2014) Gravitropism in *Phycomyces*: violation of the so-called resultant law – evidence for two response components. Plant Biol 16(suppl 1):158–166
Greening JP, Holden J, Moore D (1993) Distribution of mechanical stress is not involved in regulating stipe gravitropism in *Coprinus cinereus*. Mycol Res 97:1001–1004
Greening JP, Sanchez C, Moore D (1997) Coordinated cell elongation alone drives tropic bending in stems of the mushroom fruit body of *Coprinus cinereus*. Can J Bot 75:1174–1181
Grimaldi B, Coiro P, Filetici P, Berge E, Dobosy JR, Freitag M, Selker EU, Ballario P (2006) The *Neurospora crassa* White Collar-1 dependent blue light response requires acetylation of histone H3 lysine 14 by NGF-1. Mol Biol Cell 17:4576–4583
Grolig F, Eibel P, Schimek C, Schapat T, Dennison DS, Galland P (2000) Interaction between phototropism and gravitropism in the sporangiophore of *Phycomyces*. Plant Physiol 123:765–776
Grolig F, Herkenrath H, Pumm T, Gross A, Galland P (2004) Gravity susception by buoyancy: floating lipid globules in sporangiophores of *Phycomyces*. Planta 218:658–667
Grolig F, Döring M, Galland P (2006) Gravisusception by buoyancy: a mechanism ubiquitous among fungi? Protoplasma 229:117–123
Grolig F, Moch J, Schneider A, Galland P (2014) Actin cytoskeleton and organelle movement in the sporangiophore of the zygomycete *Phycomyces blakesleeanus*. Plant Biol 16(suppl 1):167–178
Gruen HE (1979) Control of rapid stipe elongation by the lamellae in fruit bodies of *Flammulina velutipes*. Can J Bot 57:1131–1135
Gruen HE (1982) Control of stipe elongation by the pileus and mycelium in fruiting bodies of *Flammulina velutipes* and other Agaricales. In: Wells K, Wells EK (eds) Basidium and basidiocarp. Springer, New York, pp 125–155
Hagimoto H (1963) Studies on the growth of fruit body of fungi. IV. The growth of the fruit body of *Agaricus bisporus* and the economy of the mushroom growth hormone. Bot Mag 76:256–263
Haindl E, Monzer J (1994) Elongation growth and gravitropic curvature in the *Flammulina velutipes* (Agaricales) fruiting body. Exp Mycol 18:150–158
Hart JW (1990) Plant tropisms and other growth movements. Unwin Hyman, London
Harting R, Bayram O, Laubinger K, Valerius O, Braus GH (2013) Interplay of the fungal sumoylation network for control of multicellular development. Mol Microbiol 90:1125–1145
Hatton JP, Moore D (1992) Kinetics of stem gravitropism in *Coprinus cinereus*: determination of presentation time and dosage-response relationships using clinostats. FEMS Microbiol Lett 100:81–86
He Q, Liu Y (2005) Molecular mechanism of light responses in *Neurospora*: from light-induced tran-

scription to photoadaptation. Genes Dev 19:2888–2899

He Q, Cheng P, Yang Y, Wang L, Gardner KH, Liu Y (2002) White collar-1, a DNA binding transcription factor and a light sensor. Science 297:840–843

He Q, Cha J, He Q, Lee HC, Yang Y, Liu Y (2006) CKI and CKII mediate the FREQUENCY-dependent phosphorylation of the WHITE COLLAR complex to close the *Neurospora* circadian negative feedback loop. Genes Dev 20:2552–2565

Heintzen C, Loros JJ, Dunlap JC (2001) The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. Cell 104:453–464

Helmstaedt K, Schwier EU, Christmann M, Nahlik K, Westermann M, Harting R, Grond S, Busch S, Braus GH (2011) Recruitment of the inhibitor Cand1 to the cullin substrate adaptor site mediates interaction to the neddylation site. Mol Biol Cell 22:153–164

Herrera-Estrella A, Horwitz BA (2007) Looking through the eyes of fungi: molecular genetics of photoreception. Mol Microbiol 64:5–15

Hoff B, Kamerewerd J, Sigl C, Mitterbauer R, Zadra I, Kürnsteiner H, Kück U (2010) Two components of a velvet-like complex control hyphal morphogenesis, conidiophore development, and penicillin biosynthesis in *Penicillium chrysogenum*. Eukaryot Cell 9:1236–1250

Hofmeister W (1863) Über die durch die Schwerkraft bestimmten Richtungen von Pflanzentheilen. Jahrb Wiss Bot 3:77–114

Hong Y-X, Ming Z, Gao L-D, Yang XH, Zhen M, Li DG (2001) Hyphal growth of mycorrhizal fungi in time turning vertical plate culture. Mycosystema 20:358–361

Horie T, Schimek C, Fukui J, Miyazaki A, Mihara H, Tsuru T, Maki K, Ootaki T (1998) Developmental stage-dependent response of *Pilobolus crystallinus* sporangiophores to gravitative and centrifugal stimulation. Mycoscience 39:463–470

Horwitz BA, Perlman A, Gressel J (1990) Induction of *trichoderma* sporulation by nanosecond laser pulses: evidence against cryptochrome cycling. Photochem Photobiol 51:99–104

Howard J, Roberts WM, Hudspeth AJ (1988) Mechanoelectrical transduction by hair cells. Annu Rev Biophys Biophys Chem 17:99–124

Huang G, Chen S, Li S, Cha J, Long C, Li L, He Q, Liu Y (2007) Protein kinase A and casein kinases mediate sequential phosphorylation events in the circadian negative feedback loop. Genes Dev 21:3283–3295

Huang G, He Q, Guo J, Cha J, Liu Y (2013) The Ccr4-not protein complex regulates the phase of the Neurospora circadian clock by controlling white collar protein stability and activity. J Biol Chem 288:31002–31009

Hunt SM, Thompson S, Elvin M, Heintzen C (2010) VIVID interacts with the WHITE COLLAR complex and FREQUENCY-interacting RNA helicase to alter light and clock responses in *Neurospora*. Proc Natl Acad Sci USA 107:16709–16714

Idnurm A, Heitman J (2005) Light controls growth and development via a conserved pathway in the fungal kingdom. PLoS Biol 3, e95

Idnurm A, Rodríguez-Romero J, Corrochano LM, Sanz C, Iturriaga EA, Eslava AP, Heitman J (2006) The *Phycomyces madA* gene encodes a blue-light photoreceptor for phototropism and other light responses. Proc Natl Acad Sci USA 103:4546–4551

Idnurm A, Verma S, Corrochano LM (2010) A glimpse into the basis of vision in the kingdom Mycota. Fungal Genet Biol 47:881–892

Jack TJ, Yatsu LY, Altschul AM (1967) Isolation and characterization of peanut sherosomes. Plant Physiol 42:585–597

Johanson K, Allen PL, Lewis F, Cubano LA, Hyman LE, Hammond TG (2002) *Saccharomyces cerevisiae* gene expression changes during rotating wall vessel suspension culture. J Appl Physiol 93:2171–2180

Káldi K, González BH, Brunner M (2006) Transcriptional regulation of the *Neurospora* circadian clock gene *wc-1* affects the phase of circadian output. EMBO Rep 7:199–204

Kamada T, Sano H, Nakazawa T, Nakahori K (2010) Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. Fungal Genet Biol 47:917–921

Kamisaka Y, Noda N, Sakai T, Kawasaki K (1999) Lipid body formation in an oleaginous fungus, *Mortierella ramanniana* var. *angulispora*. Biochim Biophys Acta 1438:185–198

Karniol B, Wagner JR, Walker JM, Vierstra RD (2005) Phylogenetic analysis of the phytochrome superfamily reveals distinct microbial subfamilies of photoreceptors. Biochem J 392:103–116

Kasatkina TB, Zharikova GG, Rubin AB, Palmbakh LR, Vaulina EM, Mashinsky AL (1980) Development of higher fungi under weightlessness. Life Sci Space Res 18:205–211

Kern VD, Hock B (1996a) Gravimorphogenesis and ultrastructure of the fungus Flammulina velutipes grown in space, on clinostats and under hyper-g conditions. Adv Space Res 17:183–186

Kern VD, Hock B (1996b) Gravitropismus bei Pilzen. Naturw Rdsch 49:174–180

Kher K, Greening JP, Hatton JP, Frazer LN, Moore D (1992) Kinetics and mechanics of stem gravitropism in *Coprinus cinereus*. Mycol Res 96:817–824

Kihara J, Moriwaki A, Tanaka N, Ueno M, Arase S (2007) Characterization of the BLR1 gene encoding a putative blue-light regulator in the phytopathogenic fungus *Bipolaris oryzae*. FEMS Microbiol Lett 266:110–118

Kim S, Singh P, Park J, Park S, Friedman A, Zheng T, Lee YH, Lee K (2011) Genetic and molecular characterization of a blue light photoreceptor MGWC-1 in *Magnaporthe oryzae*. Fungal Genet Biol 48:400–407

Kim H, Son H, Lee YW (2013) Effects of light on secondary metabolism and fungal development of *Fusarium graminearum*. J Appl Microbiol 116 (2):380–389

Knoll F (1909) Untersuchungen über Längenwachstum und Geotropismus der Fruchtkörperstiele von *Coprinus stiriacus*. Sitzungsber Kaiserl Akad Wiss Wien. Math-Naturwiss Klasse Abt 1 118:575–634

Kubo H (2009) Isolation of *madA* homologs in *Pilobolus crystallinus*. Mycoscience 50:400–406

Kumagai T (1989) Temperature and mycochrome system in near-UV light inducible and blue light reversible photoinduction of conidiation in *Alternaria tomato*. Photochem Photobiol 50:793–798

Kuratani M, Tanaka K, Terashima K, Muraguchi H, Nakazawa T, Nakahori K, Kamada T (2010) The *dst2* gene essential for photomorphogenesis of *Coprinopsis cinerea* encodes a protein with a putative FAD-binding-4 domain. Fungal Genet Biol 47:152–158

Lauter FR, Yanofsky C (1993) Day/night and circadian rhythm control of *con* gene expression in *Neurospora*. Proc Natl Acad Sci USA 90:8249–8253

Lauter FR, Marchfelder U, Russo VE, Yamashiro CT, Yatzkan E, Yarden O (1998) Photoregulation of cot-1, a kinase-encoding gene involved in hyphal growth in Neurospora crassa. Fungal Genet Biol 23:300–310

Lee K, Loros JJ, Dunlap JC (2000) Interconnected feedback loops in the *Neurospora* circadian system. Science 289:107–110

Lee B, Yoshida Y, Hasunuma K (2006a) Photomorphogenetic characteristics are severely affected in nucleoside diphosphate kinase-1 (*ndk-1*)-disrupted mutants in *Neurospora crassa*. Mol Genet Genomics 275:9–17

Lee K, Singh P, Chung WC, Ash J, Kim TS, Hang L, Park S (2006b) Light regulation of asexual development in the rice blast fungus, *Magnaporthe oryzae*. Fungal Genet Biol 43:694–706

Lee SR, Joo WJ, Baek YU, Kim I, Chay KO, Cho SH, Lee SJ, Kang SO (2011) Effect of light and reductones on differentiation of *Pleurotus ostreatus*. J Microbiol 49:71–77

Linden H, Macino G (1997) White collar 2, a partner in blue-light signal transduction, controlling expression of light-regulated genes in *Neurospora crassa*. EMBO J 16:98–109

Lipson ED (1975) White noise analysis of *Phycomyces* light-growth response system. III. Photomutants. Biophys J 15:1033–1045

Lipson ED, López-Díaz I, Pollock JA (1983) Mutants of *Phycomyces* with enhanced tropisms. Exp Mycol 7:241–252

Liu H, Liu B, Zhao C, Pepper M, Lin C (2011) The action mechanisms of plant cryptochromes. Trends Plant Sci 16:684–691

Lombardi LM, Brody S (2005) Circadian rhythms in *Neurospora crassa*: clock gene homologues in fungi. Fungal Genet Biol 42:887–892

López-Díaz I, Lipson ED (1983) Genetic analysis of hypertropic mutants of *Phycomyces*. Mol Gen Genet 190:318–325

Lu YK, Sun KH, Shen WC (2005) Blue light negatively regulates the sexual filamentation via the Cwc1 and Cwc2 proteins in *Cryptococcus neoformans*. Mol Microbiol 56:480–491

Ma LJ, Ibrahim AS, Skory C, Grabherr MG, Burger G, Butler M, Elias M, Idnurm A, Lang BF, Sone T, Abe A, Calvo SE, Corrochano LM, Engels R, Fu J, Hansberg W, Kim JM, Kodira CD, Koehrsen MJ, Liu B, Miranda-Saavedra D, O'Leary S, Ortiz-Castellanos L, Poulter R, Rodríguez-Romero J, Ruiz-Herrera J, Shen YQ, Zeng Q, Galagan J, Birren BW, Cuomo CA, Wickes BL (2009) Genomic analysis of the basal lineage fungus *Rhizopus oryzae* reveals a whole-genome duplication. PLoS Genet 5, e1000549

Malzahn E, Ciprianidis S, Káldi K, Schafmeier T, Brunner M (2010) Photoadaptation in *Neurospora* by competitive interaction of activating and inhibitory LOV domains. Cell 142:762–772

Merrow M, Franchi L, Dragovic Z, Görl M, Johnson J, Brunner M, Macino G, Roenneberg T (2001) Circadian regulation of the light input pathway in *Neurospora crassa*. EMBO J 20:307–315

Miyazaki Y, Sunagawa M, Higashibata A, Ishioka N, Babasaki K, Yamazaki T (2010) Differentially expressed genes under simulated microgravity in fruiting bodies of the fungus *Pleurotus ostreatus*. FEMS Microbiol Lett 307:72–79

Monzer J (1995) Actin filaments are involved in cellular graviperception of the basidiomycete *Flammulina velutipes*. Eur J Cell Biol 66:151–156

Monzer J (1996) Cellular graviperception in the basidiomycete *Flammulina velutipes* – Can the nuclei serve as fungal statoliths? Eur J Cell Biol 71:216–220

Monzer J, Haindl E, Kern V, Dressel K (1994) Gravitropism of the basidiomycete *Flammulina velutipes*: morphological and physiological aspects of the graviresponse. Exp Mycol 18:7–19

Mooney JL, Yager LN (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Moore D (1991) Perception and response to gravity in higher fungi - a critical appraisal. New Phytol 117:3–23

Moriwaki A, Katsube H, Ueno M, Arase S, Kihara J (2008) Cloning and characterization of the BLR2, the homologue of the blue-light regulator of *Neurospora crassa* WC-2, in the phytopathogenic fungus *Bipolaris oryzae*. Curr Microbiol 56:115–121

Navarro E, Peñaranda A, Hansberg W, Torres-Martínez S, Garre V (2013) A white collar 1-like protein mediates opposite regulatory functions in *Mucor circinelloides*. Fungal Genet Biol 52:42–52

Navarro-Sampedro L, Yanofsky C, Corrochano LM (2008) A genetic selection for *Neurospora crassa* mutants altered in their light regulation of transcription. Genetics 178:171–183

Novak Frazer L, Moore D (1993) Antagonists and inhibitors of calcium accumulation do not impair gravity perception though they adversely affect the gravitropic responses of *Coprinus cinereus* stipes. Mycol Res 97:11113–11118

Nsa IY, Karunarathna N, Liu X, Huang H, Boetteger B, Bell-Pedersen D (2015) A novel cryptochrome-dependent oscillator in *Neurospora crassa*. Genetics 199:233–245

Ogorodnikova U, Sineshchekov O, Galland P (2002) Blue-light perception in stage-1 sporangiophores of *Phycomyces*: a role for apical lipid globules? J Plant Physiol 159:205–209

Ogura Y, Yoshida Y, Ichimura K, Aoyagi C, Yabe N, Hasunuma K (1999) Isolation and characterization of *Neurospora crassa* nucleoside diphosphate kinase NDK-1. Eur J Biochem 266:709–714

Ogura Y, Yoshida Y, Yabe N, Hasunuma K (2001) A point mutation in nucleoside diphosphate kinase results in a deficient light response for perithecial polarity in *Neurospora crassa*. J Biol Chem 276:21228–21234

Ohm RA, Aerts D, Wosten HA, Lugones LG (2013) The blue light receptor complex WC-1/2 of *Schizophyllum commune* is involved in mushroom formation and protection against phototoxicity. Environ Microbiol 15:943–955

Olmedo M, Navarro-Sampedro L, Ruger-Herreros C, Kim SR, Jeong BK, Lee BU, Corrochano LM (2010a) A role in the regulation of transcription by light for RCO-1 and RCM-1, the *Neurospora* homologs of the yeast Tup1-Ssn6 repressor. Fungal Genet Biol 47:939–952

Olmedo M, Ruger-Herreros C, Corrochano LM (2010b) Regulation by blue light of the *fluffy* gene encoding a major regulator of conidiation in *Neurospora crassa*. Genetics 184:651–658

Olmedo M, Ruger-Herreros C, Luque EM, Corrochano LM (2010c) A complex photoreceptor system mediates the regulation by light of the conidiation genes *con-10* and *con-6* in *Neurospora crassa*. Fungal Genet Biol 47:352–363

Olmedo M, Ruger-Herreros C, Luque EM, Corrochano LM (2013) Regulation of transcription by light in *Neurospora crassa*: a model for fungal photobiology? Fungal Biol Rev 27:10–18

Palit A, Pratrap P, Lipson ED (1989) System analysis of *Phycomyces* light-growth response: *madC*, *madG*, and *madH* mutants. Biophys J 55:519–526

Parfyonov GP, Platanova RN, Tairbekov MG, Belenev YN, Olkhovenko VP, Rostopshina AV, Oigenblick EA (1979) Biological investigations aboard biosatellite Cosmos-782. Acta Astronaut 6:1235–1238

Peter E, Dick B, Baeurle SA (2012) Illuminating the early signaling pathway of a fungal light-oxygen-voltage photoreceptor. Proteins 80:471–481

Polaino Orts S, Chaudhary S, Shakya V, Miralles-Durán A, Corrochano LM, Idnurm A (2013) Map-based identification of the *mad* photosensing genes of *Phycomyces blakesleeanus*. Fung Gen Rep 60(Supplement), Abstract # 211

Pross HD, Casares A, Kiefer J (2000) Induction and repair of DNA double-strand breaks under irradiation and microgravity. Radiat Res 153:521–525

Pruss S, Fetzner R, Seither K, Herr A, Pfeiffer E, Metzler M, Lawrence CB, Fischer R (2014) Role of the *Alternaria alternata* blue-light receptor LreA (white-collar 1) in spore formation and secondary metabolism. Appl Environ Microbiol 80:2582–2591

Purschwitz J, Müller S, Kastner C, Fischer R (2006) Seeing the rainbow: light sensing in fungi. Curr Opin Microbiol 9:566–571

Purschwitz J, Muller S, Kastner C, Schoser M, Haas H, Espeso EA, Atoui A, Calvo AM, Fischer R (2008) Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. Curr Biol 18:255–259

Purschwitz J, Müller S, Fischer R (2009) Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the White Collar protein LreB. Mol Genet Genomics 281:35–42

Quail PH (1979) Plant cell fractionation. Ann Rev Plant Physiol 30:425–484

Querfurth C, Diernfellner A, Heise F, Lauinger L, Neiss A, Tataroglu O, Brunner M, Schafmeier T (2007) Posttranslational regulation of *Neurospora* circadian clock by CK1a-dependent phosphorylation. Cold Spring Harb Symp Quant Biol 72:177–183

Rawitscher F (1932) Der Geotropismus der Pflanzen. Gustav Fischer, Jena

Rockwell NC, Su YS, Lagarias JC (2006) Phytochrome structure and signaling mechanisms. Annu Rev Plant Biol 57:837–858

Rodríguez-Romero J, Corrochano LM (2004) The gene for the heat-shock protein HSP100 is induced by blue light and heat-shock in the fungus *Phycomyces blakesleeanus*. Curr Genet 46:295–303

Rodríguez-Romero J, Corrochano LM (2006) Regulation by blue light and heat shock of gene transcription in the fungus *Phycomyces*: proteins required for photoinduction and mechanism for adaptation to light. Mol Microbiol 61:1049–1059

Rodriguez-Romero J, Hedtke M, Kastner C, Muller S, Fischer R (2010) Fungi, hidden in soil or up in the air: light makes a difference. Annu Rev Microbiol 64:585–610

Röhrig J, Kastner C, Fischer R (2013) Light inhibits spore germination through phytochrome in *Aspergillus nidulans*. Curr Genet 59:55–62

Rosales-Saavedra T, Esquivel-Naranjo EU, Casas-Flores S, Martínez-Hernández P, Ibarra-Laclette E, Cortes-Penagos C, Herrera-Estrella A (2006)

Novel light-regulated genes in *Trichoderma atroviride*: a dissection by cDNA microarrays. Microbiology 152:3305–3317

Ruesch CE, Ramakrishnan M, Park J, Li N, Chong HS, Zaman R, Joska TM, Belden WJ (2015) The histone H3 lysine 9 methyltransferase DIM-5 modifies chromatin at *frequency* and represses light-activated gene expression. G3 (Bethesda) 5:93–101

Ruger-Herreros C, Rodríguez-Romero J, Fernández-Barranco R, Olmedo M, Fischer R, Corrochano LM, Cánovas D (2011) Regulation of conidiation by light in *Aspergillus nidulans*. Genetics 188:809–822

Ruger-Herreros C, Gil-Sanchez Mdel M, Sancar G, Brunner M, Corrochano LM (2014) Alteration of light-dependent gene regulation by the absence of the RCO-1/RCM-1 repressor complex in the fungus *Neurospora crassa*. PLoS ONE 9, e95069

Ruiz-Roldán MC, Garre V, Guarro J, Mariné M, Roncero MI (2008) Role of the white collar 1 photoreceptor in carotenogenesis, UV resistance, hydrophobicity, and virulence of *Fusarium oxysporum*. Eukaryot Cell 7:1227–1230

Sachs J (1879) Über orthotrope und plagiotrope Pflanzen. Arb Bot Inst Würzburg 2:226–284

Sancar G, Sancar C, Brugger B, Ha N, Sachsenheimer T, Gin E, Wdowik S, Lohmann I, Wieland F, Hofer T, Diernfellner A, Brunner M (2011) A global circadian repressor controls antiphasic expression of metabolic genes in *Neurospora*. Mol Cell 44:687–697

Sánchez-Arreguín A, Pérez-Martínez AS, Herrera-Estrella A (2012) Proteomic analysis of *Trichoderma atroviride* reveals independent roles for transcription factors BLR-1 and BLR-2 in light and darkness. Eukaryot Cell 11:30–41

Sánchez-Murillo RI, Torre-Martínez M, Aguirre-Linares J, Herrera-Estrella A (2004) Light-regulated asexual reproduction in *Paecilomyces fumosoroseus*. Microbiology 150:311–319

Sano H, Narikiyo T, Kaneko S, Yamazaki T, Shishido K (2007) Sequence analysis and expression of a blue-light photoreceptor gene, *Le.phrA* from the basidiomycetous mushroom *Lentinula edodes*. Biosci Biotechnol Biochem 71:2206–2213

Sanz C, Rodríguez-Romero J, Idnurm A, Christie JM, Heitman J, Corrochano LM, Eslava AP (2009) *Phycomyces* MADB interacts with MADA to form the primary photoreceptor complex for fungal phototropism. Proc Natl Acad Sci USA 106:7095–7100

Sarikaya Bayram O, Bayram O, Valerius O, Park HS, Irniger S, Gerke J, Ni M, Han KH, Yu JH, Braus GH (2010) LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. PLoS Genet 6, e1001226

Schafmeier T, Diernfellner AC (2011) Light input and processing in the circadian clock of *Neurospora*. FEBS Lett 585:1467–1473

Schafmeier T, Haase A, Kaldi K, Scholz J, Fuchs M, Brunner M (2005) Transcriptional feedback of *Neurospora* circadian clock gene by phosphorylation-dependent inactivation of its transcription factor. Cell 122:235–246

Schafmeier T, Káldi K, Diernfellner A, Mohr C, Brunner M (2006) Phosphorylation-dependent maturation of *Neurospora* circadian clock protein from a nuclear repressor toward a cytoplasmic activator. Genes Dev 20:297–306

Schimek C, Eibel P, Grolig F, Horie T, Ootaki T, Galland P (1999) Gravitropism in *Phycomyces*: a role for sedimenting protein crystals and for floating lipid globules. Planta 210:132–142

Schmidt W (2006) Gravity-induced absorption changes (GIACs) in *Phycomyces blakesleeanus* during parabolic flights: a spectral approach. Protoplasma 229:125–131

Schmidt W (2007) Array-spectrophotometers for measuring gravity-induced absorption changes (GIACs) during parabolic flights on the AIRBUS-300-ZERO-G. Microgr Sci Technol 19:16–21

Schmidt W (2011) Gravireception in *Phycomyces blakesleeanus* and *Arabidopsis thaliana*: hysteretic behaviour of primary reactions. Microgr Sci Technol 23:355–364

Schmidt W, Galland P (2000) Gravity-induced absorbance changes in *Phycomyces*: a novel method for detecting primary reactions of gravitropism. Planta 210:848–852

Schmidt W, Galland P (2004) Optospectroscopic detection of primary reactions associated with the graviperception of *Phycomyces*: effects of micro- and hypergravity. Plant Physiol 135:183–192

Schmoll M, Franchi L, Kubicek CP (2005) Envoy, a PAS/LOV domain protein of *Hypocrea jecorina* (anamorph *Trichoderma reesei*), modulates cellulase gene transcription in response to light. Eukaryot Cell 4:1998–2007

Schmoll M, Schuster A, Silva RN, Kubicek CP (2009) The G-Alpha protein GNA3 of *Hypocrea jecorina* (anamorph *Trichoderma reesei*) regulates cellulase gene expression in the presence of light. Eukaryot Cell 8:410–420

Schmoll M, Esquivel-Naranjo EU, Herrera-Estrella A (2010) *Trichoderma* in the light of day–physiology and development. Fungal Genet Biol 47:909–916

Schumacher J, Pradier JM, Simon A, Traeger S, Moraga J, Collado IG, Viaud M, Tudzynski B (2012) Natural variation in the VELVET gene *bcvel1* affects virulence and light-dependent differentiation in *Botrytis cinerea*. PLoS ONE 7, e47840

Schumacher J, Simon A, Cohrs KC, Viaud M, Tudzynski P (2014) The transcription factor BcLTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*. PLoS Genet 10, e1004040

Schuster A, Kubicek CP, Friedl MA, Druzhinina IS, Schmoll M (2007) Impact of light on *Hypocrea*

jecorina and the multiple cellular roles of ENVOY in this process. BMC Genomics 8:449

Schuster A, Tisch D, Seidl-Seiboth V, Kubicek CP, Schmoll M (2012) Roles of protein kinase A and adenylate cyclase in light-modulated cellulase regulation in *Trichoderma reesei*. Appl Environ Microbiol 78:2168–2178

Schwerdtfeger C, Linden H (2000) Localization and light-dependent phosphorylation of white collar 1 and 2, the two central components of blue light signaling in *Neurospora crassa*. Eur J Biochem 267:414–422

Schwerdtfeger C, Linden H (2001) Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. Mol Microbiol 39:1080–1087

Schwerdtfeger C, Linden H (2003) VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J 22:4846–4855

Seibel C, Gremel G, do Nascimento Silva R, Schuster A, Kubicek CP, Schmoll M (2009) Light-dependent roles of the G-protein alpha subunit GNA1 of Hypocrea jecorina (anamorph Trichoderma reesei). BMC Biol 7:58

Seibel C, Tisch D, Kubicek CP, Schmoll M (2012) ENVOY is a major determinant in regulation of sexual development in *Hypocrea jecorina* (*Trichoderma reesei*). Eukaryot Cell 11:885–895

Sharma AK, Spudich JL, Doolittle WF (2006) Microbial rhodopsins: functional versatility and genetic mobility. Trends Microbiol 14:463–469

Shrode LB, Lewis ZA, White LD, Bell-Pedersen D, Ebbole DJ (2001) *vvd* is required for light adaptation of conidiation-specific genes of *Neurospora crassa*, but not circadian conidiation. Fungal Genet Biol 32:169–181

Silva F, Torres-Martínez S, Garre V (2006) Distinct *white collar-1* genes control specific light responses in *Mucor circinelloides*. Mol Microbiol 61:1023–1037

Silva F, Navarro E, Peñaranda A, Murcia-Flores L, Torres-Martínez S, Garre V (2008) A RING-finger protein regulates carotenogenesis via proteolysis-independent ubiquitylation of a white collar-1-like activator. Mol Microbiol 70:1026–1036

Smith KM, Sancar G, Dekhang R, Sullivan CM, Li S, Tag AG, Sancar C, Bredeweg EL, Priest HD, McCormick RF, Thomas TL, Carrington JC, Stajich JE, Bell-Pedersen D, Brunner M, Freitag M (2010) Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for neurospora white collar complex. Eukaryot Cell 9:1549–1556

Spudich JL (2006) The multitalented microbial sensory rhodopsins. Trends Microbiol 14:480–487

Stecker T, Joiner W, Edwards KL (1990) Inhibitors affecting gravitropism of the *Phycomyces* sporangiophore. Am Soc Grav Space Biol 4:98

Steyaert JM, Weld RJ, Loguercio LL, Stewart A (2010) Rhythmic conidiation in the blue-light fungus *Trichoderma pleuroticola*. Fungal Biol 114:219–223

Stinnett SM, Espeso EA, Cobeño L, Araújo-Bazán L, Calvo AM (2007) *Aspergillus nidulans* VeA subcellular localization is dependent on the importin alpha carrier and on light. Mol Microbiol 63:242–255

Takahashi H (1997) Gravimorphogenesis: gravity-regulated formation of the peg in cucumber seedlings. Planta 203:S164–S169

Takahashi A, Ohnishi K, Takahashi S, Masukawa M, Sekikawa K, Amano T, Nakano T, Nagaoka S, Ohnishi T (2001) The effects of microgravity on induced mutation in *Escherichia coli* and *Saccharomyces cerevisiae*. Adv Space Res 28:555–561

Talora C, Franchi L, Linden H, Ballario P, Macino G (1999) Role of a white collar-1-white collar-2-complex in blue-light signal transduction. EMBO J 18:4961–4968

Tataroglu O, Lauinger L, Sancar G, Jakob K, Brunner M, Diernfellner AC (2012) Glycogen synthase kinase is a regulator of the circadian clock of *Neurospora crassa*. J Biol Chem 287:36936–36943

Tisch D, Schmoll M (2013) Targets of light signalling in *Trichoderma reesei*. BMC Genomics 14:657

Tisch D, Kubicek CP, Schmoll M (2011a) New insights into the mechanism of light modulated signaling by heterotrimeric G-proteins: ENVOY acts on gna1 and gna3 and adjusts cAMP levels in *Trichoderma reesei* (*Hypocrea jecorina*). Fungal Genet Biol 48:631–640

Tisch D, Kubicek CP, Schmoll M (2011b) The phosducin-like protein PhLP1 impacts regulation of glycoside hydrolases and light response in *Trichoderma reesei*. BMC Genomics 12:613

Tisch D, Schuster A, Schmoll M (2014) Crossroads between light response and nutrient signalling: ENV1 and PhLP1 act as mutual regulatory pair in *Trichoderma reesei*. BMC Genomics 15:425

Van Mulders SE, Stassen C, Daenen L, Devreese B, Siewers V, Van Eijsden RGE, Nielsen J, Delvaux FR, Willaerts R (2011) The influence of microgravity on invasive growth in *Saccharomyces cerevisiae*. Astrobiology 11:45–55

Veluchamy S, Rollins JA (2008) A CRY-DASH-type photolyase/cryptochrome from *Sclerotinia sclerotiorum* mediates minor UV-A-specific effects on development. Fungal Genet Biol 45:1265–1276

Volkmann D, Sievers A (1979) Graviperception in multicellular organs. In: Haupt W, Feinleib ME (eds) Encyclopedia of plant physiology, vol 7, Physiology of movements. Springer, Berlin/New York, pp 573–600

von Zeska Kress MR, Harting R, Bayram O, Christmann M, Irmer H, Valerius O, Schinke J, Goldman GH, Braus GH (2012) The COP9 signalosome counteracts the accumulation of cullin SCF ubiquitin E3 RING ligases during fungal development. Mol Microbiol 83:1162–1177

Walther I, Bechler B, Müller O, Hunzinger E, Cogoli A (1996) Cultivation of *Saccharomyces cerevisiae* in a bioreactor in microgravity. J Biotechnol 47:113–127

Watrud LS, Heithaus JJ III, Jaworski EG (1978) Geotropism in the endomycorrhizal fungus *Gigaspora margarita*. Mycologia 70:449–452

Wu C, Yang F, Smith KM, Peterson M, Dekhang R, Zhang Y, Zucker J, Bredeweg EL, Mallappa C, Zhou X, Lyubetskaya A, Townsend JP, Galagan JE, Freitag M, Dunlap JC, Bell-Pedersen D, Sachs MS (2014) Genome-wide characterization of light-regulated genes in *Neurospora crassa*. G3 (Bethesda) 4:1731–1745

Yamazaki Y, Kataoka H, Miyazaki A, Watanabe M, Ootaki T (1996) Action spectra for photoinhibition of sexual development in *Phycomyces blakesleeanus*. Photochem Photobiol 64:387–392

Yang T, Dong C (2014) Photo morphogenesis and photo response of the blue-light receptor gene Cmwc-1 in different strains of *Cordyceps militaris*. FEMS Microbiol Lett 352:190–197

Yeh YL, Lin YS, Su BJ, Shen WC (2009) A screening for suppressor mutants reveals components involved in the blue light-inhibited sexual filamentation in *Cryptococcus neoformans*. Fungal Genet Biol 46:42–54

Yoshida Y, Hasunuma K (2004) Reactive oxygen species affect photomorphogenesis in *Neurospora crassa*. J Biol Chem 279:6986–6993

Yoshida Y, Hasunuma K (2006) Light-dependent subcellular localization of nucleoside diphosphate kinase-1 in *Neurospora crassa*. FEMS Microbiol Lett 261:64–68

Yoshida Y, Ogura Y, Hasunuma K (2006) Interaction of nucleoside diphosphate kinase and catalases for stress and light responses in *Neurospora crassa*. FEBS Lett 580:3282–3286

Zharikova GG, Rubin AB, Nemchikov AV (1977) Effects of weightlessness, space orientation and light on geotropism and the formation of fruit bodies in higher fungi. Life Sci Space Res 15:291–294

Živanović B (2012) Surface tip-to-base Ca^{2+} and H^{+} ionic fluxes are involved in apical growth and graviperception of the *Phycomyces* stage I sporangiophore. Planta 236:1817–1829

Zoltowski BD, Crane BR (2008) Light activation of the LOV protein vivid generates a rapidly exchanging dimer. Biochemistry 47:7012–7019

Reproductive Processes

12 Asexual Sporulation in Agaricomycetes

Ursula Kües[1], Susanna M. Badalyan[2], Alexander Gießler[1], Bastian Dörnte[1]

CONTENTS

I. Introduction

The typical life cycle of heterothallic Agaricomycetes comprises of two alternate mycelial stages, the sterile monokaryon and the fertile dikaryon (Fig. 12.1; Kües 2000; Kothe et al. 2015). The monokaryon arises as the **primary mycelium** from germination of the **meiotic basidiospores** which in most species contain only one type of haploid nuclei. Accordingly, **monokaryons** do also have only one type of haploid nuclei in their hyphal cells (Kües 2000). A monokaryon in the strictest sense of the word is a **homokaryon** that contains just one haploid nucleus per hyphal segment, such as observed in many strains of the model basidiomycetes *Coprinopsis cinerea* and *Schizophyllum commune* (Bensaude 1918; Buller 1931; Raper and Raper 1966; Polak et al. 1997a; Kües et al. 2002a), but deviations with many more nuclei within hyphal cells (coenocytic nuclear behaviour; Boidin 1971) are also known such as in *Heterobasidion annosum* sensu lato (Korhonen and Stenlid 1998) and amongst species of the Polyporales (Rajchenberg 2011). Primary mycelia of heterothallic species are characterised by distinct **mating types**. Mating types describe physiological conditions that when different allow two individuals after hyphal fusion to form the **secondary mycelium** (Casselton and Olesnicky 1998; Kües 2000; Freihorst et al. 2016). The typical secondary mycelium, the **dikaryon**, has two distinct haploid nuclei in its hyphal cells, each one per parental monokaryon (Iwasa et al. 1998; Schuurs et al. 1998; Kües et al. 2002a). The two distinct nuclei in the dikaryotic cells divide in synchrony for cellular divisions, with **clamp cell** formation at the places of future hyphal septa as part of a coordinated cellular process that ensures the even distribution of the respective daughter nuclei to the two new hyphal cells (Bensaude 1918; Buller 1933).

In this process, a daughter nucleus of one mating type moves backwards into the becoming new subapical cell, a daughter nucleus of the other mating type will be trapped by septation in the backwards growing clamp cell while another septum laid in the main hypha separates the new subapical cell and the new hyphal tip cell with the remaining two mating-type-distinct nuclei from each other. Once the clamp cell fuses with the subapical cell, the trapped nucleus can migrate into the latter in order to pair with the already resident nucleus which renders also the subapical cell dikaryotic (Iwasa et al. 1998; Badalyan et al. 2004; Schubert et al. 2006; Raudaskoski 2015).

[1]Department of Molecular Wood Biotechnology and Technical Mycology, Büsgen-Institute, University of Göttingen, Göttingen, Germany; e-mail: ukuees@gwdg.de
[2]Laboratory of Fungal Biology and Biotechnology, Yerevan State University, Yerevan, Armenia

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

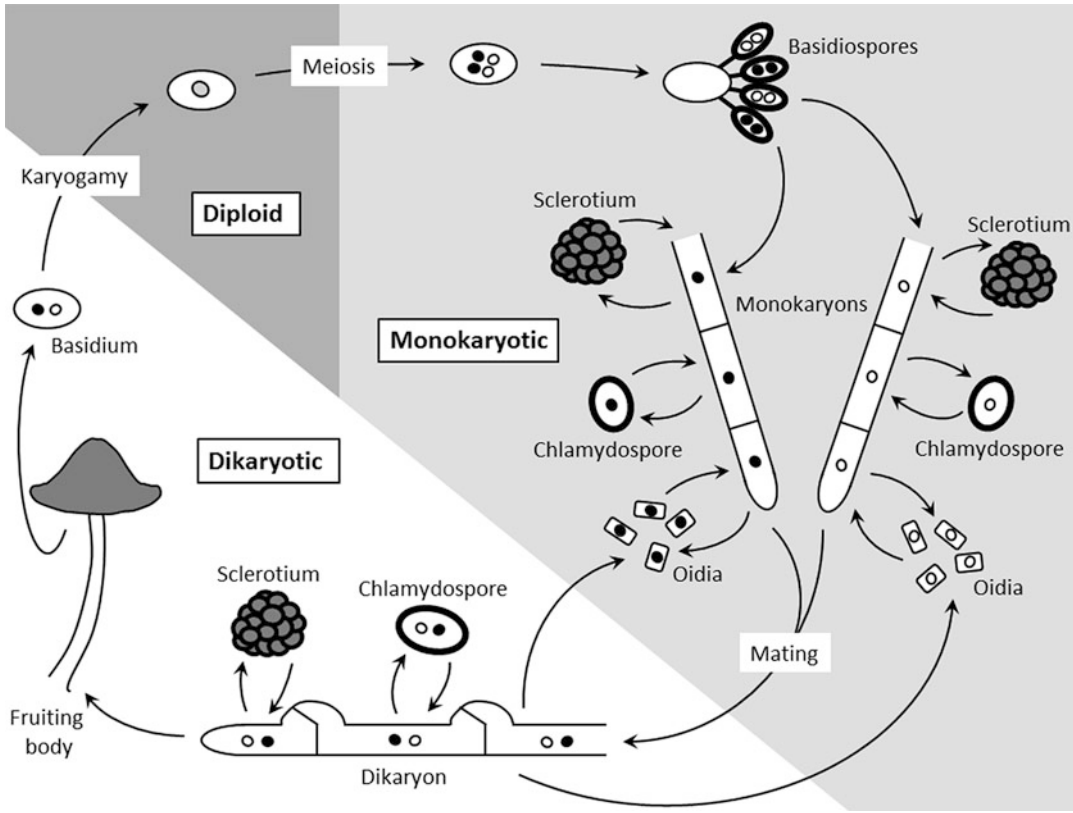


Fig. 12.1 Life cycle of the model fungus *Coprinopsis cinerea* (after Kües (2000) and Kües et al. (2002a)). Haploid meiotic basidiospores (binucleate by a postmeiotic mitosis) germinate into monokaryotic mycelia which abundantly produce uninucleate haploid oidia in the aerial mycelium. When aging, they may also produce thick-walled chlamydospores in submerged mycelium and sclerotia (multicellular brown-stained resting bodies with a melanised outer rind and an inner medulla of chlamydospore-like cells) in aerial and submerged mycelium. All these structures may germinate again into monokaryotic mycelia. When different in mating type (indicated by the *white* and *black* nuclei in the cells), monokaryons may after fusion form a dikaryon with clamp cells at hyphal septa and with two distinct nuclei in the hyphal compartments, each one per parental monokaryon. On the dikaryon, production of uninucleate haploid oidia is light induced. Also aging dikaryotic mycelia give rise to sclerotia and chlamydospores which are dikaryotic and may germinate again into dikaryotic mycelia. On the dikaryon under distinct environmental conditions (at 25–28 °C, under a day-night light-dark rhythm) fruiting bodies can develop. In the basidia, specific cells formed at the surface of the gills, the two haploid nuclei of distinct mating types will fuse by karyogamy into a diploid nucleus, and meiosis follows directly. The four haploid meiotic products will individually migrate into four basidiospores that bud off from sterigmata on the apex of the basidium. A postmeiotic mitosis follows in the basidia

However, there are also species in the Agaricomycetes with **plurinucleate heterokaryotic hyphal cells** which act less regular in synchrony of nuclear divisions and in the formation of clamp cells at hyphal septa (Boidin 1971; Butler 1972; Stenlid and Rayner 1991; Korhonen and Stenlid 1998; Rajchenberg 2011). **Fruiting bodies** can form on the secondary mycelia, given the right environmental conditions (Kües 2000; Kües and Navarro-Gonzaléz 2015; Pelkmans et al. 2016). Within the fruiting bodies in the specialised **basidia**, nuclear fusion and meiosis occur, followed by the production of four sexual **basidiospores** (**meiospores**), thereby closing the **sexual cycle** (Fig. 12.1; Kües 2000; Kües et al. 2004; Navarro-Gonzaléz 2008).

In addition to the meiotic basidiospores within the fruiting bodies, Agaricomycetes

might also form various **mitotic types of spores (mitospores)**, thus **asexual** or **vegetative spores**, in **subsidiary reproductive cycles** on the monokaryon and/or on the dikaryon, respectively (Fig. 12.1; Kües 2000; Kües et al. 2002a). There are a number of seminal early reports on asexual sporulation in Agaricomycetes (Brefeld 1877, 1889; Falck 1902; Brodie 1936). In spite of this, asexual spore formation in Agaricomycetes has mostly been neglected in research. Only few systematic studies on asexual sporulation in this subphylum are known (Walther et al. 2005). Nobles (1948) and Stalpers (1978, 1984) included characters of asexual spores in cultures as **taxonomic criteria** for wood-inhabiting non-gilled species (in the outdated artificial order of Aphyllophorales). Kendrick and Watling then draw stronger attention through 'The Whole Fungus' concept on the importance of mitotic spores produced by Agaricomycetes. These authors published a first longer literature compilation on the occasional reports of asexual sporulation by Agaricomycetes, with ~100 species of Agaricales and several hundreds of non-gilled species where occurrence of one or more types of asexual spores was noted (Kendrick and Watling 1979; Watling 1979). Clémençon (1997, 2004, 2012) in his meticulous books on anatomy of Hymenomycetes (largely corresponding to the Agaricomycetes; Hibbett et al. 2007) compiled different types of mitotic spores and **spore production modes**, whereas Walther et al. gave overviews on asexual sporulation in groups of Agaricales for taxonomic purposes (Walther and Weiß 2006, 2008; Walther et al. 2005). Other collections on occurrence of asexual spores in Agaricomycetes were presented by Buchalo and colleagues (Buchalo 1988; Buchalo et al. 2009, 2011a, b) who listed in publications in Russian and English language in total 136 different mitospore-forming species from the Agaricales, Auriculariales, Boletales, Hymenochaetales, Phallales, Polyporales and Russulales, respectively. Specifically for 77 species, the authors described types of asexual spores (**arthroconidia, blastoconidia** and **chlamydospores**; see below for definitions) in mono- and dikaryotic cultures. Reshetnikov (1991) also in Russian language gave other extensive lists of 238 species with arthroconidia, 59 with blastoconidia and 244 with chlamydospores, respectively, coming mainly from the orders of Agaricales, Boletales, Hymenochaetales, Polyporales and Russulales. A compilation of selected species known to produce vegetative spores is presented in Table 12.1.

While studies of asexual spores might be driven by interests in fungal biology and for taxonomic purposes, asexual spores can have also important implications in various types of technical applications. The unicellular, uninucleate, haploid asexual spores (**oidia**) of *C. cinerea* (Fig. 12.2) for example allow an efficient protoplast transformation procedure and make *C. cinerea* to the champion filamentous fungus in genetic transformation with several hundreds of transformants obtained per µg DNA (Binninger et al. 1987; Granado et al. 1997; Dörnte and Kües 2012). Uninucleate spores on dikaryons permit further in various species to obtain cells with individual component nuclei, for example for karyotyping and breeding purposes (Walser et al. 2001; Tanesaka et al. 2012; Kim et al. 2014). Asexual spores can serve in replica plating of fungal colonies for efficient genetic screenings (Polak et al. 1997b). Furthermore, spore inoculation procedures have been adopted for fermentations with medicinal species such as *Ganoderma lucidum* (Xu et al. 2012) and *Antrodia camphorata* (syn. *Taiwanofungus camphoratus*; Geng et al. 2013; Hu et al. 2014; Lu et al. 2014) for production of interesting bioactive secondary metabolites. Root inoculation with chlamydospores of the endophytic *Piriformospora indica* can protect host plants from root rot caused by fungal pathogens and can promote plant growth (Franken and Varma 2000; Waller et al. 2005; Harrach et al. 2013). *P. indica* chlamydospores can be produced in liquid culture (Kumar et al. 2012), have a long shelf-life and formulations for commercial applications have been developed (Tripathi et al. 2015).

Asexual spores of *Phlebiopsis gigantea* in traded registered products (Rotstop^{F}, Rotstop^{S}, PG Suspension, PG IBL) are of high economic impact in a number of North-European countries in successful biological control of *Heterobasidion* conifer root and butt rot (Asiegbu et al. 2005; Kües et al. 2007; Malecka et al. 2012). *P. gigantea* is a fast pioneer colonizer of wood (Hori et al. 2014) why commercial *Phlebiopsis* formulations are applied in high spore densities to stump surfaces in commercial forest thinning in order to block any entry into the fresh cut by airborne germinating spores of *Heterobasidion* and to subsequently avoid the spread of the pathogen from infected stumps via root contacts into living trees (Asiegbu et al. 2005; Kües et al. 2007; Malecka et al. 2012). Similar products against *Heterobasidion* infections are in tests for potential registration in other countries in order to stop the enormous yearly economic losses in the forests through

Table 12.1 Selected examples of asexual sporulation in species of Agaricomycetes to demonstrate the wide distribution of different modes of asexual sporulation and types of spores in primary and secondary mycelia and in connection with differentiated fungal structures*

Order	Family	Species	Spore type	Comments	References
Incertae sedis	Undefined	*Cenangiomyces luteus*	Holoblastic, 0–2 × septated conidia with apical appendages	Conidiomata with hyaline branched septated conidiophores; conidiogenous cells with clamp cells; conidia appear to be simple extensions of conidiogenous hypha from which they separate at basal septum; conidiogenous hypha may proliferate further spores through conidial scar or remaining portion of clamp cell	Dyko and Sutton (1979)
		Corticomyces xenasmatoides	Holoblastic conidia	Resupinate conidiomata without conidiophores; undifferentiated blastic conidiogenous cells; rhexolytic secession of terminal spores (6–9 × 4–7 μm)	Romero et al. (1989)
		Fibulotaeniella canadensis	Holoblastic binucleate conidia	Conidiophores in densely packed conidiomata or single conidiophores; conidiogenous cells clamped and conidium prior to release; sigmoid conidia (47–85 × 2–3 μm) sometimes with remains of clamp at base; conidia aggregate in slimy drops	Marvanová and Bärlocher (1988)
		Glutinoagger fibulatus	Holoblastic aseptate conidia	Sporodochial conidiomata; branched hyaline, septate conidiophores; conidiogenous cells with clamp connections sympodially proliferating; ellipsoid terminal conidia (10–17 × 5.5–8 μm) with persistent hyphal appendage (from subtending hypha) and thick mucilagineous shield; rhexolytic mode of spore release	Sivanesan and Watling (1980)
			Intercalary mitospores	Chlamydospore-like cells formed in conidiogenous hyphae or their parental hyphae, adjacent to each other or with collapsing cells in between	

		Pycnovellomyces foliicola	Holoblastic conidia with appendages	Pycnidoid conidiomata on irregularly branched hyphae with camp cells; conidiophores arising in basal cushion; holoblastic formation of cylindrical conidia with spore secession occurring at clamp cells; ends of conidia (9–22 × 2.5–3 μm) initially angular before rounding off, with 2–3 tubular appendages at apical end and 1–2 at basal end of spore	Nag Raj et al. (1989)
Agaricales	Agaricaceae	*Agaricus arvensis*	Oidia	Binucleate arthrospores (3–4 μm × 15 μm) in dikaryons formed in chains of 3 or more	Molitoris et al. (1996)
		Agaricus bisporus	Chlamydospores	Thick-walled chlamydospores in dikaryotic hyphae	
		Agaricus semotus	Arthroconidia	Pre-existing hyphae split into swollen spores	Walther et al. (2005)
		Cystoderma cinnabarinum	Arthroconidia	In dikaryotic mycelium by dissection of undifferentiated clamped hyphae	Pantidou et al. (1983)
		Cystoderma granulosum	Arthroconidia	In dikaryotic mycelium by dissection of undifferentiated clamped hyphae	
		Coprinus comatus	Conidia	Uni- or sometimes binucleate spores formed on monokaryotic mycelia without differentiated conidiophores; probably arthrospores	Kemp (1975), Walther et al. (2005), Badalyan et al. (2011a)
			Chlamydospores	Intercalary and terminal swellings and thick-walled cells within dikaryotic mycelium	
		Coprinus sterquilinus	Arthrospores	Formed in chains by septation of monokaryotic aerial hyphae	Kemp (1975)
			Spherical spores	On specialized hyphal branches	
	Amanitaceae	*Limacella illinita*	Arthroconidia	Produced in schizolytic mode from dikaryotic hyphae; dry	Buchalo (1988), Hutchison (1988)
	Fistulinaceae	*Fistulina hepatica*	Blastoconidia	Formed in short chains on simple structured conidiophores	Stalpers and Vlug (1983), Buchalo et al. (1985, 2011a, b)
			Chlamydospores	Thick-walled, large swollen terminal and intercalary lemon-shaped chlamydospores in dikaryons	
	Hydnangiaceae	*Laccaria laccata*	Chlamydospores	Abundant in dikaryotic mycelium	Pantidou et al. (1983)
			Arthrospores	Rare in dikaryotic mycelium	

(continued)

Table 12.1 (continued)

Order	Family	Species	Spore type	Comments	References
	Inocybaceae	*Tubaria furfuracea*	Oidia	Formed on monokaryons from curled oidiophores, by protoplasm portioning, vacuolation, cell wall formation and rhexolytic release	Ingold (1983)
	Lyophyllaceae	*Nyctalis lycoperdoides*	Chlamydospores	Chains of dry ornamented thick-walled chlamydospores formed within dikaryotic hyphae; spores fall apart from each other at clamp cell connections in a manner resembling arthrospore formation	Buchalo et al. (1985), Sivanesan and Watling (1980)
	Marasmiaceae	*Baeospora myosura*	Blastoconidia	Formed sympodially on sporophores; schizolytic release	Kendrick and Watling (1979), Walther et al. (2005)
		Marasmius puerariae	Oidia	Clampless conidiophores in synnemata; dry oidia (4–8 × 2–2.5 μm) develop in chains from spirally twisted branches by protoplasm portioning, vacuolation, cell wall formation and rhexolytic release	Kirschner et al. (2013)
		Marasmius oreades	Chlamydospores	Formed secondarily in basidiospores or in inflating germtubes under starvation conditions	Ingold (1988)
	Physalacriaceae	*Flammulina velutipes*	Arthrospores	Uninucleate arthroconidia formed by splitting of oidial branches in aerial mycelium of monokaryons and dikaryons; rhexolytic mode of spore liberation	Brodie (1936), Nobles (1948), Kemp (1980), Ingold (1980), Walther et al. (2005), Badalyan et al. (2006), Badalyan and Kües (2015)
			Chlamydospores	Within aging dikaryotic mycelium	
	Pleurotaceae	*Hohenbuehelia izonetae*	Blastoconidia	Produced on short denticles on clamped cells; 12.0–16.8 × 3.2–4.8 μm	Cooke and Godfrey (1964), Koziak et al. (2007a)
			Chlamydospores	Warted, ovoid; 8.8–12.8 × 4.8–7.2 μm	
		Hohenbuehelia petaloides	Blastoconidia	Single-celled, elliptical; 9–16 × 3.5–4.5 μm; formed on short denticles	Thorn and Barron (1986)
		Nematoctonus concurrens	Blastoconidia	Formed on short, conical sterigmata on clamped hyphae; colorless, cylindrical to ellipsoidal; 10–23 × 3.6–5.6 μm	Drechsler (1949)

	Nemactoctonus haptocladus	Blastoconidia	Formed on denticles on clamped hyphae; colorless, cylindrical to ellipsoidal; 11–18 × 3.3–4.5 μm	Drechsler (1946)
	Nemactoctonus tylosporus	Blastoconidia	Produced on short denticles on clamped cells; 11.2–18.4 × 1.6–3.2 μm	Drechsler (1941), Cooke and Godfrey (1964), Koziak et al. (2007a)
		Chlamydospores	Warted or smooth, ovoid or almond-shaped; 5.6–10.4 × 2.4–4.0 μm	
	Pleurotus cystidiosus	Arthrospores	Blastic conidia (3–5 μm) on simple coniodiophores resembling sterigmata; conidiophores assembled into coremia on stipes of fruiting bodies and in mono- and dikaryotic mycelia; spores on the dikaryon are dikaryotic	Šašek et al. (1986), Buchalo (1988), Stalpers et al. (1991), Esser (2000), Truong et al. (2006), Clémençon (2012)
Psathyrellacae	*Coprinellus congregatus*	Oidia	Formed on oidiophores but do not coalesce in liquid droplets	Kemp (1975)
	Coprinellus domesticus	Oidia	Formed in groups on specific aerial hyphae short oidiophores (type: *Hormographiella verticillata*) but do not coalesce in liquid droplets (dry), one oidium (13–22 × 3–4 μm) per oidial hyphae; observed on fruiting-competent mycelium	Kemp (1975), Cáceres et al. (2006), Badalyan et al. (2011a)
	Coprinellus ellisii	Oidia	Formed on aerial hyphae with multiple branched oidiophores; one oidium per oidial hyphae; observed on fruiting-competent mycelium	Badalyan et al. (2011a)
	Coprinellus micaceus	Oidia	Formed in chains by septation of monokaryotic aerial hyphae; dry	Watling (1972) cited in Kemp (1975)
	Coprinellus radians	Oidia	Rare; observed on fruiting-competent mycelium with rare pseudoclamps	Badalyan et al. (2011a)
	Coprinellus aff. *radians*	Oidia	Formed on aerial hyphae with multiple branched oidiophores; observed on fruiting-competent mycelium with rare pseudoclamps	
	Coprinellus xanthothrix	Oidia	Formed on specific aerial hyphae with short oidiophores; one oidium per oidial hyphae; on mycelium with rare pseudoclamps	

(continued)

Table 12.1 (continued)

Order	Family	Species	Spore type	Comments	References
		Coprinopsis cinerea	Oidia	Constitutive on specific oidiophores in the aerial mycelium of monokaryons, light-induced on the dikaryon; oidiophores simple structured or branched; mostly two oidia are formed per oidial hyphae; collected in liquid droplets (wet); anamorphic name: *Hormographiella aspergillata*	Brodie (1931, 1932), Polak et al. (1997a, 2001), Kertesz-Chaloupková et al. (1998), Kües et al. (1998a, 2002a, b), Kües (2000), Yoon (2004), Badalyan et al. (2011a)
			Chlamydospores	Some in submerged mycelium of aging monokaryons, in masses in aging dikaryons; dikaryotic when formed on the dikaryon	
			Blastocysts	Occasional within monokaryotic and in clamped mycelia	
		Coprinopsis gonophylla	Chlamydospores	In dikaryotic mycelium, round-oval, thick-walled	Badalyan et al. (2011a)
		Coprinopsis macrocephala	Oidia	Wet oidia released into liquid droplets at tips of oidiophores; only on monokaryons	Kemp (1975)
		Coprinopsis radiata	Oidia	Wet oidia released into liquid droplets at tips of oidiophores; only on monokaryons	
		Coprinopsis scobicola	Oidia	Wet oidia released into liquid droplets at tips of oidiophores; only on monokaryons	
		Coprinopsis strossmayeri	Chlamydopores	In dikaryotic mycelium; round-oval, thick-walled	Badalyan et al. (2011a, b)
		Coprinopsis tripsora	Dry oidia	Formed in chains by septation of monokaryotic aerial hyphae	Watling (1972) cited in Kemp (1975)
		Gymnopilus junonius	Arthroconidia	Generated from undifferentiated hyphae	Walther et al. (2005)
	Pterulaceae	*Pterula subulata*	Oidia	Arthrospores (4–5 × 9–15 μm) formed within clavaroid basidiomes as integral part of the fruiting bodies as well as on tips of hyphal branches in culture	Cripps and Caesar (1998)

Schizophyllaceae	*Schizophyllum commune*	Chlamydospores	Single or chain-like clusters of thick-walled chlamydospores in monokaryotic and dikaryotic mycelia; intercalary and terminal; 4–20 × 4–8 μm	Nobles (1948), van der Westhuizen (1958), Koltin et al. (1973), Peddireddi (2008), Badalyan and Kües (2015)
Strophariaceae	*Gymnopilus junonius*	Blastoconidia	Singly bud off from hyphal cells, with thickened cell walls	Jacobsson (1985), Walther et al. (2005)
	Hemipholiota heteroclita	Oidia	Produced on short branches in terminal or lateral brush-like clusters	Jacobsson (1985)
		Chlamydospores	Occasional intercalary or terminal swellings	
	Hypholoma fasciculare	Arthrospores	Straight or curved; 2.5–9.5 × 1.0–2.6 μm; formed on coiled conidiogenous hyphae, splitting of dikaryotic hyphae	Brefeld (1889), Garibova et al. (1986), Walther and Weiß (2008)
		Chlamydospores	Intercalary or terminal on single short sidebranches or on multiply branched short sidehyphae	
	Pholiota adiposa	Oidia	Produced on highly ramnified sidebranches; in dikaryons	Nobles (1948), Arita (1979), Buchalo et al. (1985), Jacobsson (1985)
		Chlamydospores	Terminal chlamydospores on short side branches of dikaryotic mycelium	
	Pholiota alnicola	Chlamydospores	Produced in terminal or intercalary chains of 2–3 swollen spores without protoplasmic contraction; production of similar swollen cells as outgrowth on sides of hyphae in a 'blastic' mode; swellings occur anywhere in mycelium but preferentially submerged	Jacobsson (1985), Walther et al. (2005), Walther and Weiß (2008), Badalyan and Kües (2015)
	Pholiota aurivella	Oidia	Produced on sympodially proliferating short sidebranches (conidiophores) in mono- and dikaryons; arthroconidia from dikaryons binucleate	Martens and Vandendries (1933), Brodie (1936), Reshetnikov (1982), Walther and Weiß (2008)

(continued)

Table 12.1 (continued)

Order	Family	Species	Spore type	Comments	References
		Pholiota gummosa	Oidia	Produced in monokaryons and dikaryons on simple lateral branches or on sympodially proliferating short sidebranches (conidiophores); arthroconidia from dikaryons binucleate	Jacobsson (1985), Walther et al. (2005), Walther and Weiß (2008)
			Chlamydospores	Terminal, oil-rich swellings, single or in chains	
		Pholiota highlandensis	Chlamydospores	Occasional intercalary swellings	Jacobsson (1985)
		Pholiota jahnii	Oidia	Aerial arthroconidia production as in *P. microspora*	
			Chlamydospores	Large terminal chlamydospores at sidebranches, rare; old hyphae are easily fragmented	
		Pholiota lenta	Oidia	Produced on termini of main hyphae or on slightly branched shorter sidebranches	Jacobsson (1985), Walther et al. (2005), Walther and Weiß (2008)
		Pholiota microspora (*nameko*)	Oidia	Thallic mostly uninucleate dry oidia by splitting aerial hyphae of monokaryons and dikaryons (4.9–5.2 × 1.7–1.9 μm); wet oidia in submerged mycelium	Arita (1979), Hui et al. (1999a)
		Pholiota squarrosa	Oidia	Aerial arthroconidia production as in *P. microspora* by dissection of longer hyphal branches; rarely from sympodially proliferating sidebranches	Jacobsson (1985), Walther et al. (2005), Walther and Weiß (2008)
			Chlamydospores	Terminal chlamydospores common in submerged mycelium, in dikaryons	
		Pholiota squarrosoides	Oidia	Aerial arthroconidia production as in *P. microspora* by dissection of hyphal sidebranches; spores frequently curved	Jacobsson (1985)
			Chlamydospores	Inconspicuous, probably intercalary derived or from blastic outgrowth at sides of hyphae	

		Pholiota tuberculosa	Blastoconidia (chlamydospores)	No typical arthrospore production; sporulation in monokaryons and dikaryons on short sidebranches with sympodially arising coiling conidiogenous hyphae that split into 2 or 3 strongly curved or irregularly shaped spores (6–11.5 × 2–3.5 µm); bladder-like spores swell prior to dissection and mature acropetal; single or concentrated in sporodochia	Jacobsson (1985), Walther et al. (2005), Walther and Weiß (2008)
		Psilocybe cyanescens	Oidia	Formed in monokaryons by splitting groups of curled oidial branches by vacuolation, portioning into protoplasts, septum formation and rhexolytic release; spores curved of variable length	This paper
		Psilocybe merdaria	Oidia	Formed on mono- and dikaryons; spores on dikaryons generated from clamped cells are uninucleate	Watling (1971), Valenzuela and Garnica (2000)
	Tricholomataceae	*Hemimycena conidiogena*	Thick-walled blastospores	On the surface of caps and stipes of fruiting bodies	Moreau et al. (2005)
	Typhulaceae	*Clavaria micans*	Anneloconidia	Spores bud off in blastic mode from a tip of a conidiophore with leaving rings behind at the place of spore liberation	Koske and Perrin (1971), Clémençon (1997, 2000, 2012)
		Clavaria quisquiliaris	Blastoconidia	A first blastoconidium is formed on a conidiogenous hyphal tip with further spores budding lateral from beneath; younger spores push the upper spores away	Clémençon (1997, 2004)
		Typhula incarnata	Blastoconidia	Generated in the same manner as in *C. quisquiliaris*	Remsberg (1940), Metzler (1987a)
		Typhula trifolii	Oidia	In tufts on monokaryons; 3.6 × 0.8 µm	Noble (1937)
Atheliales	Atheliaceae	*Taeniospora nasifera*	Holoblastic single-branched conidia	Simple or branched conidiophores with no clamps; conidiogenous cell with or without clamps; spore proliferations sympodial; 2-celled conidia with single branch and clamp	Marvanová and Bärlocher (1988)
Boletales	Coniophoraceae	*Coniophora puteana*	Oidia	Produced in some strains in tufts; 2.2–6.0 µm	Nobles (1948), Stalpers (1978, 1984)

(continued)

Table 12.1 (continued)

Order	Family	Species	Spore type	Comments	References
Cantharellales	Botryobasidiaceae	*Botryobasidium baicalinum*	Round chlamydospores	Formed in fruiting bodies; 13–16 μm in Ø; filled with yellowish oily contents	Kotiranta and Ryvarden (2007)
		Botryobasidium conspersum	Blastoconidia	Single spores born on denticles	Clémençon (1997, 2004)
		Botryobasidium simile	Blastoconidia	Formed in acropetal chains without denticles	
		Corticium botryosum	Blastoconidia	Single spores born on denticles	
		Hypochnus coronatus	Blastoconidia	Formed in acropetal chains without denticles	Hughes (1953), Clémençon (1997)
	Hydnaceae	*Hydnum raduloides*	Blastoconidia	Uninucleate mitospores on dikaryotic mycelium; 4.5–9.0 × 1.8–4.5 μm	Maxwell (1954)
Corticiales	Corticiaceae	*Galzinia incrustans*	Blastoconidia	Formed on denticles on monokaryotic and dikaryotic hyphae	Nobles (1937), Clémençon (1997, 2004, 2012)
Gloeophyllales	Gloeophyllaceae	*Gloeophyllum odoratum*	Oidia	Formed by fragmentation of simple hyphae in aerial mycelium; variable in length; uninucleate when on a dikaryon	Nobles (1948), Stalpers (1978)
			Chlamydospores	Numerous terminal and intercalary	
		Gloeophyllum trabeum	Oidia	Formed by fragmentation of nodose hyphae, 2.2–3.5 μm in width	
			Chlamydospores	Numerous in mycelium; ovoid to ellipsoid, intercalary or terminal, 10.5–18.0 × 6.0–10.5 μm	
Hymenochaetales	Rickenellaceae	*Resinicium furfuraceum*	Blastoconidia	Formed in monokaryons and dikaryons on *Spiniger*-type of conidiophores; uninucleate	Maxwell (1954)
Polyporales	Fomitopsidaceae	*Antrodia camphorata* (*Taiwanofungus camphoratus*)	Arthroconidia	Produced on clamped cells; rod-shaped; 2.5–3.5 × 3.0–8.5 μm	Stalpers (1978, 1984), Chang and Chou (1995)
			Chlamydospores	Produced on clamped cells in older cultures; ellipsoid to oblong; 4–7 × 12–19 μm	
		Fomitopsis pinicola	Arthroconidia	Formed by hyphal fragmentation of monokaryotic and dikaryotic hyphae into spores (5–15 × 2–3 μm)	Mukhin and Votintseva (2002), Badalyan et al. (2015)
			Blastoconidia	Single blastospores (6–7.5 × 1.1–2.5 μm) grow from monokaryotic hyphae as short side projections	

	Laetiporus sulphureus	Arthrospores	Rhexolytic mode at the dikaryon; often splitting of old conidiophores	Nobles (1948, 1965), Stalpers (1984), Badalyan and Sakeyan (2004), Buchalo et al. (2011b)
		Blastoconidia	On multiply branched conidiophores on the dikaryon; terminal only one spore on each branch; subglobose to ellipsoidal or ovoidal, thin- to thick-walled; 6–10 × 5–8 μm	
		Chlamydospores	Terminal or intercalary; 12.0–19.5 × 7.5–13 μm	
	Sporotrichum sporodochiale	Holoblastic aseptate conidia	Conidial development reminiscent of *G. fibulatus*	von Arx (1971), Sivanesan and Watling (1980)
Ganodermataceae	*Ganoderma adspersum*	Chlamydospores	On dikaryotic mycelium; oval-round; 4.4–5.3 × 6.0–9.8 μm	Badalyan et al. (2015)
	Ganoderma lucidum	Chlamydospores	Formed within fruiting bodies as gasterospores, ovoid, verrucous, brown; on dikaryotic mycelium; ellipsoid, hyaline, smooth; 4.3–5.1 × 4.5–10.2 μm	Nobles (1948), Banerjee and Sarkar (1959), Adaskaveg and Gilbertson (1986, 1989), Badalyan et al. (2015)
		Oidia	In dikaryotic mycelium; cylindrical; 3.4–5.1 × 7.8–15.6 μm	
	Tomophagus colossus	Chlamydospores	Formed within fruiting bodies (gasterospores); in dikaryotic mycelium, spherical, pigmented, and ornamented with warty projections	Stalpers (1978), Adaskaveg and Gilbertson (1989), Parihar et al. (2013)
Meruliaceae	*Ceriporia metamorphosa*	Holoblastic aseptate conidia	Conidial development reminiscent of *G. fibulatus*	Sivanesan and Watling (1980)
	Phlebia radiata	Oidia	Generated as terminal or intercalary arthroconidia in hyphae by schizolytic secession of septa	Stalpers (1978, 1984)
Phanerochaetaceae	*Phanerochaete chrysosporium*	Arthroconidia	Hyaline, cylindrical or irregular, thin-walled	Stalpers (1978, 1984); de Koker et al. (2000)
		Blastoconidia	Terminal on simple or mostly on branched conidiophores; ellipsoidal or ovoid pyriform; thick-walled; 6–10 × 5–8.5 μm	
		Chlamydospores	Terminal or intercalary; abundant in different culture media; 11–60 μm in diameter; thick-walled	

(continued)

Table 12.1 (continued)

Order	Family	Species	Spore type	Comments	References
		Phanerochaete pseudomagnoliae	Chlamydospores	Scarce in MEA cultures, many in liquid xylose medium	de Koker et al. (2000)
			Conidiospores	On xylose medium	
		Phlebiopsis gigantea	Oidia	Generated as terminal or intercalary arthroconidia in hyphae by schizolytic secession of septa	Nobles (1948), Stalpers (1978, 1984)
	Polyporaceae	*Fomes fomentarius*	Arthroconidia	Hyphal fragmentation of monokaryotic and dikaryotic hyphae into spores (5–27 × 2–3 μm) by portioning of protoplasm, septum formation and splitting	Mukhin and Votintseva (2002), Badalyan et al. (2015)
			Blastoconidia	Single blastospores (6–7.5 × 1–3 μm) budding off from monokaryotic mycelia as short side projections	
			Chlamydospores	Secondarily formed within germinating basidiospores	
		Perenniporia dipterocarpicola	Chlamydospores	Abundant on clamped mycelium; 6–13 × 3.5–7 μm	Hattori and Lee (1999)
		Perenniporia hexagonoides	Chlamydospores	Scattered in clamped mycelium; 8–10.5 × 5–7 μm	
		Polyporus badius	Thallic arthroconidia	Produced by splitting of oidial branches in aerial mycelium of monokaryons; unknown whether dikaryons can form spores	Ingold (1986)
		Polyporus squamosus	Thallic arthroconidia	Arthroconidia by successive protoplasmic retraction and rhexolytic splitting of aerial hyphae of monokaryons and dikaryons; arthrospores from a dikaryon are dikaryotic	
		Polyporus varius	Oidia	By splitting undifferentiated but length restricted dikaryotic hyphae with clamped and non-clamped septa; dry spores; variable in size (8–25 × 2.5–6.6 μm); mostly uninucleate; process on monokaryons similar with slightly smaller spores (5–22 × 2.4 μm)	Nobles (1948), Stalpers (1978), Ingold (1991)

		Pycnoporus sanguineus	Oidia	Formed by fragmentation of nodose-septate clamped hyphae; clamps disintegrate to release spores; 3.5–6.3 × 1.4–2.8 µm	Nobles (1948), Stalpers (1978)
			Chlamydospores	Intercalary and terminal in dikaryons; 6.3–11.9 × 4.9–8.4 µm	
		Trametes hirsuta	Oidia	Formed in chains of four of five; uninucleate on monokaryotic and uni- and binucleate on dikaryotic hyphae	Bose (1943), Tsukamoto et al. (2003)
Russulales	Amylosteraceae	*Amylostereum areolatum*	Oidia	Dikaryotic mycelium in culture and in mycangia splits into arthrospores consisting of one to four clamped cells	Talbot (1977), Slippers et al. (2003), Wermelinger and Thomsen (2012)
		Amylostereum chailletii	Chlamydospores	Dikaryotic mycelium splits only in mycangia into arthrospores of variable length	
	Auriscalpiaceae	*Lentinellus castoreus*	Chlamydospores	Rare in dikaryotic mycelium; terminal or intercalary; 6–11 × 4–7.5 µm	Miller and Metheven (2000)
		Lentinellus ursinus	Blastoconidia	Abundant in clamped mycelium; mostly terminal formed; thick-walled; 4–9.5 × 4–6.3 µm	
	Bondarzewiaceae	*Heterobasidium annosum* s.l.	Blastoconidia	Produced by budding on sterigmata on the terminal vesicle of long aerial club-like conidiophores (*Spiniger*-type; 300 µm); mostly binucleate; on homokaryons homokaryotic spores, on heterokaryons homo- and heterokaryotic spores; smooth; 4.5–8.0 × 3.0–6.0 µm	Brefeld (1889), Griffin and Wilson (1967), Stalpers (1974), Ahrberg (1975), Hsiang et al. (1988), Stenlid (1985), Korhonen and Stenlid (1998), Tokuda et al. (2009), Paul et al. (2012)
	Lachnocladiaceae	*Dichostereum effuscatum*	Blastoconidia	Formed on *Spiniger*-type of conidiophores	Nobles (1942)
		Dichostereum granulosum	Blastoconidia	Formed in monokaryons and dikaryons on *Spiniger*-type of conidiophores; uninucleate	Maxwell (1954)
	Peniophoraceae	*Hyphoderma mutatum*	Blastoconidia	Formed on *Spiniger*-type of conidiophores	Nobles (1935)

(continued)

Table 12.1 (continued)

Order	Family	Species	Spore type	Comments	References
	Stereaceae	*Laurilia sulcata*	Blastoconidia	Formed in monokaryons and dikaryons on *Spiniger*-type of conidiophores; uninucleate	Maxwell (1954)

[a]Note that neither the species list is complete (see the text and cited literature for further examples) nor should the table suggest for all species mentioned that there might not be other types of mitospores on one or both types of mycelia or on any other developmental structure. Often, only one type of mycelium might have been analyzed (not always clearly indicated in the publications whether being primary or secondary mycelium). Also, from the dispersed reports in the literature it is difficult to judge where observations are complete for all stages in a fungal life cycle. Spore types are assigned to descriptions within respective publications and according to the definitions given in the text. However, naming of spore types may not always be definitive and might be corrected upon further observations and more defined developmental descriptions

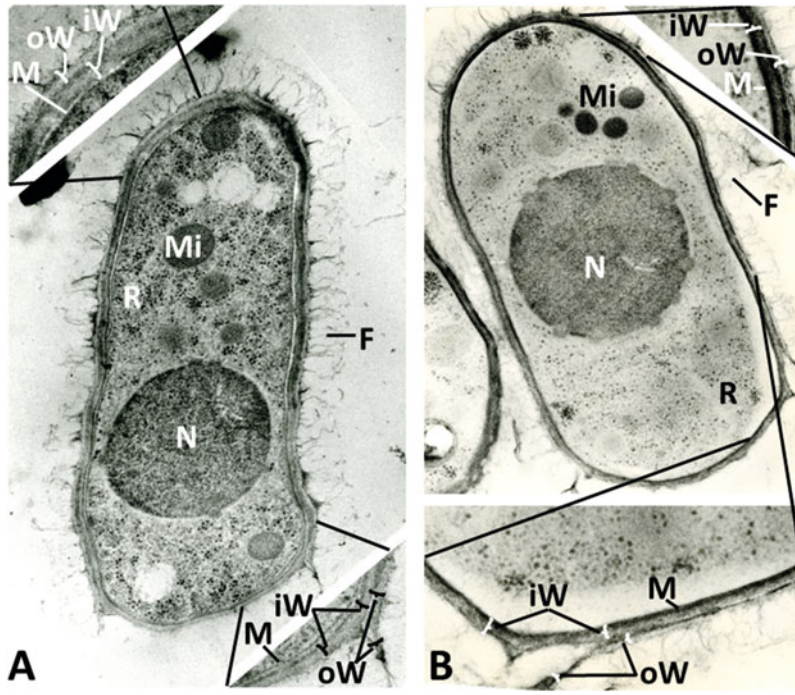


Fig. 12.2 (**a, b**) Electron microscope picture of apical wet oidia of *Coprinopsis cinerea* strain AmutBmut. The apical tip and the side walls of the spores are covered by double-layered cell walls including an outer fimbriae-like proteineous structure. The flat ends of the spores as former places of cell-cell attachment possess only a single-layered cell wall and lack the fimbrial structures indicating a schizolytic mode of spore release. Note in (**b**) the somewhat splitted double-layered cell wall of the side wall at the lower left end of the spore. Abbreviations: *F* fimbriae, *iW* inner cell wall layer, *M* membrane, *Mi* mitochondrion, *N* nucleus, *oW* outer cell wall layer, *R* ribosomes. Experimental details for sample preparation and photographing were described in Polak et al. (1997a) (Original photos by R. Hermann)

Heterobasidion wood decay (Kües et al. 2007; Dumas and Laflamme 2013) and various studies target on finding and establishing similarly successful biological control agents against various other serious fungal tree pathogens (Kües et al. 2007; Mohammed et al. 2014).

II. Types of Asexual Spores

Generally, asexual fungal spores may be produced by **thallic spore formation** through dissection of pre-existing hyphae into single cells or generated externally from a **sporogenous cell** (**exogenous spores, exospores**) by **blastic sporogenesis** (**blastospores**) or internally (**endogenous spores, endospores**) newly within **spore mother cells** (Watling 1979; Esser 2000; Fischer and Kües 2006). All three basic principles of asexual sporulation are known in the Agaricomycetes (Kendrick and Watling 1979; Clémençon 1997, 2004, 2012; Walther et al. 2005; Table 12.1). However, sporulation may further include production of specific **sporophores** on **undifferentiated vegetative hyphae**, and there are modes of spore formation that combine steps from these different basic processes of mitotic spore generation (Kües et al. 2002a). Differentiated sporophores may be free-standing as the **oidiophores** in *C. cinerea* (Polak et al. 1997a, 2001). In other species, sporogenous hyphae may group into clusters such as in the erect, brush-like ***Spiniger*-type** of *Heterobasidion* species (Brefeld 1889; Stalpers 1974). Or, they may assemble in **conidiomata** (multi-hyphal, conidia-bearing structures) such as in the **coremia** of *Dendrocollybia racemosa* (known in the literature as anamorph *Tilachlidiopsis racemosa*; Watling 1979; Stalpers et al. 1991) and in the coremia of *Pleurotus cystidiosus* (Buchalo 1988; Esser 2000; Truong et al. 2006), alternatively called **synnemata** (large erect structures bearing

compact **conidiophores** that aggregate together into a plectenchyma to form a type of stalk) and described for *P. cystidiosus* under an anamorphic name *Antromycopsis macrocarpa* (Stalpers et al. 1991). **Sporodochia** are cushion-like stromata with conidiophores and pseudoparenchyma found, for instance, in the **mitosporic** basidiomycetes *Glutinoagger fibulatus* (Sivanesan and Watling 1980) and *Sporotrichum sporodochiale* (von Arx 1971; Sivanesan and Watling 1980) and in *Termitomyces* species (Botha and Eicker 1991). Also, **conidiogenous hyphae** may not only be found in the vegetative mycelium but may also be part of fruiting bodies such as in the clavaroid basidiomes of *Pterula subulata* on which both sexual and asexual spores are formed (Cripps and Caesar 1998). *Arthrosporella ditopa* may form pileus-free clavaroid structures (described as anamorph *Nothocluvulina ditopa*) that bear **arthrospores** on their surface as do the stipes of fully developed mushrooms with pileus and basidiospores (Singer 1986; Stalpers et al. 1991). Arthrospores are also typical for cortical layers of caps and stipes of certain *Cystoderma* (Harmaja 1979; Saar 2003; Saar et al. 2009) and *Arthromyces* species (Baroni et al. 2007) and appear on the spines of basidiocarps of *Echinoporia hydnophora* (Kirschner and Wu 2005). The surface of the pileus of *Blastosporella zonata* in contrast is covered with age by blastospores born from conidial heads (Baroni et al. 2007), as are caps and stipes of *Hemimycena conidiogena* with thick-walled propagules (Moreau et al. 2005) and can be wetted basidiocarps of *Heterobasidion* species (Brefeld 1889). The mycoparasite *Squamanita odorata* produces large amounts of chlamydospore in galls ('cecidiocarps') which are formed when the fungus infects basidiocarps of other species (Bas 1965; Bas and Thoen 1998; Redhead et al. 1994). Furthermore, pileus tissues and gills, respectively, of the mycoparasites *Asterophora* (*Nyctalis*) *lycoperdoides* and *Asterophora (Nyctalis) parasitica* convert into large masses of chlamydospores (Buller 1924). Further examples of asexual spore formation linked to fruiting bodies are given in Hibbett and Thorn (2001) although basidiomes in most species are missing any such structures (Pantidou et al. 1983).

Traditionally in mycology, **anamorphs** and **teleomorphs** were distinguished as terms for asexual and sexual reproductive stages of fungi and these were often given different species names (Minnis 2015). By the restrictive interest in asexual stages for the Agaricomycetes and a long tradition by rather not naming anamorphic stages in the Basidiomycota (Seifert and Gams 2001; Kirschner and Oberwinkler 2009), there are not as many anamorphic names around for respective mitospore-producing species of Basidiomycetes as compared to the Ascomycetes (Kendrick and Watling 1979; Hibbett and Thorn 2001; Hyde et al. 2011). Nevertheless, there were some distinct anamorphic genera coined such as *Spiniger* for *Heterobasidion* and others (Brefeld 1889; Stalpers 1974), *Antromycopsis* for *Pleurotus*, the synonyms *Tilachlidiopsis* and *Sclerostilbum* for *Dendrocollybia* (*Collybia*), *Nothoclavulina* for *Arthrosporella* (Stalpers et al. 1991), *Confistulina* for *Fistulina* (Stalpers and Vlug 1983), *Nematoctonus* for *Hohenbuehelia* species (Koziak et al. 2007a, b), *Sporotrichum* among for *Ceriporia*, *Laetiporus*, *Phanerochaete* and *Pycnoporellus* species (Sivanesan and Watling 1980; Stalpers 1978, 1984), *Ovularia* for *Marasmius* (Kirschner et al. 2013), *Pseudohelicomyces* for *Psilocybe* (Valenzuela and Garnica 2000), *Hormographiella* for *Coprinopsis* and *Coprinellus*, respectively (Guarro et al. 1992; Gené et al. 1996; Cáceres et al. 2006; Badalyan et al. 2011a), and *Aegeritina* for *Subulicystidium* (Cooper 2005). In some instances, teleomorphic genera have been assigned to more than one anamorphic genus and some anamorphic genera have been assigned to species from quite distinct teleomorphic genera, both of which complicate taxonomy and understanding of evolutionary relationships within the fungi (Hyde et al. 2011). Moreover, there are also anamorphic genera such as *Cenangiomyces* (Dyko and Sutton 1979), *Chaetospermum* (Tangthirasunum et al. 2014), *Corticomyces* (Romero et al. 1989), *Ellula* (Nag Raj 1980), *Fibulochlamys* (Madrid et al. 2010), *Fibulotaeniella* (Marvanová and Bärlocher 1988), *Glutinoagger* (Sivanesan and Watling 1980), *Helicomyxa* (Kirschner and Chen 2004), *Pycnovellomyces* (Nag Raj et al. 1989), *Taeniospora* (Marvanová and Bärlocher 1988), and some others where the teleomorphs are not known of (Hibbett and Thorn 2001; Hyde et al. 2011). Policy of ICN (International Code of Nomenclature for algae, fungi and plants) for fungal naming by now is a One-Name-Per-Fungus strategy and to avoid as much as possible any double naming for species (Hyde et al. 2011; Minnis 2015).

Within the basidiomycete literature, the nomenclature of distinct spores and modes of spore formation is not too uniform. Walther et al. (2005), for example, used generally the term **conidiogenesis** for various types of asexual sporulation and included formation of any internal chlamydospores. A **conidium** in the sense of these authors is a mitotically produced

cell that becomes detached during ontogeny, independent of the shape and the thickness of the cell wall. **Spore-like swellings** of which release has never been observed are excluded from the definition by these authors.

Conidia as defined in the dictionary of fungi (Kirk et al. 2008) are asexually produced specialized spores that are non-motile, casucous (falling off readily), and not developed by cytoplasmic cleavage or free-cell formation. Kirk et al. (2008) distinguish two basic modes of conidiogenesis (**blastic** and **thallic**) and present conidium ontogenesis further in 43 different schemes. Blastic modes of conidiogenesis are characterized by a marked increase in size of a recognizable conidial initial prior to **septum formation** for spore separation from the conidiogenous cell (any cell from which or within which a conidium is directly produced). Blastic modes are further differentiated into **holoblastic** and **enteroblastic**, depending on whether both the outer and inner cell walls of the conidiogenous cell contribute to the spore cell wall or whether only the inner or no cell wall layer takes part, respectively. In thallic conidiogenesis, cross-walls are first laid to delimit a conidial initial and then the cell may enlarge. In case that the outer wall of the sporogenous cell is not involved in formation of the spore cell wall, such process is called **enterothallic**.

Highly relevant to the Agaricomycetes is further the term chlamydospores which are 1-celled endogenous spores primarily generated for perennation within pre-existing hyphal cells by contradiction of the hyphal protoplast and formation of an inner secondary, often thickened cell wall which is usually impregnated with hydrophobic material and may be pigmented (Kirk et al. 2008).

Clémençon (1997, 2004, 2012) in his books distinguished spores on the base of thallic and blastic modes. Arthrospores (or arthroconidia, in Agaricomycetes most often called oidia) are those that arise in thallic mode from simple dissection of pre-existing hyphal cells. Blastospores or blastoconidia are those that bud off exogenously on a **sporogenous** (conidiogenous) cell. Chlamydospores in the narrowest sense as endogenous thick-walled thallic spores with the mother cell wall as a loose mantle follows the definition by Kirk et al. (2008), while chlamydospores in the broad sense include chlamydospores without a loose mantle (i.e. the cell wall of the mother cell is fused with the spore cell wall), **aleuria** as terminal spores that swell after delimitation and where the cell wall eventually thickens and **blastocysts** as blastic mitospores with a thickened cell wall (Clémençon 1997, 2004, 2012). This author includes in the definition of chlamydospores also spore-like swellings that remain attached to the generating hyphae. More generally, chlamydospores may be defined as more or less swollen and thick-walled spores (Walther et al. 2005). In this report, we use the term chlamydospores more generally since details of modes of production of large thick-walled spores are often not known (Fig. 12.3). Furthermore, we try to define major modes of **sporogenesis** and major types of spores using specific Agaricomycetes as examples. However, it should be noted as pointed out by Walther et al. (2005) that fluent transitions exist between the major types of spores distinguished.

Structures identified by light microscopy as spore-like swellings might in not all instances present immature or non-released spores. In *Coprinus comatus*, the existence of uni- and binucleate spores has been mentioned in the literature (Walther et al. 2005, not further documented in the paper; Badalyan et al. 2011a). Other authors analysed by electron microscopy spore-like structures on short sporophore-like branches thought to be chlamydospores and identified them as so-called 'spiny balls', unicellular structures with hair-like tubular appendices that serve in nematode infections (Luo et al. 2004, 2007; Badalyan et al. 2005; Buchalo 1988; Buchalo et al. 2011a). As other examples, *Coprinus sterquilinus*, *Conocybe albipes*, *Climacodon septentrionalis* and *Pleurotus ostreatus* produce tiny secretory cells on vegetative hyphae which contain toxin with which they attack nematodes (Buller 1931; Kemp 1977; Barron and Thorn 1987; Hutchison et al. 1996; Tanney and Hutchison 2012; Walther and Weiß 2006; Fig. 12.4b). Such secretory vesicles in *P. ostreatus* were identified in other publications as blastospores (Reshetnikov 1991; Badalian and Melik-Khachatrian 1994). Morphological similar vesicles have been reported, often also as blastospores, in *Agrocybe pediades*, *Agrocybe firma*, *Agrocybe vervacti*, *Conocybe appendiculata* and further *Conocybe* species, *Gymnopilus pampeanus*, *Pleurotus eryngii*, *Pleurotus smithii*, *S. commune* and more (Brefeld 1889; Stalpers et al. 1991; Badalian and Melik-Khachatrian 1994; Sede and Lopéz 1999; Walther and Weiß 2006; Fig. 12.4a). According to Walther and Weiß (2006), any such swollen structures may mistakenly be believed to be blastospores when the carrying hyphae break of underneath the swollen terminal cells. As another interesting example, *Leucoagaricus gongylophorus* forms in clusters large swollen cells at ends of hyphae as symbiontic organs (called gongylida or bromatia) for feed of leaf-cutting ants and for spread of proteolytic enzymes and laccases by the ants onto chewed-up plant material (De Fine Licht et al. 2014;

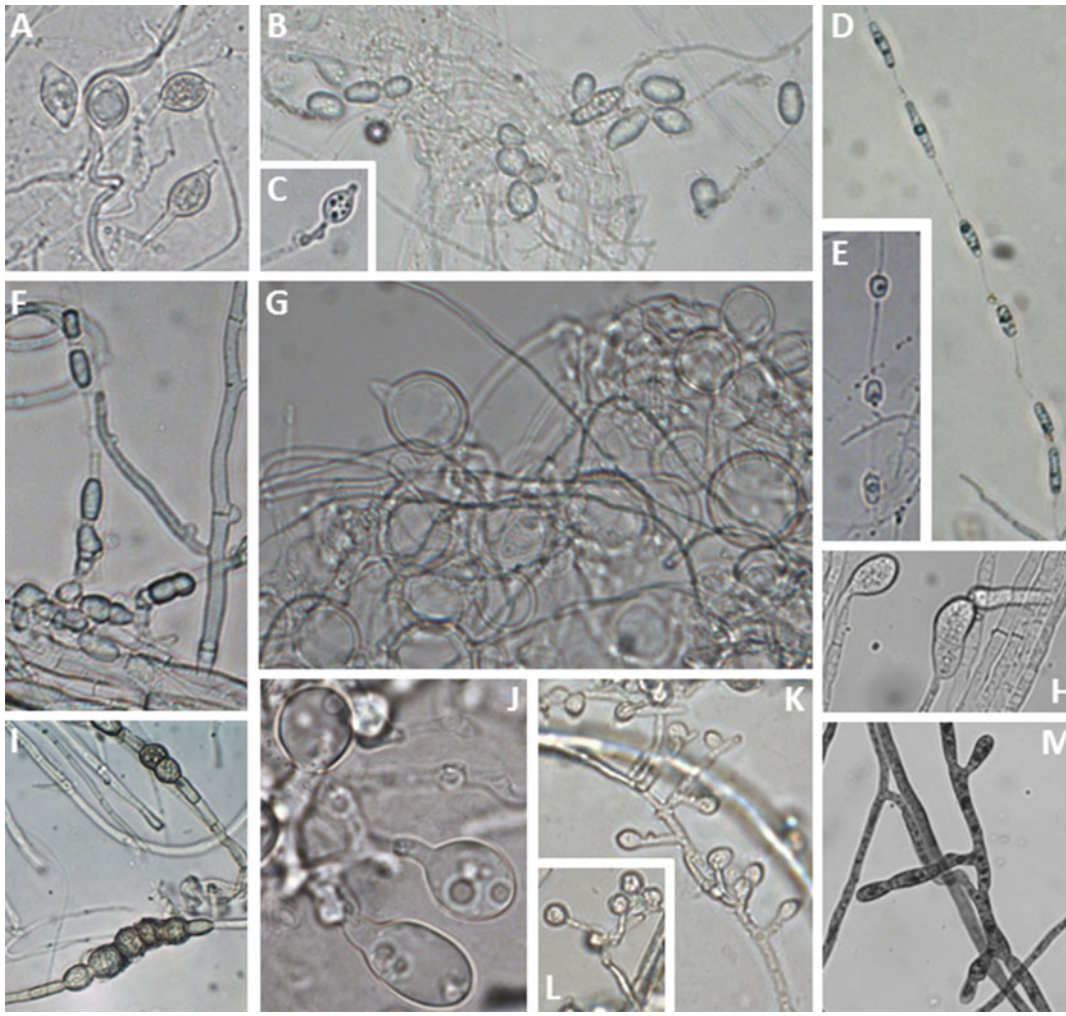


Fig. 12.3 Chlamydospores or chlamydospore-like swellings of a selection of dikaryotic strains of Agaricomycetes. (**a–c**) *Ganoderma resinaceum* strains Gr-7, Gr-5 and F-1. (**d, e**) *Ganoderma lucidum* strain GLU1. (**f**) *Schizophyllum commune* strain V-1. (**g**) *Ganoderma adspersum* strain 1016. (**h**) *Coprinellus radians* strain C22. (**i**) *Coprinellus disseminatus* strain 3-2S. (**j**) *Coprinellus bisporus* strain C406. (**k, l**) *Laetiporus sulphureus* strain Ls-3. (**m**) *Coprinopsis cothurnata* strain C145. Strains were grown on 1.5 % MEA medium with chloramphenicol (100 mg/L) at 25 °C and observed under a light microscope as described by Badalyan et al. (2011a) (All photos were taken using a 40× objective. Note that the sample of *C. cothurnata* in (**m**) was stained with a 0.1 % methylene-blue aqueous solution for better contrasting. Photos were all taken by SMB)

Kooij et al. 2014; Lange and Grell 2014). Likewise, large globular cysts in aging cultures of *Termitomyces* and *Attamyces* species might serve in nourishing their termite hosts (Kreisel 1972; Watling 1979; Botha and Eicker 1991). Examples of multiple other types of specialized swollen cells (cysts) with often unknown function that may or may not break off from the mycelium are collected by Clémençon (1997, 2004, 2012). Intrinsic to the definition of spores is their reproductive component (Kirk et al. 2008).

A. Thallic Arthrospore Formation by Dissection of Pre-existing Undifferentiated Hyphae

Thallic sporogenesis in its simplest case has been reported by Brodie (1936) for monokaryons and dikaryons of *Flammulina velutipes*. In this fungus, vegetative hyphae of monokaryons and dikaryons fragment secondarily into chains

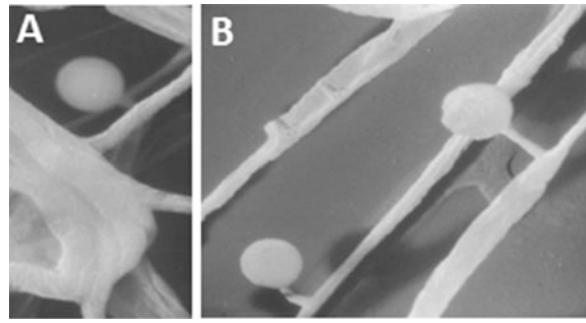


Fig. 12.4 Scanning electron microscope pictures of secretory cells in (**a**) *Pleurotus eryngii* dikaryon MS-27 and (**b**) in *Pleurotus ostreatus* dikaryon 004 (Reprinted with permission from Badalian and Melik-Khachatrian (1994))

of single uninucleate cells (Brodie 1936; Nobles 1948; Takamaru 1954; Reshetnikov 1991; Badalyan et al. 2006). Examples for chains of oidia on a *F. velutipes* dikaryon and on a dikaryon of the related species *Flammulina rossica* can be seen in Fig. 12.5b, c, respectively. Individual chains in *F. velutipes* may count only a few to over 40 arthrospores (Brodie 1936; Ingold 1980). Released spores assemble into larger clumps of 100–200 oidia (Kemp 1980). In the dikaryon, all uninucleate oidia within a chain and within a larger clump are of the same mating type. While dikaryotic colonies produce uninucleate arthrospores of both kinds of parental mating types, there is however often a strong bias within dikaryotic colonies towards spore production of only one mating type (Brodie 1936; Aschan 1952; Aschan-Åberg 1960; Kinugawa 1979; Kemp 1980; Kitamoto et al. 2000). The process of thallic sporulation in the dikaryon thus provides a specific developmental programme for **dedikaryotization** and **monokaryotization**, respectively (Aschan 1952; Ingold 1980; Kitamoto et al. 2000; see also Sect. III).

Oidia clumps in aerial mycelium of *F. velutipes* dikaryons are born on a single clampless aerial hypha (Kemp 1980). Standard dikaryotic hyphal branching in growing *F. velutipes* colonies occurs at positions slightly below clamps, whereas clampless hyphae are produced in irregular frequencies by a first branching at fixed positions above (unfused) clamp cells, possibly due to some irregularities in nuclear distributions during clamp cell formation (Ingold 1980; Kemp 1980). Some further clampless side branches can emerge on the first clampless hypha, all of which will extend to long, sparingly branched hyphae. Two to three neighbouring clampless aerial hyphae may thereby form **mycelial strands** by adhering to each other. From the long clampless aerial hyphae, complex systems of multiple lateral **oidial branches** eventually arise. Oidial branches may loosely coil around each other. They have initially no cross-walls, but some scattered simple cross-walls appear with time (Ingold 1980). In aging dikaryotic colonies, clampless branches for spore formation can in addition arise at low frequencies from both-sided clamped intercalary hyphal cells which may have increased their number of nuclei beyond two. Processes in the production of oidial branches in monokaryons are similar to those observed in the dikaryons (Brodie 1936; Ingold 1980). In both types of mycelia, release of oidia starts in oidial branches at the hyphal apexes and proceeds **basipetally** and can also continue from oidial branches into the parental hyphae (Brodie 1936; Ingold 1980; Kemp 1980). Spore formation involves alternative **vacuolation** and **portioning of protoplasm** over the length of a hypha for subsequent **splitting** into discrete oidia (Brodie 1936; Reshetnikov 1991). Vacuolic spaces between portions of protoplasm are very narrow in oidial branches (compare Fig. 12.5b) and irregular extensive in cases of rare intercalary spore formation in older hyphal segments (Ingold 1980). Transverse cell walls will form to delimitate the oidia within the parental hyphae that with time disintegrate (Brodie 1936; Ingold 1980). Hyphal cell walls rupture **rhexolytically** in the regions in between the spores (Walther et al. 2005;

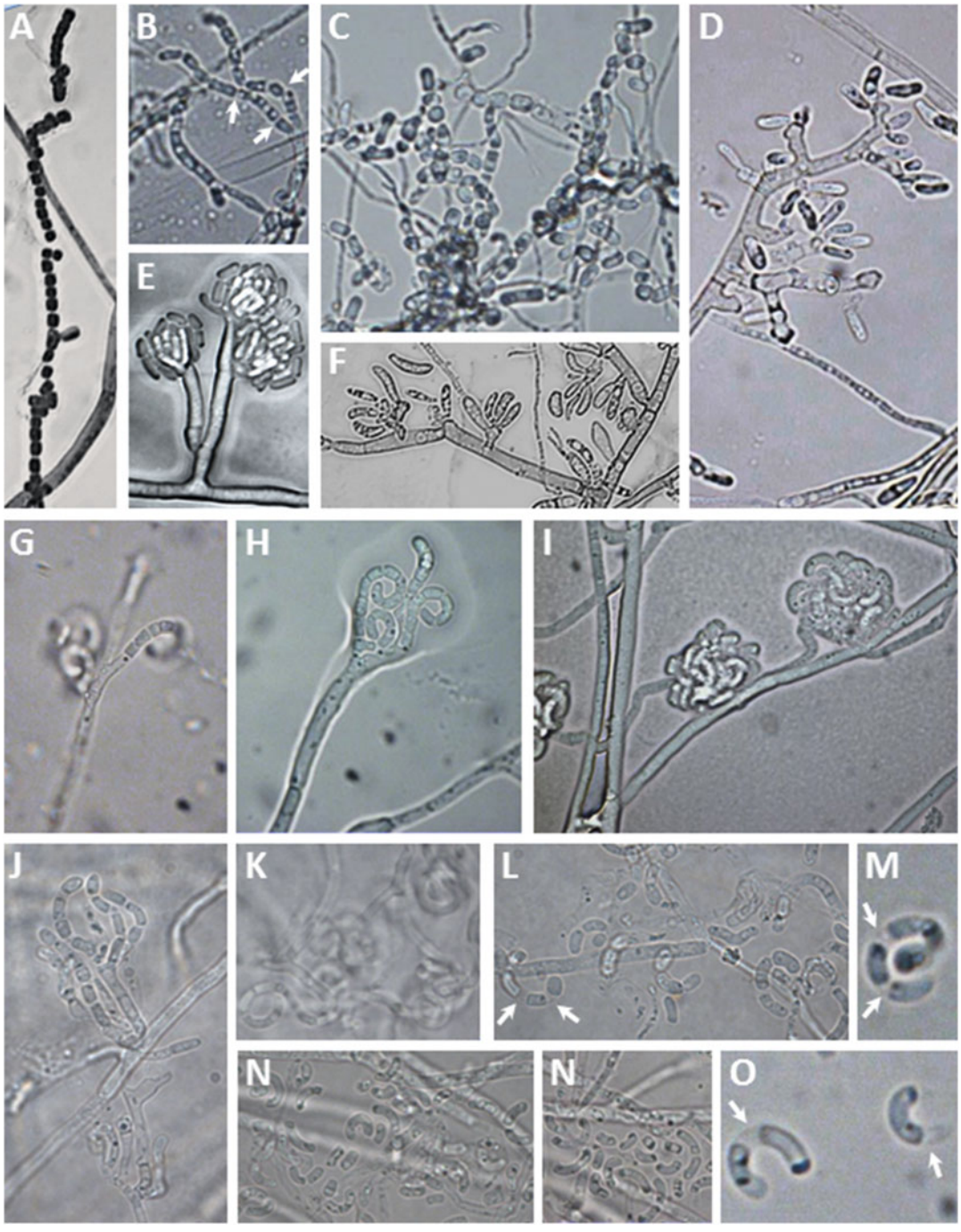


Fig. 12.5 Arthrospore formation in selected species of Agaricomycetes. Vegetative hyphae split into chains of arthrospores in (**a**) *Coprinopsis lagopus* dikaryon C6B, (**b**) *Flammulina velutipes* dikaryon SBII-2 and (**c**) *Flammulina rossica* dikaryon SBR-12. Oidiophores of *Hormographiella* types are produced in (**d**) *Coprinellus ellisii* dikaryon C140, (**e**) *Coprinopsis cinerea* monokaryon 5026 and (**f**) *Coprinellus* aff. *radians* dikaryon C35. (**g–o**) Arthrospore formation in *Psilocybe cyanescens* monokaryons Bad Düben 1-3 (**g, h, j**), KA1-4 (**i, m–o**) and Diss 3-1 (**k, l**). (**g–k**) Stages of conidiophore production, successive outgrowth of enrolling oidial branches and vacuolisation and protoplasm portioning in oidial hyphae. (**l–o**) Rhexolytic mode of spore

Reshetnikov 1991), i.e. in intermediate, usually reduced cells (Clémençon 1997, 2004, 2012). Resulting free spores are dry and can be blown away by wind (Brodie 1936; Ingold 1980). Most oidia of *F. velutipes* are cylindrical to oval and of a size of 5–8 × 10–20 µm. However, there are also longer ones (up to 80 µm), curved ones (from coiled hyphal segments) and Y-shaped ones (from points of hyphal branchings), and there might be spores staying connected in blocks of two or three (Ingold 1980).

Rhexolytic refers to a mode of secession of spores by circumscissile splitting the periclinal cell wall of hyphal mother cells apart of the basal septa of spores, thereby destroying the reproductive cell. **Schizolytic** splitting in contrast refers to secession of spores by splitting the delimiting (double-layered) septum so that only half of the cross wall remains as outer shield of the spore (Descals 1985; Kirk et al. 2008). According to Walther et al. (2005), most modes of splittings between arthroconidia in Agaricales are rhexolytic. In contrast, the mode of splitting in the Tricholomataceae is mostly schizolytic (Watling 1979; Walther et al. 2005) and Hutchison (1988) reports this for arthrospore formation in dikaryons of *Limacella illinita* (Amanitaceae). However, the mode of splitting is not always easy to observe purely by light microscopy when two spores are so close together so that potential protoplasm contractions and vacuolizations in between as preparation for the rhexolytic mode of separation might be overlooked (Walther et al. 2005). Electron microscopy may help to observe the fine structure of the spore cell walls (one-layered or double-layered; are residues from the parental hyphal cell protruding from the spore?) at the sites of spore release in order to decide on a splitting mode (Heintz and Niederpruem 1970, 1971; Jurand and Kemp 1972; Gardiner and Day 1988; Polak et al. 1997a; Kües et al. 2002a; Dörnte and Kües 2012; Fig. 12.2).

Many species of the Agaricales undergo similar simple thallic arthrospore formation by usually rhexolytic splitting of more or less straight growing, morphologically not or little differentiated hyphae into individual arthrospores, such as seen mono- and dikaryons of *Agrocybe aegerita*, *Agrocybe dura*, *Agrocybe firma* and *Agrocybe praecox* (Walther and Weiß 2006; Buchalo 1988; Buchalo et al. 2009, 2011a, b); in monokaryons of *Gymnopilus junonius*, *Gymnopilus sapineus* and *Gymnopilus spectabilis* (Reshetnikov 1991; Walther et al. 2005) and *Cystoderma amianthium* (Walther et al. 2005); in clampless hyphae of the homothallic mycoparasites *A. lycoperdoides* and *A. parasitica* (Brefeld 1889; Buller 1924; Thompson 1936); and in dikaryons of *Amauroderma rude* (Furtado 1966), *Cystoderma granulosum* (Pantidou et al. 1983), *Moniliophthora perniciosa* (Delgado and Cook 1976), *Coprinopsis lagopus* (Fig. 12.5a; note similar presentations given under the likely false labelled species name *C. cinerea* in Watling 1979), *Hypholoma fasciculare* (Brefeld 1889; Garibova et al. 1986; Fig. 12.6), *Lepista nuda* (Buchalo et al. 1985, 2009, 2011a, b), *Oudemansiella canarii* (Sede and Lopéz 1999), *T. camporatus* (Chang and Chou 1995) and *Agaricus arvensis* (Molitoris et al. 1996). Oidia from splitting undifferentiated hyphae were observed in monokaryons of *Gloeophyllum odoratum* (Nobles 1948) and dikaryons of *Bjerkandera fumosa* (Clémençon 1997, 2004, 2012), *Laetiporus sulphureus* (Buchalo 1988; Buchalo et al. 2009, 2011b), *Lentinus suavissimus* (Peterson et al. 1997), *Mycena metuloidifera* (Valenzuela et al. 1997), *Polyporus adustus*, *Polyporus umbellatus*, *Polyporus varius* and *P. giganteа* (Nobles 1948; Stalpers 1978; Peterson et al. 1997) from the Polyporales and *Punctularia strigosozonata* (Stalpers 1978) from the Corticales. *Fomes fomentarius*, *Fomitopsis pinicola*, *Polyporus elegans*, *Polyporus squamosus*, *Phlebia radiata* and *Phlebia rufa* form arthrospores on both types of mycelia (Brefeld 1889; Boddy and Rayner 1983b; Peterson et al. 1997; Mukhin and

Fig. 12.5 (continued) release and free C-shaped spores of variable lengths. *Arrows* point to empty spaces in oidial branches where spores will be separated from each other by rhexolytic splitting of the parental cell wall. Strains were grown on glass slides and photographed as described in Polak et al. (1997a), using a 40× objective (**a–d, f**) or a 100× objective (all others). Growth media and temperatures were either YMG/T and 37 °C (*C. cinerea*; Granado et al. 1997) or MEA and 25 °C (all others). Note that the samples of *C. lagopus* and *C.* aff. *radians* were stained with a 0.1 % methylene-blue aqueous solution for better contrasting. Photos (**a**, **b**, **d–f**) were taken by SMB, photo (**e**) by E. Polak, photos (**g–o**) by AG

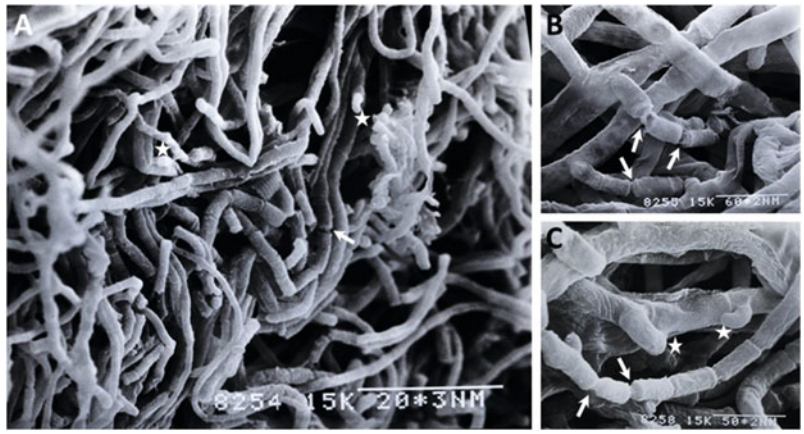


Fig. 12.6 Scanning electron microscope pictures of aerial mycelium and arthrospores generated in rhexolytic mode from hyphae of the dikaryotic *Hypholoma fasciculare* strain 0395. *Arrows* point to spores separating from each other by splitting the empty spaces of parental hyphae in between. *Stars* mark positions of clamp cells (Photos were taken by SMB. Photos **b**, **c**) reprinted from Garibova et al. (1986) with permission)

Votintseva 2002; Badalyan et al. 2015). However, species may differ in frequency of arthrospore formation (compare the different reports listed in this chapter; Boddy and Rayner 1983b). While the majority of competent species apparently produces uninucleate arthrospores from dikaryons (Reshetnikov 1991; Walther and Weiß 2006, 2008), oidia from dikaryons of *M. perniciosa* are, for example, binucleate (Delgado and Cook 1976). Outgrowth of clampless monokaryotic hyphae precedes arthrospore formation in dikaryons of *B. fumosa* (Clémençon 1997, 2004, 2012) and *P. adustus* (Nobles 1948), respectively.

Monokaryons of *Polyporus badius* produce in narrow distances ellipsoidal arthroconidia (7–8 × 2.5–3 μm) in rhexolytic manner from loose tufts of length-restricted oidial branches that are slightly thicker than their parental hyphae. Whether dikaryons in this species give also rise to arthroconidia remains to be established (Ingold 1986). Arthrospore formation in *Polyporus squamosus* was observed on mono- and dikaryons. Main hyphae and shorter side branches may convert into oidia in the monokaryon, whereas in the dikaryons, it is preferentially specific branches that grow out from clamp cells. The processes in the different types of mycelia are similar in that there is successive protoplasmic retraction with repetitive retraction-septum formations as the sizes of the protoplasts within the parental hyphae shrink. Spores are released by rhexolytic periclinal hyphal cell wall rupture and somewhat swell; those obtained from dikaryons are wider than those of monokaryons. Depending on the place of rhexolytic rupture, mature spores may carry empty chambers at their ends originating from the repetitive septum formation during successive protoplasmic retraction. Spores obtained from dikaryons are dikaryotic and germinate into new dikaryons (Ingold 1986).

Dikaryotic sporulating hyphae in aerial mycelium of *Polyporus varius* show limited length growth. They are wider towards their tips, and their clamp connections are closer together than those in the parental hyphae and in between are also non-clamped septa. The ovoid to rod-shaped arthrospores resulting from splitting of these hyphae are dry and very variable in size (8–25 × 2.5–6.6 μm). Many are monokaryotic and some are dikaryotic (10 % of spores which will germinate into new dikaryons). The process of sporulation in monokaryons is similar but results in smaller spores sized 5–22 × 2–4 μm (Ingold 1991). A recent report documents oidia (7–10 × 3–4.2 μm) also for dikaryons of *Polyporus umbellatus*. The authors suggest that these arise from short outgrowths from clamp cells (Xing and Guo 2008).

Pholiota microspora (*nameko*) produces abundant **dry oidia** from aerial hyphae in monokaryons and dikaryons and in low quan-

tities also **wet oidia** in submerged mycelium (Arita 1979; Hui et al. 1999a). Aerial oidia are globular, oval or rod shaped and the average size in monokaryons is 4.9 × 1.9 µm and in dikaryons 5.2 × 1.7 µm. Spores of both types of mycelia are mostly uninucleate (about 80 % of oidia from monokaryons; about 70 % of oidia from dikaryons), and smaller parts are binucleate (about 12–15 % in monokaryons; 15–25 % in dikaryons) with the remaining being polynucleate (up to 6 nuclei per spore).

Dry oidia are hydrophobic, wet oidia are hydrophilic spores (Kemp 1975). Hui et al. (1999a) show that aerial hydrophobic oidia of *P. microspora* are covered by a rodlet layer, likely of assembled **hydrophobins**, while submerged wet oidia miss such layer. Hydrophobins are short secreted cysteine-rich amphipathic fungal proteins that assemble on surfaces of aerial cells into films with an outer hydrophobic side and an inner hydrophilic side that contacts the cell walls (Wösten 2001; Walser et al. 2003; Linder et al. 2005). Ásgeirsdóttir et al. (1997) documented in *C. cinerea* that the wet oidia from aerial mycelium miss hydrophobins. The surfaces of primary cell walls of wet oidia in different species are covered by fimbria-like proteineous structures and they are encapsulated in sticky mucilage (Jurand and Kemp 1972; Castle and Boulianne 1991; Polak et al. 1997a; Hui et al. 1999a; Kües et al. 2002a; Dörnte and Kües 2012; Fig. 12.2). There is possibly a certain tendency that oidia generated by splitting of undifferentiated hyphae within the mycelium (Fig. 12.4a–c) are dry and that oidia generated on specific carrier hyphae (conidiophores, oidiophores; Fig. 12.4d–f) are wet and assemble in globose mucoid masses at the tips of the sporophores (Kemp 1975; Walther et al. 2005; Walther and Weiß 2006, 2008). In *Pholiota squarrosoadiposa*, dikaryotic arthroconidia assemble abundantly in slimy droplets within the aerial mycelium (Clémençon 2004, 2012) as do spore masses in mono- and dikaryotic mycelium of *Pholiota tuberculosa* (Walther and Weiß 2008).

About 80 % of aerial oidia on both types of *P. microspora* mycelia arise from shorter lateral branches that developed perpendicular to the main hyphae and that might have further vertically be branched. Portions of protoplasts separate in such secondary branches by large vacuoles, cell walls are formed around the protoplast, and the original hyphal cell wall is believed to be dissolved (rhexolytic mode of secession). The other 20 % of spores in aerial *P. microspora* mycelia generate sequentially from the termini of the main vegetative hyphae. Thereby, a first terminal arthrospore develops while the tip of the mother hyphae continues to grow which can lead to **acropetal** formation of a second or more oidia. Since such temporary terminal oidia in *P. microspora* tend to somewhat swell, they may more specifically be referred to as **aleurioconidia** (Arita 1979; Hui et al. 1999a). Walther et al. (2005) considered such terminally formed spores as intergradations between arthrospores and blastoconidia.

Between different *Pholiota* species, the degree of swellings of such terminal types of spore varies and there might be further intercalary swollen cells giving rise to chlamydospores (Table 12.1). Swelling might happen prior or after septum formation. It can therefore be difficult to clearly distinguish in these species between (transitions in) spore types by mode of production and by morphologic shapes and sizes and in denominations between simple arthrospores, blastoconidia and chlamydospores. Accordingly, different authors in the literature use different terms for the same type of spores (Jacobsson 1985; Walther et al. 2005; Walther and Weiß 2008).

Many other *Pholiota* species (Strophariaceae) undergo similar modes of thallic oidia production on mono- and dikaryotic mycelia (for some examples, see Table 12.1), while species differ in degree of length and ramification of the spore-forming hyphae (Reshetnikov 1991; Walther et al. 2005; Walther and Weiß 2008). In some species, for example, *Pholiota jahnii*, the sporogenous hyphae are moreover coiled (Arita 1979; Jacobsson 1985; Walther et al. 2005; Walther and Weiß 2008). A special case is represented by monokaryons and dikaryons of *Pholiota tuberculosa*. In this species, sporogenous hyphae proliferating sympodially from a short hyphal side branch strongly coil. Coiled sporogenous hyphae might give in acropetal manner 2–3 spores which swell prior to maturation and release. Spores are curved or irregularly formed of sizes of 6–11.5 × 2–3.5 µm. Spores assemble in masses into slimy drops (Walther and Weiß 2008).

Also in species from other genera in the Strophariaceae, oidial branches are more distinct in shape since they enrol in young stage into loops and intermingle into clews and

finally result in large clusters of more or less C-shaped oidia (Brefeld 1889; Walther et al. 2005; Walther and Weiß 2008). Such types of oidia have, for instance, been reported from monokaryons of *Tubaria furfuracea* (Ingold 1983) and from mycelia of *Psilocybe coprophila*. While Brodie (1935) observed oidia production only on *P. coprophila* monokaryons, other researchers reported arthrospore formation in the species from both the monokaryons and the dikaryons (Gilmore 1926; Reshetnikov 1991; Walther and Weiß 2006). In this species, single side hyphae strongly coil up while extending in length by hyphal tip growth to later split by rhexolytic mode into a collection of small groups of interlinked spores and of individual spores, respectively (Reshetnikov 1991; Walther and Weiß 2006). More often for arthrospore production, oidial hyphae sympodially branch off from the tip of a short side branch, coil up and dissect into spores in basipetal order. Parts of the apex of the side branch might be included (Reshetnikov 1991). Another example is represented by monokaryons of *Psilocybe cyanescens* where groups of enrolling oidial branches arise in sympodial manner mainly from tips of carrier hyphae within the aerial mycelium (Fig. 12.5g, h, j) and ball together (Fig. 12.5i, k). Within oidial branches, vacuolation, portioning of protoplasm and septum formation happen (Fig. 12.5h, j, k) for subsequent rhexolytic fragmentation into smaller groups of spores (Fig. 12.5l, m, o) and finally into single curved spores of variable lengths (Fig. 12.5m, o) that assemble in larger clumps of spores at the tips of the carrier hyphae (Fig. 12.5i). Arthroconidia in this species seem to be exclusively formed on the monokaryons and are not found on dikaryons so far (AG, unpublished observations). However, production of arthrospores on curling oidial branches of conidiophores has been observed on mono- and dikaryotic mycelium of *Psilocybe merdaria*. Spore-producing hyphae in dikaryons of *P. merdaria* have clamp cells, and generated spores are uninucleate and dry. Septation within oidial branches is first acropetal and later more random (Watling 1971; Valenzuela and Garnica 2000). Valenzuela and Garnica (2000) believe that the spores in *P. merdaria* are released by schizolytic splitting of septa. According to Watling (1971), oidia formation on dikaryons in the species is rare as compared to monokaryons. *Psilocybe inquilina* and an undefined *Psilocybe* sp. are reported to sporulate only on the monokaryons (Walther and Weiß 2006).

Within the Strophariaceae, the thallic mode of conidiogenesis seems to predominate. Walther and Weiß (2008) distinguish two basic types of thallic conidiogenesis. In mode I, arthroconidia are formed by fragmentation of straight or coiled conidiogenous hyphae that arise as lateral or terminal branches (mode Ia) or slightly sympodial (mode Ib). The lateral or terminal branches in mode 1a are considered as undifferentiated conidiophores (examples: *Hypholoma capnoides*, *Kuehneromyces mutabilis*, *Pholiota lenta*, *Stropharia rugosoannulata*). A slightly sympodial branching pattern in mode 1b results in a first primitive conidiophore differentiation in a sterile stalk and a conidiogenous apical part (examples: *Pholiota lucifera*, *Psilocybe* species). In mode II, the spore-forming structures are more complex, distinguish by morphology from normal side-branches, and adopt a clear conidiophore function. The conidiophores in the more advanced mode II sympodially proliferate short branches on their apexes that develop into single spores or chains of arthroconidia. The morphological plasticity in spore formation in mode II species makes it difficult to define the sporulation as (purely) thallic or also blastic spore generation. Spores will swell and may become coloured (examples: *Pholiota aurivella*, *Pholiota gummosa*, *Pholiota squarrosa*). Note that swollen spores generated by mode II are sometimes by other authors (e.g. in Martens and Vandendries 1933; Hübsch 1978) considered as chlamydospores (further discussion in Walther and Weiß 2008 and see also Hughes 1953 and Reshetnikov 1991). The majority of analysed Strophariaceae can be assigned in asexual sporulation to mode I of arthrospore formation while a few species (*Pholiota alnicola*, *P. tuberculosa*; see Sect. II.D) deviate in asexual sporulation from both modes I and II. In most sporulating species of the Strophariaceae however, arthrospore formation is observed on both, monokaryons and dikaryons. Arthrospore release in both modes usually starts at the apices of oidial branches and continues either basipetally or random (Walther and Weiß 2008).

Curved mitospores born in spiral chains have been further described for *Panaeolus campanulatus* from the Psathyrellaceae (Brefeld 1889; Clémençon 1997) and *Simocybe sumptuosa* from the Inocybaceae (Clémençon 2000, 2004, 2012). In *S. sumptosa*, thin bean-shaped spores are born from ends of hyphae or from short conidiogenous side hyphae. The spores in

S. sumptosa are binucleate and assemble in the aerial mycelium of dikaryons into slimy droplets (Clémençon 2000, 2004, 2012), often together with swollen thick-walled chlamydospores (Clémençon 2000; see Sect. II.E).

B. Production of Arthrospores on Special Differentiated Sporophores

We apparently see in arthrospore formation within the Agaricomycetes an increasing complexity in spore-generating hyphae, from simple undifferentiated hyphae that are morphologically alike to the standard vegetative hyphae (Fig. 12.5a, c) over an outgrowth of simple or slightly modified side branches that proliferate possibly at distinct places (Fig. 12.5b) to the generation of primitive (Fig. 12.5g, h) and also more advanced conidiophores, respectively, oidiophores (Fig. 12.5d–f).

Further, the arthrospores themselves may maintain the width of their generating hyphae (Fig. 12.5a, b, d–o) or may possibly swell (Fig. 12.5c; Walther et al. 2005; Walther and Weiß 2008) and they may be curved from coiling of the sporogenous hyphae (Fig. 12.5l–o). Walther et al. (2005) discuss these spore features, schizo- and rhexolytic modes of spore releases as well as degrees of differentiation of conidiogenous hyphae and conidiophores in terms of evolution within the Agaricales. Typically, arthrospores of Agaricaceae may swell unlike spores of Bolbitiaceae and Psathyrellaceae. Also other characters in sporulation follow up major clades in the Agaricales (see also Hibbett and Thorn 2001).

Arthrospores in mono- and in dikaryons of *P. aurivella* are in larger numbers produced, in chains of up to 4–6 spores on distinct structures formed by short multiply branched side hyphae. The arthrospores may be cylindrical (8–13 × 1.7–2.2 µm), thin-walled and of widths defined by their conidiogenous hyphae. Slightly swollen spores are produced from short side branches in chains of two. Others arise in the species singly from short single side branches, swell more strongly, have thick cell walls (Reshetnikov 1991; Walther and Weiß 2008) and, depending on authors, are distinguished as chlamydospores (Martens and Vandendries 1933; Hübsch 1978). If from dikaryons, spores in *P. aurivella* are binucleate (Reshetnikov 1982, 1991; Walther and Weiß 2008). Tree-like structures with a number of conidiogenous hyphae dissecting into up to 8 thin-walled not or only slightly swollen arthroconidia occur on mono- and dikaryons of *Pholiota gummosa* (Walther and Weiß 2008). Irregularly branched complex structures which assemble in heaps up to 150 spores are reported from monokaryons of *Favolus tenuiculus* from the Polyporales. The terminal arthroconidia are clavate; all others in subjacent positions are barrel shaped to cylindrical (Peterson et al. 1997).

Many *Bolbitius* and *Conocybe* species (both genera from the Bolbitiaceae) produce specific conidiophores on the aerial vegetative mycelium of monokaryons but usually not on dikaryons. *Conocybe subovalis* is an exception of a species with conidiophores produced also on the dikaryon. Conidiophores of Bolbitiaceae are often slender long stalk cells (exception of short stalks: *C. albipes*) that may or may not separate from the hyphal foot cell by septation, and frequently, they are slightly swollen. In some species, the stalks are further branched (*Bolbitius vitellinus*, *Conocybe appendiculata*) or may be further divided into more stalk cells (*Conocybe magnicapitata*). Conidiogenous hyphae proliferate sympodially on the often swollen and with proceeding branching enlarged apex of conidiophores. Conidiogenous hyphae often adopt a coiled morphology. After delimitation of a conidiogenous hypha from a stalk cell by septa formation, plasma in the conidiogenous hyphae will be concentrated into portions and septa will be laid. At first, septa formation runs in basipetal succession, but later septation is random and splits already delimited hyphal segments into two or more further cells. Conidiogenous hyphae mostly dissect in rhexolytic mode into in total 4–8 straight cylindrical or curved arthroconidia. The unicellular spores are thin-walled and will not further swell. Released spores in most species are mucous and assemble into clumps at the tips of the conidiophores (Watling 1979; Walther et al. 2005; Walther and Weiß 2006).

Agrocybe arvalis develops conidiophores of a similar simple type as *Bolbitius* and *Conocybe* species (Walther

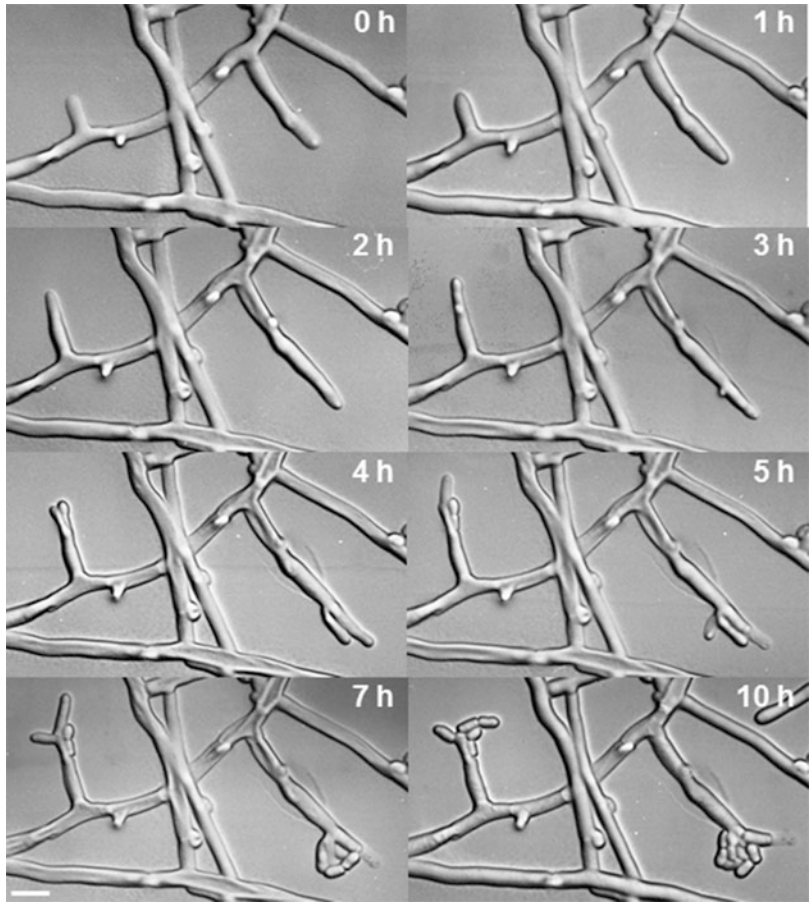


Fig. 12.7 Development of oidiophores of *Coprinopsis cinerea* homokaryon AmutBmut with time. In the images to the *left*, a young bulge is seen on an aerial hypha which, during the following 3 h, elongates into a structure starting to successively produce oidial hyphae. Two hours later, the first two spores are released, followed by more within the next 5 h. In the images to the *right*, development of a larger oidiophore with more oidial hyphae and spores can be seen. The scale bar represents 10 μm (Photos were taken by E. Polak as described (Polak et al. 1997a). The figure is reprinted from Fischer and Kües (2006) from the second edition of The Mycota Vol. I)

and Weiß 2006) whereas several other *Agrocybe* species form abundant arthrospores by fragmentation of undifferentiated mono- and dikaryotic hyphae (see Sect. II. A). The conidiophores in *A. arvalis* sympodially proliferate oidial branches which split in rhexolytic mode into 4–8 spores. In low frequency, such type of conidiophores can also be found in mono- and dikaryotic cultures of *A. aegerita* as a minor mode of oidia production (Walther and Weiß 2006). However, the genus *Agrocybe* has recently been excluded from the Bolbitiaceae on base of phylogenetic data. Species named in the genus seem not to be of monophyletic origin and some species seem to nest in the Strophariaceae (Gulden et al. 2005; Walther et al. 2005; Matheny et al. 2006; Tóth et al. 2013).

A case of advanced conidiophore development is presented by the well-studied *C. cinerea*. This fungus produces specific oidiophores (Figs. 12.5e, 12.7 and 12.8) in the aerial mycelium of monokaryons and in low frequency on dikaryons. On the tips of the oidiophores, short oidial branches (termed **oidial hyphae**) bud off to eventually split into two or rarely also into three or even more distinct uninucleate oidia (Bensaude 1918; Brodie 1931; Reshetnikov 1991; Gené et al. 1996; Polak et al. 1997a, 2001; Verweij et al. 1997;

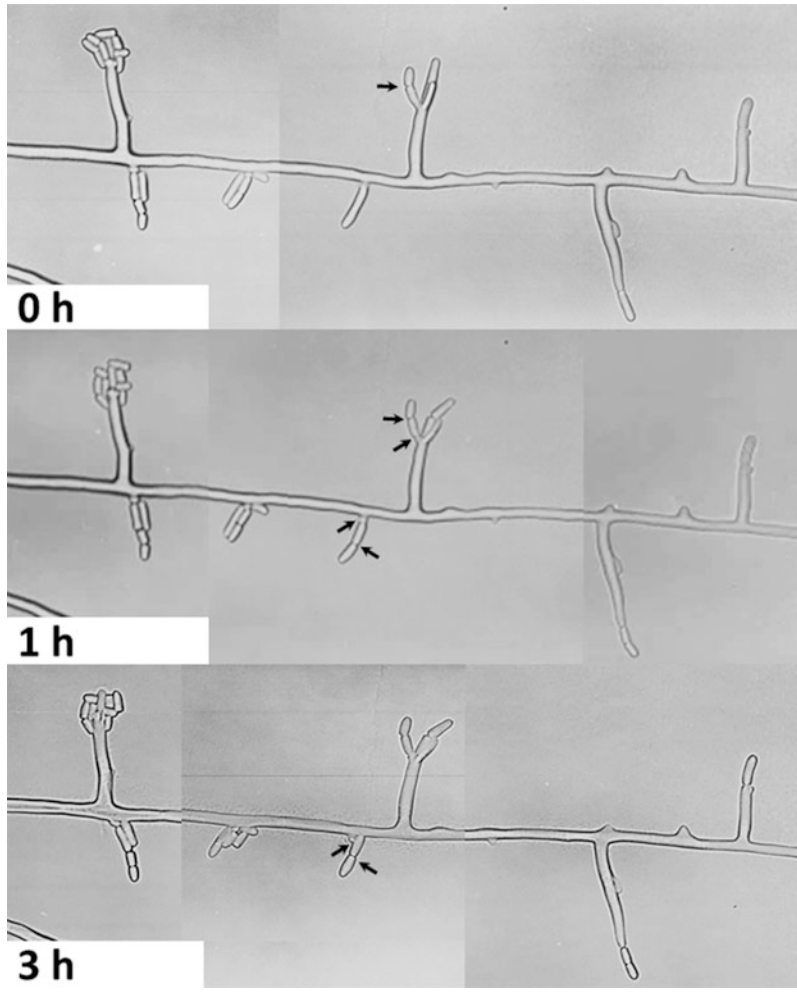


Fig. 12.8 Development of oidiophores of *Coprinopsis cinerea* monokaryon FA2222 with time. *Arrows* point to places of spore-spore and of spore-oidiophore splitting. Photos were taken by E. Polak as described in Polak et al. (1997a)

Kües et al. 2002a; Yoon 2004; Figs. 12.7, 12.8 and 12.9). Oidia formation in *C. cinerea* involves aspects of budding (production of the oidial hyphae) and thallic spore release by splitting the established oidial hypha into individual equally sized cells (Kendrick and Watling 1979; Watling 1979; Reshetnikov 1991; Polak et al. 1997a).

Oidiophores mainly arise in the aerial mycelium but oidiophores have occasionally also been observed submerged within the agar phase in cultures on artificial medium (Brodie 1931; Polak et al. 2001).

At 37 °C as favoured growth temperature of the fungus, the whole process of oidiation takes between 12 and 24 h (Polak et al. 1997a; Fig. 12.7). Using the self-compatible homokaryon AmutBmut (*Amut, Bmut*), a specific mutant where oidiation is light dependent by the *Amut* defect in the *A* mating-type locus (Polak et al. 1997a; Kertesz-Chaloupková et al. 1998), it was possible to document the complete developmental process on individual structures over the time. At first, a small lateral bulging appears from a main hypha that distinguishes in appearance from lateral outgrowths of normal side

branches (Polak et al. 1997a; Fig. 12.7, 0 h, the oidiophore on the left side of the photos). Its base is broader (3–7 μm) than the width of vegetative side hyphae (~2–4 μm), and the branching angle is ~80–85° (Fig. 12.7) and wider as compared to ~70–75° of monokaryotic branches and to ~10–45 °C of dikaryotic branches (Polak et al. 1997a; Polak 1999; Kües et al. 2002a). A nucleus within the parental hyphal cell (now the becoming footcell) will divide, one of the daughter nuclei migrates laterally into the growing oidiophore, and a septum is laid to separate the young oidiophore from its footcell (Fig. 12.7, 1 h, the oidiophore on the left side of the photos). The oidiophore will further elongate in length and it tapers with length (Fig. 12.7, 0–3 h; both oidiophores). At the apex of a fully grown oidiophore, short oidial hyphae bud off, one after each other (Fig. 12.7, 3–10 h; both oidiophores). Consecutive divisions of the nucleus within the oidiophore provide each new oidial hypha with a nucleus. An oidial hypha separates afterwards by septum formation from the oidiophore, another nuclear division follows in the oidial hypha, another septum formation divides it into two rod-shaped cells of equal length (usually about 2 × 4–6 μm in length), and finally the oidial hypha breaks up into two separate uninucleate hyaline cells (Polak et al. 1997a; Fig. 12.7, 4–10 h; both oidiophores). These free oidia will assemble in sticky liquid droplets at the tip of the oidiophore (Brodie 1931; Polak et al. 1997a). More oidial hyphae and oidia are produced over the time until up to 200 spores are collected at the tip of the oidiophore (Polak et al. 1997a). The processes on oidiophores of monokaryons are alike (Fig. 12.8). The 'wet' oidia of *C. cinerea* are strongly hydrophilic, and by their surrounding gelatinous layer, they are also sticky (Brodie 1931; Kemp 1975; Reshetnikov 1991; Guarro et al. 1992; Polak et al. 1997a, b). Due to the spore-containing liquid droplets, aerial mycelium of sporulating cultures appears as if speckled with dewdrops (Brodie 1931; Polak et al. 1997a; Kües et al. 1998a, 2002a).

Reshetnikov (1991) and Yoon (2004) observed stages in oidiation of a normal monokaryon of *C. cinerea* and reported similar observations to those of Polak et al. (1997a, 2001) on the self-compatible homokaryon AmutBmut and various monokaryons, respectively. According to Yoon (2004), splitting of terminal and intercalary oidia might not be simultaneous and the upper oidium might be released prior to the lower one. The order of events over the time on different oidial hyphae shown in Fig. 12.8 suggests this can be the case but that it also does not always occur in that order, consistent with observations by Guarro et al. (1992). As a special addition, Yoon (2004) report outgrowth of oidiophores also from multi-fold coiled hyphae (hyphal loops). Such loop structures are encountered in many related species but their function remains elusive (Badalyan et al. 2011b).

Brodie (1931) and Walther et al. (2005) state that oidia release in *C. cinerea* is rhexolytic. In contrast, Yoon (2004) compared the dissection of oidial hyphae into individual spores to schizolysis in cell divisions in the ascomycetous fission yeast *Schizosaccharomyces pombe*. Polak concluded that septa are laid in between mitotically dividing nuclei in a shared protoplast and that the mode of spore release is schizolytic by septum splitting. Electron microscope pictures were taken as support for the view on a schizolytic mode of separation of oidial hyphae into individual spores. Different from the double-layered spore side walls, the sides of the splits have only a single cell wall layer as is expected for schizolysis through double-layered septa (Polak et al. 1997a; Polak 1999; see also the model of schizolytic secession as presented in Walther et al. 2005). Spores coming from the apex of oidial hyphae have only one flat side with a single-layered cell wall as consequence of spore separation. Other spores have two flat sides with a single-layered cell wall, owing to splitting between spores within the oidial hyphae and to splitting from the oidiophore (Fig. 12.2; Heintz and Niederpruem 1970, 1971; Guarro et al. 1992; Polak et al. 1997a; Kües et al. 2002a; Yoon 2004; Dörnte and Kües 2012). The flat ends with the single-layered cell walls will be used for oidia germination (Brodie 1931; Heintz and Niederpruem 1971).

Polak et al. (1997a) noted as first step towards spore delimitation only single septa being laid between oidial hyphae and the stalk and between becoming spores within oidial hyphae that split according to the interpretation of the authors into two individual single-layered cell walls during the process of spore release. Brodie

(1931) however describes appearance of a single fine line at first at places of later spore splitting but does not further comment on the observed fine lines in his concluding process descriptions and leaves open what happens to them in later development. He summarizes as events in the process of oidiation 1. the division of protoplasm into two (or more) separate masses, 2. shrinking or condensation of the protoplasmic masses with the formation of gaps in between, 3. the development of a wall at the ends of portions of shrunken protoplasm and 4. the dissolution of the wall of the oidial hyphae in between the two new cells.

How do the single fine lines of Brodie (1931) relate to the single septa mentioned by Polak et al. (1997a)? Figure 12.9 shows the head of a young oidiophore of strain AmutBmut with four oidial hyphae (numbered here 1–4 from left to right in the photos) at different stages of development. One is at the state of mitosis with the nucleus not yet separated into two daughter nuclei (no. 2). The other three oidial hyphae have all two nuclei. One of these seems to have across the middle a single septum positioned seen as a fine line (no. 1) and the protoplasts in the lower and upper parts of the oidial hyphae appear to have shrunken. Another one seems to have started splitting into two completed spores with a clearly recognisable gap in between (no. 3). The fourth one has not yet a septum but apparently two separated portions of protoplasm with a gap in between (no. 4). Thus, details in this figure support specific observations made by Polak et al. (1997a) and made by Brodie (1931) while the process may need further attention for clarification in future studies.

Walther et al. (2005) present three submodes of rhexolytic splitting for spore release. In the 1st submode, portions of contracted protoplasts are each delimited by individual double-layered septa and splitting of spores occurs in hyphal gaps in between. In the 2nd submode, a septum is formed before plasma contraction. Upon subsequent shrinkage of the protoplasts, secondary septa are laid and spores split somewhere in between in the protoplasma-free hyphal zones. In the 3rd submode, single septa are formed between portions of protoplasm and every second cellular segment will be lost as place of rhexolytic splitting. Neither of these three descriptions appear to fit all the observed situations in *C. cinerea* and they would not explain why the *C. cinerea* spores at their flat ends possess only a single cell wall layer as shown in Fig. 12.2. In Ingold's model of rhexolytic hyphal dissection however, crosswalls between portions of protoplasm separated within hyphae by empty spaces are presented only single-layered (Ingold 1980) and this could explain the cytological observations on oidia in *C. cinerea*.

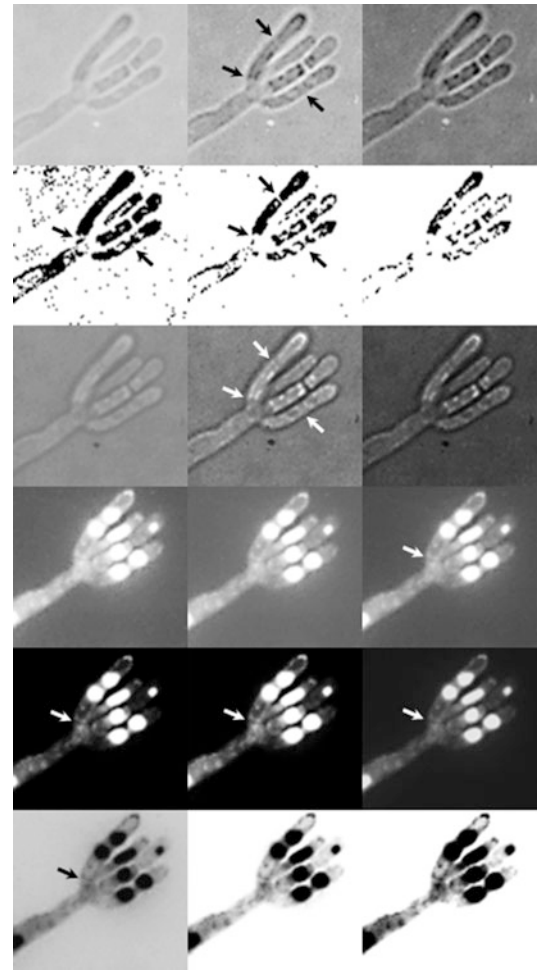


Fig. 12.9 Oidiophore of *Coprinopsis cinerea* homokaryon AmutBmut stained with DAPI (4′,6-diamidin-2-phenylindol). Positive light microscopy photo after image editing: from *left* to *right* with the contrast enhanced and brightness reduced (1st row), positive light microscopy photo with 100 % contrast and slight changes in brightness (2nd row), negative light microscopy photo (inverse *grey scale*) with increasing contrast and reduced brightness (3rd row), positive photo of DAPI staining under different brightness (4th row), positive photo of DAPI-stained nuclei under reduced brightness and enhanced contrast (5th row), and negative photo of DAPI-stained nuclei (inverse *grey scale*) with increasing contrast and brightness (6th row). *Arrows* mark positions of septum formation and protoplast partitions (Original photos taken by E. Polak as described (Polak et al. 1997a))

Oidiophores in *C. cinerea* are persistent and will not dissociate themselves into spores. A simple oidiophore in *C. cinerea* (known also as anamorph *Hormographiella aspergillata*) consists of just one single stem cell (type 1 oidiophores; the oidiophore on the left side of the photos in Fig. 12.7; e.g. the upward growing oidiophores in Fig. 12.8) and measures about 20–30 μm in length (Brodie 1931; Reshetnikov

1991; Gené et al. 1996; Polak et al. 1997a; Verweij et al. 1997). However, oidiophores divided by two or three stem cells exist with one, two or rarely also more side branches (type 2A oidiophores; Fig. 12.5e) that all will produce oidia at their apical ends (Reshetnikov 1991; Guarro et al. 1992; Polak et al. 2001; Yoon 2004). Some oidiophores produce oidial hyphae also from short lateral bulges on subapical stem cells (type 2B oidiophores; Fig. 12.7, the oidiophore on the right side of photos); others have only a stunted stem cell (type 3 oidiophores). In other instances, no stem cells are produced and oidial hyphae bud off directly from a vegetative hypha, singly (type 4A oidiophores; the downwards growing oidiophore in the middle of the photos shown in Fig. 12.8) or in groups (type 4B oidiophores; the downwards growing oidiophore on the right side of the photos in Fig. 12.8), (Reshetnikov 1991; Polak et al. 2001; Kües et al. 2002a; Badalyan et al. 2011a). While oidial hyphae generated on tips of oidiophores usually split up into only two spores (Reshetnikov 1991; Polak et al. 1997a, 2001; Yoon 2004), oidial hyphae generated directly at a vegetative hypha can give 2, 3, 4 or more spores (Polak et al. 2001; Kües et al. 2002a; Badalyan et al. 2011a; see oidial hyphae at the lower left side in Fig. 12.8). Analysis of more than 20 different strains revealed that each strain usually forms all types of oidiophores, but the frequencies per type differ between the strains. Usually, there is a preference in each strain for one or two types of oidiophores (Polak et al. 2001).

The most abundant branching pattern of emergence of oidial hyphae at tips of oidiophore stem cells results in an umbel-like appearance (Figs. 12.5e, 12.7, 12.8, and 12.9). In rare cases, oidial hyphae at tips of oidiophores branch themselves prior to splitting up into individual spores which gives a panicle-like appearance. When oidial hyphae bud off in line one behind the other at only one side of an oidiophore, the appearance is cyme-like (Reshetnikov 1991; Polak et al. 2001). Further variations are encountered in sizes of oidia. Although oidia in most strains measure 2×4–6 µm, there can be higher variation in length (Brodie 1931; Polak et al. 1997a, 2001). Regardless of whether formed on monokaryons or on dikaryons, oidia are nearly exclusively uninucleate (Bensaude 1918; Brodie 1931; Hollenstein 1997; Polak et al. 1997a, 2001). Oidiophores on dikaryons can occur on cells with clamp cells at both ends (Kües 2000; Badalyan et al. 2011a).

Hormographiella-type of oidiophores characterise monokaryons of species within the family of Psathyrellaceae (Walther et al. 2005; Badalyan et al. 2011a). Oidiophores of *Hormographiella*-types have been reported for *Coprinellus domesticus* (identified as *Hormographiella verticillata*), *Coprinellus ellisii* (Fig. 12.5d) and *Coprinellus* aff. *radians* (Fig. 12.5f), *Coprinellus* aff. *xanthothrix* and a yet unknown species termed as anamorph *Hormographiella candelabrata* (Guarro et al. 1992; Gené et al. 1996; Cáceres et al. 2006; Badalyan et al. 2011a). The complexity of oidiophores differs between the species. *C. domesticus* and *C.* aff. *xanthothrix* form multiple dense clusters of oidial hyphae, either directly on cells of little branched parental hyphae or on very short stalks that may or may not deliminate from the parental hyphae by a septum (Reshetnikov 1991; Guarro et al. 1992; Cáceres et al. 2006; Badalyan et al. 2011a). These two species resemble in branching pattern to a certain degree the simple types 3, 4A and 4B oidiophores defined by Polak for *C. cinerea* (Polak et al. 1997a; Cáceres et al. 2006; Badalyan et al. 2011a). Similarly, *Coprinellus congregatus*, *Coprinellus hiascens* and *Coprinellus sassii* produce also tufts of oidia on shortest stalks (Watling 1979).

Two distinct types of ITS sequences are given in NCBI (http://www.ncbi.nlm.nih.gov/) for the species *C. domesticus* (HQ847052 for strain SZMC-NL 1292; KP132301 for strain CNRMA10.949 linked as a barcode of the species to the International Society of Human and Animal Mycology (ISHAM)-ITS-reference barcoding database; Irinyi et al. 2015). The two sequences share only a level of 96 % identity, strongly supporting that these come from two distinct species. The sequence of the *Hormographiella verticillata* voucher strain (AY663836) of the large ribosomal RNA gene is to 99 % identical to that of strain SZMC-NL-1292 (HQ847132) that in course of delimitation of species within the genus *Coprinellus* has been renamed to *Coprinellus* aff. *xanthothrix* (Nagy et al. 2012). The strain C482 (EU168106) identified by Naumann et al. (2007) on base of 100 % sequence identity to AF361228 as *Coprinellus xanthothrix* and described by Badalyan

et al. (2011a) to produce oidia in *C. domesticus* similar manner shares 99 % sequence identity in the ITS region with KP132301. Consequently to current understanding, C482 should possibly be renamed as *C. domesticus*. Nevertheless of the vice versa exchange of species attribution, there are still two closely related species with *H. verticillata* type of oidiophores (Cáceres et al. 2006).

Oidiophores in *C. ellisii* and *C.* aff. *radians* in contrast are much more branched into tree-like structures (Guarro et al. 1992; Cáceres et al. 2006; Badalyan et al. 2011a; Fig. 12.5d, f). Their branching resembles in part the more complex type 2A oidiophores of *C. cinerea* (Polak et al. 1997a; Kües et al. 2002a; Badalyan et al. 2011a). However, compared to *C. cinerea*, less oidia are produced per oidiophore tip cell in the *Coprinellus* species, and every oidial hypha results usually only in one single spore (Reshetnikov 1991; Guarro et al. 1992; Cáceres et al. 2006; Badalyan et al. 2011a), whereas *C. cinerea* and *H. candelabrata* split oidial hyphae mostly into two (Polak et al. 1997a, 2001) and 2–7 arthrospores, respectively (Guarro et al. 1992). Whether the number of spores generated per oidial hyphae will be a taxonomic character to distinguish the genera *Coprinellus* and *Coprinopsis* needs further study. However, drawings of *Coprinellus ephemerus*, *Coprinellus callinus*, *Coprinellus subimpatiens* and *Coprinellus subdisseminatus* show all oidiophores with oidial hyphae dividing into 2 or 3 spores (Nichols 1904; Clémençon 1997). In *Coprinopsis verticillata*, oidiophores arise in high densities on swollen clubbed ends of thin hyphae with very short stalks that produce 1–3 oidial hyphae each at the upper tips which fall apart into probably 2 or also 3 individual spores. Released spores are collected in large slimy masses at the hyphal ends (Thielke 1984). Unlike *C. cinerea* and *C. verticillata*, oidia in *C. domesticus* and in *H. candelabrata* are dry and do not collect in slimy conidial heads (Guarro et al. 1992).

Species in the genus *Psathyrella* also have oidiophores of the *Hormographiella*-type (Kühner 1978). *Psathyrella candolleana* (Reshetnikov 1991), *Psathyrella corrugis* (Walther et al. 2005) and *Psathyrella spadiceogrisea* (Brefeld 1889) have all short stalks and their oidial hyphae split mostly into 2 oidia. Oidia of *P. coprophila* are wet and assemble in slimy heads within the aerial mycelium of monokaryons (Brefeld 1889; Kendrick and Watling 1979; Jurand and Kemp 1972; Kemp 1975).

Different from the coprinoid species belonging to the Psathyrellaceae, a desert coprinoid species *Coprinus xerophilus* from the Agaricaceae (Keirle et al. 2004) is homothallic and produces on clamped mycelium chains of multiple binucleate rod-shaped oidia (5–15 µm long), by segmentation of upright growing aerial hyphae that occasionally might be branched. The oidia germinate at one or both ends to give rise to a clamped mycelium (Barnett 1945). Although arthrospores are not observed in the type species of the genus, *C. comatus*, *C. sterquilinus* as another species of the genus might also produce arthroconidia (Kemp 1977).

C. Arthrospore Production in Conidiomata

Coremia of *P. cystidiosus*, *Pleurotus smithii*, *Pleurotus australis* and allies may occur on stipes, caps and lamellae of fruiting bodies, respectively (Buchalo 1988; Guzmán et al. 1991; Clémençon 1997, 2004, 2012; Segedin et al. 1995; Peterson et al. 1997; Zervakis 1998), and also on mono- and on dikaryotic mycelia in culture (e.g. Buchalo 1988; Guzmán et al. 1991; Petersen 1992; Capelari 1999; Esser 2000; Truong et al. 2006; Clémençon 2004, 2012). The coremia are bundles of parallel growing erected hyphae (forming a prosenchyma) that together form a stalk and that release large numbers of chains of brown-coloured arthroconidia (12–20 × 4–7 µm) from the upper hyphal tips into a mucous liquid at the loosened apex of the coremia (the **capitellum**) which by the masses of spores results in shiny black heads. Apical hyphal cells somewhat swell to become ovoid to subcylindrical in shape upon spore maturation. Arthroconidia are produced basipetally by sequential schizolytic secession of apical hyphal cells (Šašek et al. 1986; Stalpers et al. 1991; Capelari 1999; Walther et al. 2005; Truong et al. 2006; Clémençon 2012). For spore release, the parental cell starts to circumferentially break at the place of a hyphal septum, while the septa centripetally split and the ends of the spores round up, including in clamped dikaryotic cells the respective portions of the clamps. Formation

of a conidial cell wall initiates at the doliporus. The doliporus degenerates and the pore between two cells will be fully closed by a conidial cell wall (Moore 1977; Clémençon 2012). While the cell wall of the parental hypha becomes gelatinous, the mature brown spores have double-layered conidial cell walls with an outer layer being fibrous and smooth and an inner thickened layer with pore-like channels as the principal cell wall (Moore 1977; Stalpers et al. 1991). Since the spores swell during development and have a thickened cell wall, Clémençon (2012) refers to them as chlamydospores rather than as arthroconidia as it is more common in literature. After release of spores, conidiogenous hyphae within the coremia continue to grow for further spore production (Moore 1977; Truong et al. 2006). An average coremium may contain 20,000 conidiogenous hyphae. Every hypha may produce 20 spores per day, up to 20 days (Truong et al. 2006; Clémençon 2012). Dikaryotic mycelia exclusively produce dikaryotic arthrospores from clamped conidiogenous hyphae, and monokaryotic mycelia produce uninucleate spores from clampless hyphae. Moisture is required at the capitellum for spore production and an unknown signal component released by the fungus into the mucous liquid. Light is an environmental signal required for induction of coremia formation (Truong et al. 2006).

Very similar as in *Pleurotus* species, arthroconidia form in synnemata of *A. macrocarpa* basipetally on diverged clamped hyphae in the capitellum, round up and stain brownish-black, while in addition blastoconidia arise on denticles on hyphae of the synnemata stipe (Stalpers et al. 1991). Synnemata on stipes of *D. racemosa* produce masses of slimy hyaline arthroconidia at terminal ends of clamped divergently branching hyphae. Arthroconidia are at first angular but will become with time ellipsoidal to cylindrical, and they can have remnants of clamp connections (Watling 1979; Stalpers et al. 1991; Clémençon 1997, 2004, 2012). Hydnoid spines and cushions with clamped hyphae breaking up into arthrospores have been described as conidiomata on wood samples for the tropic species *Echinoporia hydnophora* (Clémençon 1997, 2004, 2012). Analysis of sporodochia of *Termitomyces* species taken from termitaria (nests of termites) revealed chains of a few arthrospores produced in basipetal succession on distinct long undifferentiated conidiogenous hyphae. Chains of arthroconidia may be sympodially branched (Botha and Eicker 1991).

D. Blastic Sporogenesis

Blastoconidia arise by budding from parental cells, either from undifferentiated hyphal cells, often on short thin sterigma-like **denticles** (a small tooth-like projection on which a spore is born), or also from specific sporophores (Stalpers 1988; Fig. 12.10). Some of such spores are thin-walled, while others may strengthen their cell walls and the morphological transition to thick-walled blastocysts as special subgroup of chlamydospores can be fluent (Clémençon 1997, 2004, 2012; Walther et al. 2005; see also Sect. II.E). An important cytological distinction between obvious blastospores (whether thin-walled or with thickened cell walls) and blastocysts in the sense of chlamydospores however concerns the origin of the spore nuclei. Blastospores obtain a daughter nucleus after mitosis in the conidiogenous cell unlike blastocysts that take over the resident nuclei from the mother cells (Clémençon 1997, 2000, 2012).

Blastic sporulation appears to be more common in early lines of Basidiomycetes than in the Agaricomycetes where strict blastic conidiogenesis is possibly seldom (Kendrick and Watling 1979). The rare examples documented in the Agaricales include *Gymnopilus junonius* with thick-walled single blastoconidia born on short sterigmata on undifferentiated hyphae (Walther et al. 2005), *Hohenbuehelia* and related anamorphic *Nematoctonus* species that form on denticles singular cone-shaped conidia for passive distribution (Drechsler 1941, 1946, 1949; Cooke and Godfrey 1964; Giuma and Cooke 1972; Thorn and Barron 1986; Reshetnikov 1991; Koziak et al. 2007a) and *Laccaria laccata* which produces terminal and intercalary solitary spherical smooth hyaline spores (7 µm Ø) on 5–8 µm long sterigmata born on clamped cells (Pantidou et al. 1983) which according to Walther et al. (2005) might however be non-germinable secretory cells. All these species follow the simple *Galzinia*-type

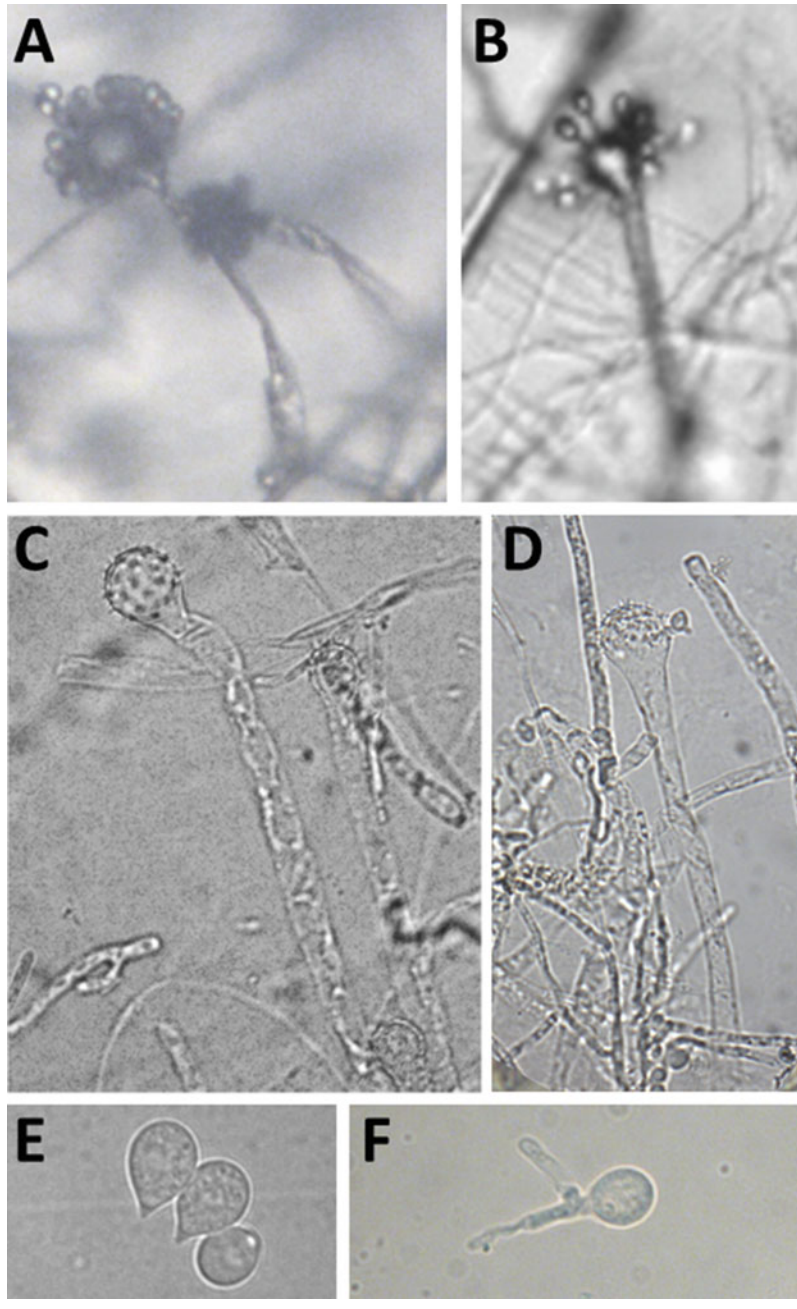


Fig. 12.10 Conidiophores and blastospores of *Heterobasidion irregulare* homokaryon TC32-1, a reference strain with an established genome sequence (Olson et al. 2012). (**a, b**) Conidiophores with spores still attached, and (**c, d**) conidiophores with mace-like appearance after spores broke off from their sterigmata. (**e, f**) Mature and germinated blastospores. The strain was grown on MEA at room temperature (Photos in (**a, b**) were taken without cover slip, photos in (**c, d**) with cover slip, using a 40× objective. For photos in (**e, f**) a 100× objective was used. All photos were taken by BD)

of blastospore formation on denticles, singular or in tufts, on morphologically undifferentiated hyphal cells, as described first for monokaryotic and dikaryotic mycelia of *Galzinia incrustans* from the Corticales. Blastospore formation in *G. incrustans* starts with a small peg that extends into a tube, the end of which blows up into the spore. The spore separates from the denticle by cell wall formation and splitting. Denticles in dikaryons may form on clamp cells. Over 80 % of blastoconidia from a dikaryon are binucleate and germinate into a dikaryon, whereas >10 % are monokaryotic and the rest is trinucleate (Nobles 1937; Clémençon 1997, 2000, 2012). *Corticum botryosum*, *Botryobasidium conspersum* and other Botryobasidiaceae also produce single blastoconidia on denticles, whereas blastoconidia in *Hypochnus coronatus* and in *Botryobasidium simile* arise in long chains of inflated spores without denticles (Hughes 1953; Clémençon 1997, 2004, 2012).

Other species appoint distinct conidiophores for blastoconidia production (Stalpers 1978, 1988). In *Clavaria quisquiliaris*, a first blastoconidium is generated at the apex of a conidiogenous hyphal tip. Subsequently, the next conidium initiates by lateral budding underneath the first spore. With increase in spore size, the new spore stretches in position into the axis of the conidiogenous hypha, while the first spore is pushed away. Another sideward budding can follow (Clémençon 1997, 2004). In the same manner, blastospores of *Baeospora myosura* appear sympodially on differentiated sporophores and secede schizolytically (Kendrick and Watling 1979; Walther et al. 2005). Also in the same manner as in *C. quiquiliaris*, holoblastic conidia are generated on tip cells of conidiophores in *Typhula incarnata* (Remsberg 1940; Metzler 1987a) and *Typhula graminum* (Olariaga et al. 2008).

Mitospores produced on *Typhula trifolii* monokaryons in contrast have been described as oidia (3.6×0.8 µm) born in clusters of single spores on tips of very short and thin sidebranches (Noble 1937).

Uninucleate blastoconidia arise sympodially from conidiophores that grow out from hyphal cells or from clamps of dikaryotic mycelia of *Hydnum raduloides*. Only a single nucleus migrates from a parental dikaryotic cell into a conidiophore (Maxwell 1954). *Fistulina hepatica* also has a blastic mode of mitotic spore formation. Spores are generated at random, in seemingly retrogressive sequence or in dense clusters at the tips of conidiophores. Some immature blastoconidia at lowest position may grow out into further conidiogenous branches on which then more spores are formed. Cell walls of the round inflated blastospores may thicken, even to degrees as expected to be found for thick-walled chlamydospores (Stalpers and Vlug 1983; Buchalo 1988; Buchalo et al. 1985, 2011b). *Clavaria micans* is special since it is one of the very few known cases in Agaricomycetes with **anneloconidia**. Its mitospores bud off one after the other from a tip of conidiophore, each leaving behind a collar-like ring on the conidiogenous cell at the place of spore liberation (Koske and Perrin 1971; Clémençon 1997, 2000, 2012).

Eye-catching are species of the Russulales with *Spiniger*-types of conidiophores (Stalpers 1974; Kendrick and Watling 1979; Fig. 12.10). *Corticium effuscatum* (Nobles 1942), *Hyphoderma mutatum* (Nobles 1935), *Hyphoderma populneum*, *Bondarzewia berkeleyi*, *Paullicorticium curiosum* (Stalpers 1974), *Dichostereum effuscatum* (Clémençon 1997, 2004, 2012), *Dichostereum granulosum*, *Laurilia sulcata* and *Resinicium furfuraceum* (Maxwell 1954) have such types of conidiophores. The best described case of blastic sporogenesis on *Spiniger*-type conidiophores concerns however *H. annosum* s.l. (Brefeld 1889; Stalpers 1974; Fig. 12.10). *Spiniger* conidiophores are abundantly generated on homokaryotic and heterokaryotic mycelia of *Heterobasidion* species (Brefeld 1889; Stalpers 1974, 1978; Korhonen and Stenlid 1998; Tokuda et al. 2009; Paul et al. 2012). The club-like conidiophores are long erect aerial hyphae of variable lengths (up to 300 µm; Tokuda et al. 2009; Paul et al. 2012) with an inflated vesicle at the top on which multiple denticles arise (Greig 1998; Fig. 12.10a–d), each of which produces a single ovoid to pyriform smooth blastospore (Fig. 12.10a, b, e, f). Conidiophores might arise singly as unbranched structure or they might carry at the upper ends short side branches ending in inflated vesicles producing

also blastospores on short denticles. Multiple conidiophores can also group into brush-like conidiomes (Brefeld 1889). Blastospores are of similar sizes (about 4.5–8.0 × 3.0–6.0 µm) than the encrusted basidiospores but have smooth surfaces (Stalpers 1974; Korhonen and Stenlid 1998; Tokuda et al. 2009; Paul et al. 2012). They may have only one nucleus but usually carry two or more nuclei. When coming from a heterokaryon, they can be either homokaryotic or heterokaryotic (Griffin and Wilson 1967; Ahrberg 1975; Stenlid 1985; Hsiang et al. 1988; Korhonen and Stenlid 1998). Spore populations from heterokaryons can in statistical random manner roughly divide into 25 % of one mating type, 25 % of the other mating type and 50 % of heterokaryotic cells (Hsiang et al. 1988), but large variations between different strains have been reported including preferences for recovery of spores of only one distinct mating type (Ramsdale and Rayner 1994, 1996; James et al. 2008, 2009).

Nobles (1935) reported for *H. mutatum* that one nucleus migrates into vesicles of conidiophores of homokaryons and a pair of nuclei into vesicles of conidiophores of heterokaryons. Several mitoses follow to give 16 or more nuclei that will individually migrate through denticles into the blastospores. Accordingly, in this species all blastospores will be uninucleate.

E. Chlamydospore Production

Thick-walled swollen chlamydospores are broadly distributed types of asexual spores in the Agaricomycetes (Nobles 1948; Stalpers 1978; Kendrick and Watling 1979; Buchalo 1988; Buchalo et al. 2009; Reshetnikov 1991; Clémençon 1997, 2004, 2012; Fig. 12.3; Table 12.1) although Watling (1979) and Walther et al. (2005) reckon that in the Agaricales arthrospores are the most prominent types of mitospores.

Often, chlamydospores are intercalarily produced within existing hyphae, singly in hyphal cells or also in chains over the length of a hypha (Fig. 12.3a–i), but there are also terminally produced thick-walled swollen solitary spores (Kendrick and Watling 1979; Figs. 12.3j–l; 12.11) or single swollen chlamydospores or short chains of 2 or 3 swollen cells arising from conversion of very short side branches (Fig. 12.3m). Terminal chlamydospores by some authors are distinguished as aleuria or aleuriospores (Barron 1968; Clémençon 1997, 2004, 2012), whereas others reject this term as confusing and obsolete (Kendrick and Watling 1979; Kirk et al. 2008). Another subtype of chlamydospores is presented by blastocysts (blastic chlamydospores) born from hyphal cells by budding, transfer of the whole cell content including the residing nuclei (without extra mitosis) from the mother cell into the bud, swell growth and subsequent cell wall strengthening (Clémençon 1997, 2004, 2012). Cytological descriptions of chlamydospores and even more their modes of production in most species tend to be poor (Kendrick and Watling 1979; Reshetnikov 1991). Typically, there are no special mechanisms for release of chlamydospores from the mycelium, unlike for other mitospores that are liberated by breaking off from the parental mycelium in rhexolytic or schizolytic secessions. Chlamydospores in contrast may stay within their hyphae until these degenerate with time.

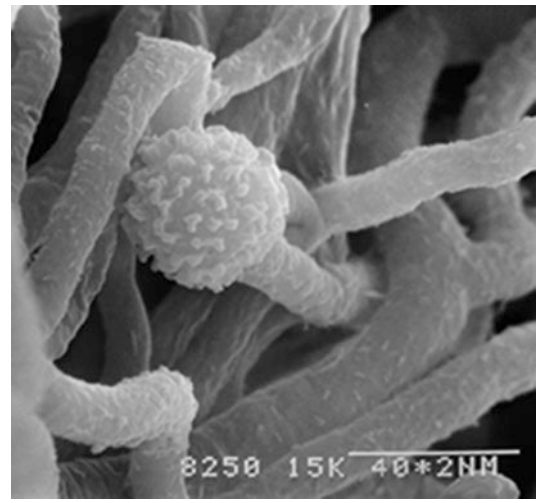


Fig. 12.11 Scanning electron microscope picture of a terminal chlamydospore of *Hypholoma fasciculare* dikaryon 0395 (Photo taken by SMB. Reprinted from Garibova et al. (1986) with permission)

Chlamydospores were first apparent in history on fruiting bodies of the mycoparasite *A. lycoperdoides* and *A. parasitica* where pileus tissues and gill trama, respectively, are converted

into chlamydospores. Similar chlamydospores can be produced in cultures of these homothallic species, as single spores on hyphae that possess clamp cells (Brefeld 1889; Buller 1924; Thompson 1936; Watling 1979; Koller and Jahrmann 1985). The chlamydospores are binucleate and contain glycogen granules and lipid droplets (Brefeld 1889; Clémençon 1997, 2004, 2012) and will germinate into clamped mycelia (Buller 1924). The process of chlamydospore production involves the steps of swelling of the mother cell with stretching of its cell wall, cytoplasm condensation, secretion of lipids and production of the thickened inner spore cell wall. Mature chlamydospores thus are covered by two cell wall layers, by the outer wall of the mother cell (the 'mantle') and by the inner thickened wall formed during spore production (Watling 1979; Clémençon 1997, 2004, 2012). Chlamydospores of *A. parasitica* have a smooth surface (Redhead et al. 1994; Clémençon 1997, 2004, 2012). The stellate chlamydospores of *A. lycoperdoides* in contrast have characteristic blunt projections irregularly distributed over the spore surface (Koller and Jahrmann 1985; Redhead et al. 1994). These projections originate from upfolding of the hyphal cell wall during swelling of the clamped mother cell which happens prior to the cytoplasm condensation (Clémençon 1997, 2004, 2012). A stellate form of chlamydospores as in *A. lycoperdoides* is rare and further only known from a few other species including *Tomophagus colossus* (Stalpers 1978; Adaskaveg and Gilbertson 1989; Parihar et al. 2013), *Lepista flaccida* (Clémençon 2003) and *Sarcodon imbricatus* (Agerer 1991). Stellate chlamydospores of *L. flaccida* arise in swollen dikaryotic hyphae with granular content in a similar mode as described for *A. lycoperdoides*. The resulting thick-walled spores are binucleate (Clémençon 2003).

Chlamydospores as apical and/or intercalary elements were observed in the pileipellis and pileitrama of fruiting bodies of species from various orders of Agaricomycetes. Examples are *Albatrellus ellisii*, *Albatrellus flettii*, *Antrodia pulverulenta*, *Boletus chlamydosporus*, *Botryobasidium baicalinum*, *Crinipellis pedemontana*, *Ganoderma subamboinense* and other *Ganoderma* species, *Inonotus rickii*, *Inonotus nidus-pici*, *Lentinellus* species, *T. colossus* and *Squamanita* species (Reshetnikov 1991; Segedin 1996; Gottlieb et al. 2002; Hong and Jung 2004; Petersen and Hughes 2004; Plątek and Cabała 2005; Albee-Scott 2007; Kotiranta and Ryvarden 2007; Vizzini et al. 2007; Douanla-Meli and Langer 2009; Dai et al. 2010; Parihar et al. 2013; Spirin et al. 2013). The *Inonotus* species give rise to normal basidiomes with hymenia and in addition to imperfect fruiting-body-like hymenium-less assemblies that are semi-spherical or cushion-like, soft and fleshy at first and that with age disintegrate into a mass of globose to ovoid golden-brown chlamydospores (Barnard 1993; Gottlieb et al. 2002; Melo et al. 2002; Plątek and Cabała 2005; Annesi et al. 2010) which also form in chains (1–20 × 8–12.5 μm) in cultures on clampless hyphae (Davidson et al. 1942; Stalpers 1978, 2000; Gottlieb et al. 2002; Ramos et al. 2008). Verrucous chlamydospores are typically found in the context of fruiting bodies of *Postia brunnea*, *Postia ptychogaster*, *Postia renneyi* and *Postia stellifera* sp. nov., whereas chlamydospores in cultures of these species are smooth except in *P. stellifera* (Ryvarden and Gilbertson 1994; Rajchenberg and Buchanan 1996; Stalpers 2000; Hattori et al. 2010). Thick-walled blastosporal structures on denticles, interstages between blastoconidia and thick-walled chlamydospores, arise in *Hemimycena conidiogena* fruiting bodies on the surface of the stipes (Moreau et al. 2005).

Chlamydospores within fruiting bodies can have similar morphologies and ornamentations as basidiospores (Albee-Scott 2007). In addition to the verrucose meiotic basidiospores, secondary apomitic spores (9–11 × 6–8 μm) occur in conks of *Ganoderma* species. These thick-walled chlamydospores of *Ganoderma* are known under a specific name gasterospores. They are ovoid with truncated apex, brown, verrucose, and double-walled with a hyaline epispore and can be of alike appearance of the basidiospores or sometimes also of more simple look (Banerjee and Sarkar 1959; Steyaert 1972, 1980). Gasterospores arise terminally on peg-like protrusions on thick-walled, hyaline or brown-colored hyphae (Banerjee and Sarkar 1959). As another special case, Ingold (1988) reports for *Marasmius oreades* that round chlamydospores arise as single secondary spore type within germinating basidiospores and in inflating germtubes when required nutrients are too few for further mycelial growth. Similar observations exist for *F. fomentarius* (Mukhin and Votintseva 2002).

Chlamydospores of mycoparasitic *Squamanita* species generate as transformations of

clamped hyphal mother cells in galls formed from parasitised fruiting bodies of other basidiomycetous species. Species distinguish in spore surfaces. *Squamanita contortipes* has rugose walls, *Squamanita pearsonii* pitted walls and *Squamanita paradoxa* smooth surfaces (Redhead et al. 1994).

Chlamydospores have moreover been detected in nature as dense mats on the ectomycorrhizal mantle of *Hydnellum peckii* (intercalarily or subterminal produced warty spores, 11–13 µm in Ø) and on the mantle of *Hydnellum caeruleum* (single-walled, spherical to ellipsoid smooth spores) and on rhizomorphs of *Phellodon niger* (terminally or mostly intercalarily formed round smooth endospores, 8–10 µm in Ø), *S. imbricatus* (intercalarily produced in thin-walled, easily collapsing hyphae as solitary stellate dark-brown endospores with hollow warts, 3–5 µm in Ø with 3–5 µm long outgrowths), *Boletopsis leucomelaena* (formed in oidia-like cells), *Bankera fuligineoalba* and other ectomycorrhizal Bankeraceae (Danielson 1983; Agerer 1991, 1992, 1993, 2006; Kernaghan 2001). An example with chlamydospores on rhizomorphs from the ectomycorrhizal Entolomataceae is *Entoloma clypeatum* f. *hybridum* (Kobayashi and Yamada 2003). *Tricholoma* species form terminal chlamydospore-like round thick-walled swellings on hyphae on mycorrhizal root tips (Gill et al. 1999; Yamada et al. 1999). Chlamydospores of *Tricholoma bakamatsutake* were observed on the surface of shiro (mycelial blocks in soil from which fruiting body development starts; Terashima et al. 1993) and of *Tricholoma matsutake* within the root cortex of artificially infested *Pinus* species (Yun et al. 1997). Similar structures are known from laboratory cultures of distinct *Tricholoma* species (Shimazono 1979).

Masses of smooth thick-walled chlamydospores are generated by the endophytic root coloniser *P. indica* in planta. The pear-shaped spores are formed in root hairs where the fungus enters a host and inside and outside of host cells in the root cortex tissue (Verma et al. 1998; Peškan-Berghöfer et al. 2004; Waller et al. 2005). Pear-shaped chlamydospores (16–25 × 10–17 µm) arise in culture as terminal propagules on clusters of short straight hyphal branches or on hyaline somewhat inflated moniliform hyphae characterised by irregular swellings near the point of first spore formation. With cell wall thickening, the spores adopt a yellow colour. They have multiple nuclei, between 8 and 25, and can germinate in culture into small hyphae (Sun and Oelmüller 2010; Kost and Rexer 2013).

Within laboratory cultures, abundant chlamydospores are generated in dikaryons of *Leucopaxillus amarus*. As in *Asterophora* species, the spore-forming process starts in clamped cells by stretching of the hyphal cell wall, swelling of the mother cell and condensation of the cytoplasm. The cell content will be retracted, retraction-septa are formed at both ends of the condensed protoplast to separate the spore from the hyaline part of the hyphal mother cell, and finally cell walls will be thickened (Pantidou et al. 1983). Similar courses of development may be undertaken in dikaryotic mycelium of *Ganoderma* species (Badalyan et al. 2015; Fig. 12.3a–e), *Agrocybe cylindrica* and *Gymnopilus pampeanus* (Sede and Lopéz 1999) and *Rhodotus palmatus* (Doguet 1956). Chains of smooth chlamydospores are produced from short clamped cells of multiply branched side hyphae in *Postia* species, or they generate terminally or intercalarily within longer somatic hyphae (Brefeld 1889; Ryvarden and Gilbertson 1994; Rajchenberg and Buchanan 1996; Hattori et al. 2010). When produced within longer hyphal cells, retraction-septa are formed upon protoplast condensation (Hattori et al. 2010). In *Lentinus tigrinus*, chlamydospore production is mainly intercalary with steps of retraction of cytoplasm, retractive septum formation, local hyphal swelling and thick cell wall production. This species in addition forms on short thin hyphae chlamydospores in blastic mode (Clémençon 1997).

Glycogen-rich chlamydospore-like structures appear further in filaments of *Cryptococcus* yeasts (Kurtzman 1973; Lin and Heitman 2005). Morphological similar shaped-round cells have recently been observed in widest hyphae within the gelatinous loosely interwoven inner zone in a fruiting body of *Auricularia auricula-judae* (Kües and Navarro-Gonzaléz 2015).

In *S. commune*, ellipsoid chlamydospores are formed in monokaryotic and dikaryotic

mycelium by intercalary septations of deformed hyphal cells (Nobles 1948; van der Westhuizen 1958; Wesssels 1965; Koltin et al. 1973; Fig. 12.3f). Intercalary septa are devoid of the typical basidiomycetous septal dolipore apparatus. When spores are formed adjacent to a pre-existing septum, this will thicken to close the doliporus first into a narrow channel and eventually also fully. The cell wall components of chlamydospores do not differ from those of the parental vegetative hyphae (Koltin et al. 1973). Chains of short rounded cells arise with condensed cytoplasm and thick cell walls which with time will detached from the hyphal network (Nobles 1948; Wesssels 1965; Koltin et al. 1973). Chlamydospores originating from monokaryons and from dikaryons might be uni-, bi- or seldom also trinucleate. Chlamydospores from dikaryons germinate into monokaryons of either one of the parental mating types (Koltin et al. 1973). Chlamydospores of *S. commune* have been detected in vessels of infected wood (Peddireddi 2008). Other wood-inhabiting species (*Antrodia carbonica*, *L. sulphureus*, *R. palmatus*, *Rhodonia (Postia) placenta*) also produce chlamydospores within wood vessels (Marryat 1908; Powell 2002; Schwarze et al. 2004; Schwarze 2007) or in larger amounts on surfaces of wood (e.g. *Ganoderma* species and *Leucogyrophana mollis*; Fernando 2008; Mattsson et al. 2010).

Thick-walled chlamydospores in vicinity of clamped mycelium of a fossil fungus *Palaeofibulus antartica* have been observed in a lacunar canal of a fossil plant stem (Dennis 1969; Osborn et al. 1989).

Chlamydospores for long-term survival of the dung fungus *C. cinerea* are found in brown-coloured patches at the air-agar interface of mycelial mattings in aging cultures of both monokaryons and dikaryons, while chlamydospore production on dikaryons is more common (Bensaude 1918; Lewis 1961; Andersen 1971; Kües et al. 1998a). The nuclear condition of chlamydospores from dikaryons is binucleate (Day 1959). Chlamydospores in *C. cinerea* are variable in form; they may be round or oval or also irregularly shaped (Lewis 1961; Andersen 1971; Kües et al. 2002a). There appear to be two distinct modes of generation: In the first more common mode, thick-walled inflated spores develop endogenously within cells of aging hyphae, following the compression of the cytoplasm (Lewis 1961; Andersen 1971; Kües et al. 2002a). Not as often, swollen thick-walled blastocysts appear as an inflated bud at the side of a hyphal cell into which the compressed cytoplasm from the hyphal cell is transferred (Kües et al. 2002a).

Swollen thick-walled hyphal segments resembling stages of chlamydospore development are multiply observed in mattings of mono- and dikaryons of *C. cinerea*. In the dikaryons, these swollen segments are connected to glycogen storage as repositories for food for preparation of fruiting (Madelin 1960; Waters et al. 1975). Appearance of such inflated glycogen-rich cells in mycelial mattings correlates further with production of the multicellular brown-rinded sclerotia serving as resting bodies (Fig. 12.1). Glycogen-rich cells play also a role in the differentiation process of sclerotia: Glycogen stored in cells of the central region of the developing sclerotia will be mobilized in favour for the formation of thick hyaline secondary cell walls in the outer rind that will cover the rounded swollen cells filling the inner medulla of the mature sclerotia (Volz and Niederpruem 1970; Waters et al. 1972, 1975). These medullary cells are morphologically alike to chlamydospores (Volz and Niederpruem 1970; Waters et al. 1972, 1975; Kües et al. 2002a), inferring further a close linkage of production of sclerotia with its large thick-walled medullary cells and of chlamydospores in the free mycelium (Kües 2000; Kües et al. 2002a; Badalyan et al. 2011a).

Terminal holothallic thick-walled propagules or short chains of yellow spores occur in *Fibulochlamys ferruginosa* as transitions of short clamped cells on unbranched sporogenous hyphae (Romero et al. 1989) and in *Fibulochlamys chilensis* on branched hyphae (Madrid et al. 2010). Spores are freed by a rhexolytic mode. Spores in *F. ferruginosa* are smooth, while those from *F. chilensis* are wrinkled with age (Romero et al. 1989; Madrid et al. 2010). In *Pholiota adiposa*, terminal thick-walled spores are produced on unbranched and branched stunted side hyphae. The short sporogenous propagations arise on clamped parental hyphae in submerged and aerial mycelium and are often not separated by septa from the parental cells (Cartwright 1929; Nobles

1948; Buchalo et al. 1985). Thick-walled spores in *P. adiposa* however are produced by mode II of conidiogenesis with thallic and also blastic features (Sect. II.A) defined by Walther and Weiß (2008) for the Strophariaceae.

Other examples for production of mainly terminal chlamydospores on short single side hyphae or on bunches of multiply branched short side hyphae are *F. velutipes* (Badalyan et al. 2006), *L. sulphureus* (Nobles 1948; Stalpers 1984; Clémençon 1997, 2004, 2012; Badalyan and Sakeyan 2004; Fig. 12.3k, l), *H. fasciculare* (Garibova et al. 1986), *Lentinellus cochleatus* (Clémençon 1997, 2004, 2012) and *Phanerochaete chrysosporium* (Burdsall and Eslyn 1974). Terminal ovoid chlamydospores in *S. sumptuosa* are produced in clusters on multiply branched clamped hyphae, and they can collect in slimy heads together with the thin bean-shaped arthroconidia (Clémençon 2000; Sect. II.A). Thick-walled mitospores in *Volvariella volvaceae* are blastocysts laterally formed on hyphal cells that donate their complete cell content with many nuclei into the spores (Clémençon 1997, 2004, 2012).

III. Regulation of Asexual Spore Formation

Principally, asexual spores might be formed on the monokaryon or on the dikaryon or on both (Table 12.1; Sect. II). Such latter situation is best known for *C. cinerea* (Fig. 12.1). This mushroom forms abundant oidia in the aerial mycelium of the monokaryon (Sect. II.C; Fig. 12.5e) and may produce smaller numbers of oidia on the dikaryon when growing in light (Bensaude 1918; Brodie 1931; Swamy et al. 1984; Kertesz-Chaloupková et al. 1998; Kües 2000; Polak et al. 2001). Moreover, the monokaryon may produce some chlamydospores in the aging submerged mycelium, but the dikaryon produces many more (Andersen 1971; Kües et al. 1998a, 2002a). In this species, some insight exists on the regulation of formation of the different types of spores. Oidia production on the monokaryon is constitutive, whereas it is regulated by mating-type genes and by light in the dikaryon (Kertesz-Chaloupková et al. 1998). Mating-type genes and light also take part in the dikaryon in the regulation of chlamydospore formation (Kües et al. 1998a, b, 2002b). Chlamydospore production in dikaryons happens more regular and copious than in monokaryons and preferentially in the dark (Andersen 1971; Kües et al. 1998a).

C. cinerea as a typical tetrapolar basidiomycete has two distinct mating type loci, *A* and *B (matA* and *matB)* which contain the master genes for regulation of dikaryon development and sexual reproduction. For successful dikaryon formation, two fusing monokaryons must be different at both loci. The *A* locus (or *HD* locus) encodes two types of homeodomain transcription factors (HD1 and HD2), the *B* locus (or *P/R* locus) pheromones and pheromone receptors. For dikaryon formation, an HD1 protein encoded in one *A* allele (from one mating monokaryon) has to interact with an HD2 protein of another *A* allele (from the other mating monokaryon) in order to form a functional transcription factor complex that can migrate into the nucleus and bind to promoters of *A*-regulated genes for control of downstream *A*-regulated pathways. Likewise, a pheromone encoded in one *B* allele must interact with a receptor coming from the other *B* allele in order to start a pheromone signalling pathway that culminates in the nucleus in tunings of expression of genes of the *B* pathway. In dikaryon formation and maintenance, the *A* genes regulate nuclear pairing, clamp cell production and synchronized nuclear division whereas the *B* genes control the reciprocal nuclear migration into mycelium of opposite mating type for establishing the dikaryon and subsequently in dikaryon growth the clamp cell fusion (Casselton and Olesnicky 1998; Kües et al. 2011; Kües 2015; Raudaskoski 2015; see also Kothe et al. 2016).

In the monokaryon, the *A* mating-type genes are not required. A knock-out of the *A* locus grows as a normal monokaryon and produces abundantly oidia (Pardo 1995; Polak 1999). Knock-outs for the *B* locus are not yet available, but also their genes are not expected to have an essential function in the growth and development of monokaryons (Pardo 1995).

The *A* mating-type genes in *C. cinerea* repress oidiation in the dikaryon when growing in the dark (Tymon et al. 1992; Kües et al. 1994, 1998a). Light signals override this *A*-mediated repression, while the *B* mating-type genes counteract the negative effect of light on *A* regulation (Kües et al. 1998a, 2002b). These observations were confirmed in repetitive experiments (Srivilai et al. 2009). Notably, activation of the *B* mating-type pathway alone has no effect on oidia numbers. Oidiation in *Bmut* strains

(self-activated by mutations within the *matB* locus) and in *B*-activated transformants (monokaryons transformed with *B* genes from foreign *matB* alleles) remains high and constitutive (Kertesz-Chaloupková et al. 1998; Kües et al. 2002b).

Light in the blue range (360–490 nm) is active in controlling developmental processes on the *C. cinerea* dikaryon (Borriss 1934; Madelin 1956; Tsusué 1969; Lu 1974; Kertesz-Chaloupková et al. 1998; Kües et al. 1998a). The effect of light on oidiation within cultures is local and not a systemic event (Kertesz-Chaloupková et al. 1998). Light targets at induction of oidiophore development (Polak et al. 1997a) while oidiophore maturation can occur fully in dark (Kertesz-Chaloupková et al. 1998). An established mycelium keeps competence for light-induction of oidiation for several days (6 days complete, up to 20 days partial). Brief illumination of 1 min already activates oidiation, 10-min illumination of 0.1 μE $m^{-2}s^{-1}$ (blue light) or 0.5 μE m^{-2} s^{-1} (white light) is sufficient for full response. Temperature influences the amount of spores produced per plate. Spore yields at higher temperatures (37 °C, 42 °C) are about 10-fold higher than at 25 °C (Kertesz-Chaloupková et al. 1998; Kües et al. 1998b). Light induction of oidiation is not restricted to defined nutritional conditions as is fruiting body development (Madelin 1956, 1960; Rao and Niederpruem 1969; Kertesz-Chaloupková et al. 1998; Kües et al. 2004) although available metabolizable nutrients will influence absolute spore numbers produced on a mycelium. Nutritional effects on absolute oidia numbers are observed in light-induced oidiation on dikaryon-like strains (*Amut Bmut* homokaryons activated in both mating type pathways) as well as in constitutive oidiation in monokaryons. Too much glucose in a medium leads to reductions in spore numbers (catabolite repression) while amounts of nitrogen are less influential (Walser 1997a, b).

In sum, when growing on complete medium in the dark, a dikaryon may produce 10^6–10^7 spores per 9 cm Ø Petri dish; in total 100- to 1000-fold less than monokaryons do by constitutive spore production. In light, oidiation production on the dikaryon is about tenfold enhanced, while spore numbers as high as at the monokaryon are not reached (Hollenstein 1997; Kertesz-Chaloupková et al. 1998). Moreover, when only the *A* mating-type pathway is activated in a strain (such as by mutation in the *A* locus or such as by transformation of cloned *A* genes into compatible monokaryons), it produces only low numbers of oidia when growing in the dark but as many oidia as the monokaryons when growing in light and *A*-mediated repression on oidiation is fully released (Kertesz-Chaloupková et al. 1998; Kües et al. 1998a, 2002b; Srivilai et al. 2009). In accordance, mutants being blind by defects in genes for putative blue light receptors (*dst1*/*wc1* for a white-collar 1-type receptor; *dst2* for a protein with FAD-binding domain) and other defects in the light signalling pathways do not show an effect in releasing *A*-mediated repression of oidiation (Terashima et al. 2005; Kamada et al. 2010; Kuratani et al. 2010; Chaisaena 2008).

Also genes acting within the *A* mating-type pathway affect oidiation in dikaryons of *C. cinerea*. Gene *clp1* encodes a putative transcription regulator that induces clamp cell formation in the dikaryon (Inada et al. 2001). The gene is possibly induced by the *A* mating-type proteins (Brown and Casselton 2001; Kamada 2002; Kües 2015). A mutation in gene *clp1* resulted in somewhat elevated levels of oidia in the dark although in light there was still some increase in spore numbers (Inada et al. 2001). Intact Clp1 in dikaryons negatively affects the functions of gene *pcc1* (Brown and Casselton 2001; Kamada 2002; Kües 2015) for a HMG box transcription factor that negatively regulates clamp cell production (Murata et al. 1998). Defects in *pcc1* cause clamp cell production and allow also fruiting in mutant monokaryons (Uno and Ishikawa 1971; Murata et al. 1998; Murata and Kamada 2009). Furthermore, oidiation in respective mutants renders to become positively light-controlled (Kües 2000). Pcc1 in the wild-type monokaryon blocks thus possibly a dikaryon-specific repressor gene of oidiation (Kües et al. 2002a). Repressor Pcc1 is expressed in both monokaryons and dikaryons of *C. cinerea*, and regulation of the protein must therefore be posttranslational (Murata et al. 1998). Defects in gene *pcc1* in monokaryons lead further to increased cellular cAMP values (Uno and Ishikawa 1971, 1973a, b) and related enzyme activities (adenylate cyclase, phosphodiesterase, cAMP-dependent protein kinase, glycogen synthetase, glycogen phosphorylase; Uno and Ishikawa 1974, 1976, 1978, 1981; Uno et al. 1974), another phenotype known in the dikaryon to be coordinately controlled by the mating-type genes (Swamy et al. 1985a, b) and light (Uno et al. 1974). cAMP levels and cAMP-linked enzyme activities decrease with increasing glu-

cose amounts in a growth medium, while any fruiting abilities disappear in dikaryons and in *pcc1* mutants (Uno and Ishikawa 1974). Nutritional control is thus one possibility of regulating the activities of the Pcc1 transcription factor as well as of regulating any crosstalk between the *A* and *B* mating-type pathways. Nutritional conditions, both C and especially N, appear to influence specifically the *B* mating-type pathway (Kües et al. 2004). Little molecular detail is yet established in *C. cinerea* as how the mating-type pathways interlink with each other and further with the light and nutritional signalling pathways (Kües et al. 2004; Kües 2015). More insight in molecular interconnections exists in the smut fungus *Ustilago maydis* (Brefort et al. 2012). Here, the Clp1 protein is one of the factors that orchestrate mating-type pheromone signalling and homeodomain transcription factor activities. Clp1 binds to and thereby inactivates the HD2 proteins for regulation of *HD*- and *PR*-dependent cell cycle control (Heimel et al. 2010a, b). Another modulator of interest in *U. maydis* is Ubc2 that interacts with MAPKK as one of the upstream kinases in the MAP kinase cascade (Mayorga and Gold 2001; Klosterman et al. 2008) which is triggered by cAMP signalling and the pheromone-activated signalling cascade, respectively (Brefort et al. 2012). The Ubc2 homolog in *C. cinerea*, Cc.ubc2, is a mediator in the crosstalk between the *A* and *B* mating-type pathways. A defect in Cc.ubc2 blocks phosphorylation of a presumptive kinase of the MAP kinase cascade and disturbs *A* and *B* mating-type regulation of developmental processes in the dikaryon. Amongst the observed defects, oidia production in the dark is 100-fold increased (Nakazawa et al. 2011).

Cc.snf5, a gene encoding a putative component of the SWI/SNF chromatin remodeling complex, is essential for sexual development in *C. cinerea*. Gene disruption results in loss of fruiting and blocks clamp cell formation (Ando et al. 2013). Similarly, mutants of gene *Cc.rmt1* for an arginine methyltransferase fail in initiation of fruiting body development and clamp cell production (Nakazawa et al. 2010). A dikaryon-specific defect in regulation of oidia production in *Cc.snf5* and in *Cc.rmt1* mutants was not tested but is quite likely. However, silencing in *C. cinerea* of genes *atr1* and *chk1* acting in DNA damage checkpoint control did still enable the first specific step in fruiting body development (secondary hyphal knot formation), did not fully block clamp cell formation and, of interest here, did not affect levels of oidia formation in dark and in light (de Sena-Tomás et al. 2013).

Oidia produced on a dikaryon of *C. cinerea* are usually uninucleate and tend to be mostly of only one of the two possible mating types. Importantly, alleles of the *A* mating-type locus are influential on the decision which type of spores will be released in higher numbers (Hollenstein 1997). In *C. cinerea*, the dominance in frequency of one mating type in spore recovery can be reversed by insertion of foreign *A* mating-type genes into the respective favoured nucleus (Hollenstein 1997). These transformation data support further an influence of the HD transcription factors on the choice of nuclei released. A model proposes that functional HD1-HD2 transcription factor complexes formed in close vicinity of a nucleus are required to suppress the genes for oidiation in the respective nucleus. Accordingly, it will be the nucleus which is less repressed by the HD1-HD2 transcription factor complexes that is found in the oidia generated on the dikaryon (Polak 1999; Fischer and Kües 2006; Kües et al. unpublished).

Similar hierarchical recoveries of nuclei in dependence of *HD* mating-type genes were reported for uninucleate spores from dikaryons of the tetrapolar *F. velutipes* (Kitamoto et al. 2000). Ninety percent of oidia obtained from *P. microspora* dikaryons germinate into monokaryons indicating that many of the binucleate and polynucleate spores are homokaryotic. Also in this bipolar species, there is a hierarchy of preferential spore recovery by mating type (Hui et al. 1999a, b) as well as for outgrowth of types of monokaryotic hyphae from a dikaryon (Arita 1979; Masuda et al. 1995). Blastoconidia from heterokaryons of *H. annosum* s.l. are in large parts uninucleate, and there is also a strong preference for recovery of only one mating type from a heterokaryon (Ramsdale and Rayner 1994, 1996; James et al. 2008). In these species, mating-type control of dikaryon and heterokaryon development will thus be given up in favour for escape of one type of nucleus from a nuclear partnership. When mitospores

with only one type of nuclei are distributed to new environments, this would give them a chance to find a new mating partner for possibly forming a better, probably more fit dikaryon (Kües 2002; Kües et al. 2002a).

The various morphological observations on spore production on dikaryotic mycelia of Agaricomycetes described in the sections above which involve local production of clamp-less hyphae or outgrowth of conidiogenous hyphae from clamp cells of dikaryotic hyphae might indicate that mating-type control is somehow also given up for spore formation in such species. The nuclear states of spores will need to be analysed to proof this assumption for such species. However, other species seem to proceed a different route and stabilise also for spore production the heterokaryotic situation. For example, once a heterokaryon is formed in *Termitomyces* sp., asexual spore production will be enhanced as compared to homokaryons and only heterokaryotic spores were observed for the species (Nobre et al. 2014).

Aging of mycelia causes loss of light-induced oidia production of *C. cinerea* (Walser 1997b). On the other hand, chlamydospores of *C. cinerea* appear in aging cultures (Andersen 1971; Kües et al. 1998a), and regulation of chlamydospores is quite opposite to regulation of oidia formation (Kües et al. 1998a, 2002b). Chlamydospore production in *C. cinerea* is thus likely a reaction on nutritional depletion. Chlamydospores on the dikaryon are binucleate (Andersen 1971; Esser 2000), and for production of these spores, nuclear interaction is thus not given up. Not surprisingly therefore, more chlamydospores are produced in the dark when the *B* mating-type reaction on *A* mating-type control of development is stronger (Kües et al. 1998a, 2002b). In line with an importance of *B* mating-type genes, it might be further noted from work in *S. commune* that nuclear distances and loss of reciprocal mating-type control of gene expression in the two nuclei in dikaryons are controlled on the one hand by the *B* mating-type genes and on the other hand by nutrition (Schuurs et al. 1998).

Other than that we have some insight in mating type regulation on dikaryons, our understanding of regulation of asexual spore formation is generally rather limited. There are the occasional notes that nutrient sources can play a role, conditions of aeration or also presence of light (Whitney and Bohaychuk 1975; Ingold 1988; Walser 1997a, b; de Koker et al. 2000; Saxena et al. 2001; Geng et al. 2013; see sections above). Xu et al. (2012) isolated 147 differentially expressed genes from sporulating cultures of *G. lucidum* that might have roles in asexual sporulation. The products of 86 of the unigenes possess similarity to known functions in cell organization, signal transduction, cell metabolism and protein biosynthesis and transcription regulation. 13 others had similarities to hypothetical proteins in the databank whereas the remaining 46 appear to be all new. A mutant screening approach in *C. cinerea* identified many morphological changes in oidiophore and spore production and also in spore morphology. Most of the mutations (85 %) simultaneously affect also the fruiting process. However, a number of morphological mutants have altered shapes of oidiophores, or shapes and lengths of oidial hyphae are affected or spores might be much longer or shorter than normal, or they are swollen, shrivelled or branched. In a flocculating strain, the outer fimbriae and the gelatinous outer layer were found to be absent. In another mutant, spores are released through rhexolytic ripping the cells below. Oidia in another mutant are not released. Interesting are further regulatory mutants including constitutive producers and spore-negative strains unable to initiate oidiophore production (Polak 1999). The pool of available mutants holds promise for future work to identify many interesting genes (Fischer and Kües 2006) as do large scale molecular approaches on expressed genes such as started in *G. lucidum* (Xu et al. 2012). In *Heterobasidion irregulare*, transcription of genes for expansins of the cerato-platanin-type has been studied and one gene (*HiCP2*) was specifically expressed in sporulating mycelium (Baccelli et al. 2015).

IV. Conclusions and Prospect

Asexual spore formation in Agaricomycetes is still a largely neglected field of research, although some attention has been given to it in the past, often for taxonomic purposes. The account in this chapter on the multitude of species where one or more types of mitospores have been observed can provide an impression that asexual sporulation in Agaricomycetes is widespread, possibly, much more than has been perceived so far. Descriptions are not necessarily collected from systematic species analysis so that the complete distribution range of abilities for mitospore formation within the Agaricomycetes remains open. Also, different

developmental stages (primary and secondary mycelium, developmental structures) are often not all analysed. For example, the original claim that arthrospore formation on dikaryons is rare (Brodie 1936; Watling 1979; Walther et al. 2005) may be of need of some correction considering the multiple examples of species with oidia production on dikaryons that we found in the literature (see Sects. II.A–II.C). Similarly, the ability for chlamydospore generation might be more differentially perceived since in some species their production can be abundant and thus very obvious (see, e.g. Nobles 1948; Kües et al. 1998a; Hattori and Lee 1999; Saxena et al. 2001; Douanla-Meli and Langer 2009; Badalyan and Sakeyan 2004; Badalyan and Kües 2015), whereas in others it is only scarce (for examples, see Nobles 1948; Valenzuela et al. 1997; Miller and Metheven 2000; Badalyan et al. 2011a, 2015). Formation of blastoconidia appears to be possibly less distributed over the range of Agaricomycetes. Does this restricted distribution reflect a common phylogenetic background, multiple gains or losses of asexual sporulation abilities in evolution? As a support for possibly early gain of distinct modes of sporulation in evolution, in more basal lines of basidiomycetes such as in *Dacrymyces stillatus* (Dacrymycetes), all basic types of mitospores can be generated on vegetative hyphae in multiple modes (Mossebo et al. 2001).

Modes of production are described in some species to certain detail, whereas regulation mechanisms of sporulation in most instances are unknown. At the time being with little knowledge on the cytological and genetic processes of asexual sporulation, it appears best for global overview to continue to distinguish between thallic arthrospore formation, blastic sporulation and endogenous chlamydospore formation as main modes of mitotic sporulation (Watling 1979; Stalpers 1988; Reshetnikov 1991; Clémençon 1997, 2000, 2012), although between different fungal species features in the production of asexual spores are not always as sharply separated (Walther et al. 2005; this report). Spore-type interpretations can be a matter of debate with much space open also for potential misinterpretations (Kendrick and Watling 1979; Clémençon 1997, 2004, 2012) which we are not free from also in this chapter. Only with deeper insight into the cytologies, we might be able to recognise the extent of true overlaps between modes of sporulation or whether they are indeed (all) fully distinct processes.

What we know about regulation focuses mostly around the mating-type genes, thus on the decisions whether asexual spores are formed on the primary or on the secondary mycelia or on both, in the same or in different frequencies, whether mitospores from dikaryons are still heterokaryotic or whether they have escaped into a homokaryotic state. So far, we have no definitive clue in any fungus, not even in the best analysed species *C. cinerea*, on any gene that might be directly involved in the process of sporulation and might be specific for spore formation.

Spore types might be explained by mode of generation, by spore release and by function (Kendrick and Watling 1979). In our spore descriptions, we concentrated in first instance on modes of generation. Where considered, spore release was taken secondarily and functions were treated even further subordinate or were so far ignored. Therefore, a further remaining question addresses the spore functions. Asexual spores serve mainly three not mutually exclusive lines of functions: distribution within the environment, fertilisation of mycelia of different mating type and duration under unfortunate environmental conditions. An outlook on spore functions emphasising their positions for the fungal reproduction, distribution and survival, for modulating population structures and for suppression of competitors in a shared environment therefore finishes this chapter.

Thick-walled chlamydospores with condensed cytoplasm are generally believed to serve the purpose of duration. Usually, they will remain in a substrate until environmental conditions become more favourable for new growth. In this sense, a dikaryotic nature of the spores can be more favourable. Dikaryotic chlamydospores in *C. cinerea* can grow out with one germination hypha that will give rise to a new dikaryotic mycelium. Alternatively, the spores might germinate at both ends to form monokaryotic hyphae, but since these are of

different mating types, they will soon fuse to form again a dikaryon (Andersen 1971; Esser 2000). In wood-inhabiting fungi, there is a tendency that species with chlamydospores survive longer when buried in soil and under flooding (Chang 1996, 2003; Chang et al. 2002) and they resist any heat stress better than species without (Powell 2002; Allen et al. 2014). Fungal competition in wood can be high in complex hierarchies (Boddy 2000). Extremely fluctuating moisture content of wood and desiccation periods favour survival of chlamydosporic species such as *R. palmatus* and *Schizopora paradoxa*, respectively, which can then outcompete in wood those decay species that under other conditions have growth preferences (Boddy and Rayner 1983a; Rayner and Boddy 1988). Species with chlamydospores better survive cryopreservation and lyophilisation (Croan et al. 1999), further supporting their biological role in persistence under adverse environmental conditions. Chlamydospores of *Piptoporus quercinus* germinated well even after longer exposures (for up to 14 d) to extreme cold or heat (–20 °C or 40 °C) and to desiccation (Crockatt et al. 2010). Chlamydospores function not in long-range dispersal in space, but they apparently have a passive role for dispersal in time (Crockatt et al. 2010). Amongst the community of fungal wood colonisers, there can be also mycoparasites (Boddy 2000) that may produce chitinases and glucanases to attack basidiomycetous wood decay fungi (Murmanis et al. 1988; Bruce et al. 1995; Lee et al. 2012; Ujor et al. 2012). Possibly, formation of thick-walled chlamydospores might offer some degree of defence against such attack.

A very interesting case of a rather specialized function, i.e. of capturing amoebae as prey by immature stalked chlamydospores surrounded by sticky mucilage during delayed spore maturation has been reported by Drechsler (1960) for the species *Pagidospora amoebophila*. Once the round thick-walled chlamydospores are matured, the amoeba-capturing ability will be lost. Mature chlamydospores detach from the mycelium by slight disturbances.

In contrast to their chlamydospores, the uninucleate oidia in *C. cinerea* are only short lived and lose within a few days the ability of germination (Hollenstein 1997), and light of shorter wavelength reduces the oidia lifetime even further (Rahman and Cowan 1972). Particularly older literature reported on negative attempts of germination of arthrospores in several other species of Agaricomycetes (Falck 1902; Nichols 1904; Kemp 1975). Whether this is due to a general lack of germination ability of such spores or whether the results were influenced by fast aging of spores such as that observed in *C. cinerea* remains to be tested. However, studies on various wood-rotting species showed that their arthrospores do not help species in long-term survival in soil and under flooding (Chang 1996; Chang et al. 2002).

The biological functions of the oidia are thus different. Oidia production in monokaryons in *C. cinerea* appears to follow up a circadian oscillation (Walser 1997b), and also the light-induced oidiation process in mating-type controlled strains ensures maturation of spores (Polak et al. 1997a; Polak 1999) at times when insects are possibly active and when humidity is high. The elegant experiment by Brodie (1931) with flies and sporulating monokaryotic *C. cinerea* mycelium grown on horse dung showed that the wet arthrospores in sticky liquid attach to insects in order to be distributed onto new horse dung (ectozoochory). There, the short-lived oidia may serve as spermatia for mating with monokaryons already present on the new substrate (Bensaude 1918; Brodie 1931, 1932; Kemp 1977), or in the case of a prevailing dikaryon, there might also be a possibility for the Buller phenomenon to happen, i.e. a mating reaction between a monokaryon and a dikaryon that can result in the formation of a new dikaryon (Kües 2000, 2002; Nieuwenhuis et al. 2013). However, according to Kemp (1975), *C. cinerea* dikaryons appear not to react on the ungerminated spores.

Another fascinating function is the homing reaction of the wet *C. cinerea* oidia with which they attract hyphae of other individuals for cellular fusion. These spores can induce a change in direction of growth of hyphae from the own species independent of their mating type and, importantly, also of hyphae of foreign species. In the first case, a mating reaction might follow the fusion when mycelia are com-

patible; in the latter case, a somatic incompatibility occurs upon fusion and a killing reaction of the hyphae of the unwanted competitor for space and resources starts (Kemp 1975, 1977). Oidial homing has been observed also for other coprinoid species, with wet and with dry oidia, for oidia of *Psathyrella* species, for oidia of *Clitocybe truncicola*, for oidia of *F. velutipes* and conidia of *T. incarnata* (Bistis 1970; Kemp 1977; Metzler 1987b). Oidia seem to secrete a signalling compound upon which other fungi in the closer environment react. In most species, monokaryons react more readily on oidial homing than their dikaryons, with *F. velutipes* being an exception. In species, where spore germination into monokaryons could not be induced, oidial homing with the purpose of interspecies killing is assumed to be the main spore function (Bistis 1970; Kemp 1977). Spermatia function has been demonstrated in *T. incarnata* (Metzler 1987b).

Some spores of other species enable an intimate symbiosis with insects such as in the cases of the wood-rotting *Amylostereum* species whose oidia are either collected into or produced and then transported within mycetangia (intersegmental sacs as special insect organs) by female siricid woodwasps to the fungal substrate where the insects oviposit their eggs (Thomsen 1998; Slippers et al. 2003). A consequence of the obligate mutualistic relationship between the fungi and the woodwasps is that propagation of the fungus to new wooden substrate is largely clonal, resulting in a low overall genetic diversity of the species in nature (Bergeron et al. 2011). Upon infection of living trees by a pathogenic fungus, small spores such as hydrophilic oidia might easily be distributed further in the living trees, such as through the rising liquid in the sapwood (Boddy and Rayner 1983a).

Other symbiotic insect relationships relate to termites. Termites in new colonies start fungal gardens from the sexual basidiospores of *Termitomyces* species, while asexual homokaryotic and heterokaryotic spores serve the fungal propagation by workers within termite colonies (Nobre et al. 2014). Blastoconidia of nematode-capturing *Nematoctonus* and *Hohenbuehelia* species in contrast function in parasitism of nematodes. They can vigorously adhere to eelworms for invasion of the animals upon conidial germination (Drechsler 1941, 1946, 1949; Barron and Dierkes 1977; Koziak et al. 2007a, b).

Many oidia and blastospores of Agaricomycetes are dry spores and are likely to be distributed by wind. High spore numbers are then particularly important for species that are in need to infect trees, stumps and dead wood (Edman et al. 2004a, b). While many of the species employ basidiospores for the dispersal (Edman et al. 2004a, b), *Heterobasidion* species and their antagonist *P. gigantea* might use both airborne sexual and airborne asexual spores for stump infection (Risbeth 1959; Kuhlman and Hendrix 1964; Thor et al. 1997; Redfern and Stenlid 1998; Gunulf et al. 2012; Oliva et al. 2013). While blastic mitospores have been observed in nature on barks of stumps, dead trees and infected roots and in insect galleries in wood (suggesting possible transmission via insects) and while they have reasonable lifetimes (Korhonen and Stenlid 1998; Stenlid and Rayner 1989, 1991; Redfern and Stenlid 1998), basidiospores of *Heterobasidion* are given higher importance in stump infection in nature (Hsiang et al. 1988; Stenlid and Rayner 1989; Möykklen 1997; Redfern and Stenlid 1998), by a possible better growth rate of mycelium originating from basidiospores as compared to blastoconidia (Kuhlman and Hendrix 1964) and by the longevity and high spore-producing capacity of basidiocarps (Redfern and Stenlid 1998). However, systematic studies on this need to be conducted (Gunulf et al. 2012). Both types of spores in *Heterobasidion* are vectors for viruses and transmissions between species are possible (Ihrmark et al. 2002, 2004; Vainio et al. 2011, 2015). Viruses can take influence on population dynamics, with beneficial, cryptic or detrimental effects on individual hosts in dependence of environmental and ecological conditions (Hyder et al. 2013).

Multicellular spores are rare in the Agaricomycetes. There are however exceptional freshwater species that for distribution when in contact with water form on their mycelial surfaces multicellular aero-aquatic propagules referred to in the literature as conidia or conidial bodies. The propagules of *Peyronelina glomerulata* contain 20–30 smooth globular inner cells covered by 7 to 17 incurved spinose outer hyphal arms.

When immersed, the propagules float on the surface of the water due to air that becomes enclosed by the hyphal arms (Fisher et al. 1976; Wongsawas et al. 2009; Yamaguchi et al. 2009). The globose conidial bodies of *Subulicystidium longisporum* are 50–150 mm in Ø and consist of interwoven hyaline clamped hyphae that can retain air between the cells. Characteristic encrusted cystidia project from the surface of conidial balls of *S. longisporum* (Abdullah et al. 1997; Hyde and Goh 1999; Cooper 2005). Propagules of *Bulbillomyces farinosus* up to 400 µm in Ø are formed on clamped stalk cells. They consist of smooth subglobose cells (12–20 × 10–15 µm) surrounded by a hyphal cortex (Cooper 2005). In some other aquatic species, multicellular conidia for distribution in water are generated by breaking off branched hyphae or branched ends or conidiophores (Nawawi 1985; Marvanová and Bärlocher 1988).

Acknowledgements Eline Polak is gratefully acknowledged for the wealth of photos she left from her PhD work on oidiation in *C. cinerea*, René Hermann for the EM pictures of *C. cinerea* oidia and Mónica Navarro-González for ITS sequencing of *Coprinellus* strains. SMB acknowledges a DAAD fellowship for a research stay at the University of Göttingen.

References

Abdullah SK, Guarro J, Figueras MJ, Descals E (1997) Spanish hyphomycetes. XVI Some aero-aquatic conidial fungi. Mycotaxon 61:311–318

Adaskaveg JE, Gilbertson RL (1986) Cultural studies and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* complex. Mycologia 78:694–704

Adaskaveg JE, Gilbertson RL (1989) Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *G. lucidum* and *G. tsugae*. Mycol Res 92:182–191

Agerer R (1991) Ectomycorrhizae of *Sarcodon imbricatus* on Norway spruce and their chlamydospores. Mycorrhiza 1:21–30

Agerer R (1992) Ectomycorrhizae of *Phellodon niger* on Norway spruce and their chlamydospores. Mycorrhiza 2:47–52

Agerer R (1993) Ectomycorrhizae of *Hydnellum peckii* on Norway spruce and their chlamydospores. Mycologia 85:75–83

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Prog 5:67–107

Ahrberg HE (1975) Untersuchungen zur Cytologie und zum Wachstum verschiedener Stämme von *Fomes annosus* (Fr.) Cooke. I. Kernzahlen und DNS-Gehalt in verschiedenen Isolierungen und Entwicklungsstadien. Eur J Forest Pathol 5:287–330 (in German)

Albee-Scott S (2007) The phylogenetic placement of Leucogastreales, including *Mycolevis siccigleba* (Cribbeaceae), in the Albatrellaceae using morphological and molecular data. Mycol Res 111:653–662

Allen S, members of the NAPPO Forestry Panel (2014) ST 05: review of heat treatment of wood and wood packaging. NAPPO (North American Plant Protection Organization, Ottawa, Canada, http://www.nappo.org/en/data/files/download/Science_and_technology_documents/ST%205HeathTreatment-e.pdf

Andersen GE (1971) The life history and genetics of *Coprinus lagopus*. Harris Biological Supplies, Weston-super-Mare

Ando Y, Nakazawa T, Oka K, Nakahori K, Kamada T (2013) *Cc.snf5*, a gene encoding a putative component of the SWI/SNF chromatin remodelling complex, is essential for sexual development in the agaricomycete *Coprinopsis cinerea*. Fungal Genet Biol 50:82–89

Annesi T, D'Amico L, Bressanin D, Motta E, Mazza G (2010) Characterization of Italian isolates of *Inonotus rickii*. Phytopathol Mediterr 49:301–308

Arita I (1979) Cytological studies on *Pholiota nameko*. Rept Tottori Mycol Inst 17:1–118

Aschan K (1952) Studies on dediploidisation mycelia of the basidiomycete *Collybia velutipes*. Svensk Bot Tidskr 46:366–392

Aschan-Åberg K (1960) Studies on mono- and dikaryotic mycelia of *Collybia velutipes*. Phys Plant 13:280–297

Ásgeirsdóttir S, Halsall JR, Casselton LA (1997) Expression of two closely linked hydrophobin genes of *Coprinus cinereus* is monokaryon-specific and down-regulated by the *oid-1* mutation. Fungal Genet Biol 22:54–63

Asiegbu FO, Adomas A, Stenlid J (2005) Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. s.l. Mol Plant Pathol 6:395–409

Baccelli I, Gonthier P, Bernardi R (2015) Gene expression analyses reveal a relationship between conidiation and cerato-platanin in homokaryotic and heterokaryotic strains of the fungal plant pathogen *Heterobasidion irregulare*. Mycol Prog 14:40

Badalian SM, Melik-Khachatrian JH (1994) Investigation of edible agarics mushrooms on not standard cultural media (algae-agar, algae-grave). Proceed YSU 182:103–108

Badalyan SM, Kües U (2015) Morphological characteristics and anamorphs in different collections of xylotrophic basidiomycetous mushrooms. In: Muchin VA, Kovalenko AE, Kurakov AV, Veselkin DV, Shyryaev AG, Paukov AG, Tretyakova AS, Shyryaeva OS (eds) Biodiversity and ecology of fungi and fungiform organisms of the Northern Eurasia. Proceedings of all-Russian conference with international participation. Ural University Press, Yekaterinburg, pp 300–302

Badalyan SM, Sakeyan CZ (2004) Morphological, physiological, and growth characteristics of mycelia of several wood-decaying medicinal mushrooms (Aphyllophoromycetidae). Int J Med Mushrooms 6:347–360

Badalyan SM, Polak E, Hermann R, Aebi M, Kües U (2004) Role of peg formation in clamp cell fusion of homobasidiomycete fungi. J Basic Microbiol 44:167–177

Badalyan SM, Gharibyan NG, Sakeyan CZ (2005) Catalogue of the fungal culture collection at the Yerevan State University, Armenia. Youngstown State University Press, Yerevan

Badalyan SM, Hughes KW, Sakeyan CZ, Helmbrecht E (2006) Morphology, growth characteristics, and genetic variability of the edible medicinal mushroom *Flammulina velutipes* (W. Curt.: Fr.) Singer collections. Int J Med Mushrooms 8:263–278

Badalyan SM, Navarro-González M, Kües U (2011a) Taxonomic significance of anamorphic characteristics in the life cycle of coprinoid mushrooms. In: Savoie J-M, Foulongne-Oriol M, Largeteau M, Barroso G (eds) Proceedings of the 7th international conference on mushroom biology and mushroom products (ICMBMP7). INRA, Bordeaux, pp 140–154

Badalyan SM, Szafranski K, Hoegger PJ, Navarro-González M, Majcherczyk A, Kües U (2011b) New Armenian wood-associated coprinoid mushrooms: *Coprinopsis strossmayeri* and *Coprinellus* aff. *radians*. Diversity 3:136–154

Badalyan S, Shnyreva AV, Iotti M, Zambonelli A (2015) Genetic resources and mycelial characteristics of several medicinal polypore mushrooms (Polyporales, basidiomycetes). Int J Med Mushrooms 17:371–384

Banerjee S, Sarkar A (1959) Spore-forms in sporophores of *Ganoderma lucidum* (Leyss.) Karst. Proc Indiana Acad Sci 49:94–98

Barnard EL (1993) *Inonotus rickii* (anamorph: *Ptychogaster cubensis*): a-wood-decaying fungus of importance to Florida hardwoods. Plant Pathol Circ 357. Florida Department of Agriculture & Consumer Services, Tallahassee, FL. https://www.freshfromflorida.com/content/download/11364/144480/pp357.pdf

Barnett HL (1945) Notes on the culture of *Coprinus asterophorus*. Mycologia 37:194–196

Baroni TJ, Franco-Molano AE, Lodge JD, Lindner DL, Horak E, Hofstetter V (2007) *Arthromyces* and *Blastosporella*, two new genera of conidia-producing lyophylloid agarics (*Agaricales*, *Basidiomycota*) from the neotropics. Mycol Res 111:572–580

Barron GL (1968) The genera of hyphomycetes from soil. Williams and Williams, Baltimore

Barron GL, Dierkes Y (1977) Nematophagous fungi: *Hohenbuehelia*, the perfect state of *Nematoctonus*. Can J Bot 55:3054–3062

Barron GL, Thorn RG (1987) Destruction of nematodes by species of *Pleurotus*. Can J Bot 65:774–778

Bas C (1965) The genus *Squamanita*. Persoonia 3:331–359

Bas C, Thoen D (1998) *Squamanita citricolor*, a new species from Central Africa. Persoonia 17:135–139

Bensaude M (1918) Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes. Imprimerie Nemourienne. Henri Bouloy, Nemours (in French)

Bergeron M-J, Leal I, Foord B, Ross G, Davis C, Slippers N, de Groot P, Hamelin RC (2011) Putative origin of clonal lineages of *Amylostereum areolatum*, the fungal symbiont associated with *Sirex noctilio*, retrieved from *Pinus sylvestris*, in eastern Canada. Fungal Biol 115:750–758

Binninger DM, Skrzynia C, Pukkila PJ, Casselton LA (1987) DNA-mediated transformation of the basidiomycete *Coprinus cinereus*. EMBO J 6:835–840

Bistis GN (1970) Dikaryotization in *Clitocybe truncicola*. Mycologia 62:911–923

Boddy L (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Ecol 31:185–194

Boddy L, Rayner ADM (1983a) Ecological roles of basidiomycetes forming decay communities in attached oak branches. New Phytol 93:77–88

Boddy L, Rayner ADM (1983b) Mycelial interactions, morphogenesis and ecology of *Phlebia radiata* and *P. rufa* from oak. Trans Br Mycol Soc 80:437–448

Boidin J (1971) Nuclear behaviour in the mycelium and the evolution of basidiomycetes. In: Petersen RH (ed) Evolution in the higher basidiomycetes. University of Tennessee Press, Knoxville, pp 129–148

Borriss H (1934) Über den Einfluß äusserer Faktoren auf Wachstum und Entwicklung der Fruchtkörper von *Coprinus lagopus*. Planta 22:644–684 (in German)

Bose SR (1943) Binucleate oidia on binucleate mycelium of *Polystictus hirsutus* Fr. Nature 152:508–509

Botha WJ, Eicker A (1991) Cultural studies in the genus *Termitomyces* in South Africa. Mycol Res 95:444–451

Brefeld O (1877) Botanische Untersuchungen über Schimmelpilze. III. Heft: Basidiomyceten I. Verlag von Arthur Felix, Leipzig (in German)

Brefeld O (1889) Untersuchungen aus dem Gesamtgebiete der Mykologie. Basidiomyceten III, vol 8. Verlag von Arthur Felix, Leipzig (in German)

Brefort T, Doehlemann G, Mendoza-Mendoza A, Reissmann S, Djamei A, Kahmann R (2012) *Ustilago* as a pathogen. Annu Rev Phytopathol 47:423–445

Brodie HJ (1931) The oidia of *Coprinus lagopus* and their relation with insects. Ann Bot 45:315–344

Brodie HJ (1932) Oidial mycelia and the diploidization process in *Coprinus lagopus*. Ann Bot 46:727–732

Brodie HJ (1935) The oidia of *Psilocybe coprophila* and the pairing reactions of monosporus mycelia. Can J Bot 12:661–667

Brodie HJ (1936) The occurrence and function of oidia in the hymenomycetes. Am J Bot 23:309–327

Brown AJ, Casselton LA (2001) Mating in mushrooms: increasing the chances but prolonging the affair. Trends Genet 17:393–400

Bruce A, Srinivasan U, Staines HJ, Highley TL (1995) Chitinase and laminarinase production in liquid culture by *Trichoderma* spp. and their role in biocontrol of wood decay fungi. Int Biodeterior Biodegrad 35:337–353

Buchalo AS (1988) Higher edible basidiomycetes in pure culture. Naukova Dumka, Kiev (in Russian)

Buchalo AS, Šašek V, Zakordonec OA (1985) Scanning electron microscopic study of anamorphs of some *basidiomycetes* in culture. Folia Microbiol 30:506–508

Buchalo A, Mykchaylova O, Lomberg M, Wasser SP, Volz PA, Nevo E (eds) (2009) Microstructures of vegetative mycelium of macromycetes in pure culture. Alterpress, Kiev

Buchalo AS, Wasser SP, Mikchaylova OB (2011a) Micromorphological characteristics of edible and medicinal macromycetes in pure culture. In: Buchalo AS, Babitskaya WG, Bisko NA, Wasser SP, Dudka IA, Mitropolskaya NY, Mikchaylova OB, Negreiko AM, Poedinok NL, Solomko EF (eds) Biological characteristics of medicinal macromycetes in culture, vol 1. Alterpress, Kiev, pp 105–134 (in Russian)

Buchalo AS, Wasser SP, Mykchaylova OB, Bilay VT, Lomberg ML (2011b) Taxonomic significance of microstructures in pure cultures of macromycetes. In: Savoie J-M, Foulongne-Oriol M, Largeteau M, Barroso G (eds) Proceedings of the 7th international conference on mushroom biology and mushroom products (ICMBMP7). INRA, Bordeaux, pp 50–57

Buller AHR (1924) Researches on fungi, vol 3. Longmans Green & Company, London

Buller AHR (1931) Researches on fungi, vol 4. Longmans Green & Company, London

Buller AHR (1933) Researches on fungi, vol 5. Longmans Green & Company, London

Burdsall HH Jr, Eslyn WE (1974) A new *Phanerochaete* with a *Chrysosporium* imperfect state. Mycotaxon 1:123–133

Butler GM (1972) Nuclear and non-nuclear factors influencing clamp connection formation in *Coprinus disseminatus*. Ann Bot 36:263–279

Cáceres O, Kirschner R, Piepenbring M, Schöfer H, Gené J (2006) *Hormographiella verticillata* and an *Ozonium* stage of *Coprinellus domesticus*. Antonie Van Leeuwenhoek 89:79–90

Capelari M (1999) First record of *Antromycopsis macrocarpa* for Brazil. Mycotaxon 72:101–105

Cartwright KSG (1929) Notes on basidiomycetes grown in culture. Trans Br Mycol Soc 14:300–305

Casselton LA, Olesnicky N (1998) Molecular genetics of mating recognition in basidiomycete fungi. Microbiol Mol Biol Rev 62:55–70

Castle AJ, Boulianne RB (1991) Fimbrial proteins of *Ustilago violaceae*, *Coprinus cinereus*, and *Schizophyllum commune*. MSA Newslett 4:8

Chaisaena W (2008) Light effects on fruiting body development of wildtype in comparison to light-insensitive mutant strains of the basidiomycete *Coprinopsis cinerea*, grazing on mites (*Tyrophagus putrescentiae*) on the strains and production of volatile organic compounds during fruiting body development. PhD thesis, University of Göttingen, Göttingen, Germany

Chang T-T (1996) Survival of *Phellinus noxius* in soil and in roots of dead host plants. Phytopathology 86:272–276

Chang TT (2003) Effect of soil moisture content on the survival of *Ganoderma* species and other wood-inhabiting fungi. Plant Dis 87:1201–1204

Chang TT, Chou WN (1995) *Antrodia cinnamomea* sp. nov. on *Cinnamomum kanehirai* in Taiwan. Mycol Res 99:756–758

Chang TT, Wu ML, Fu CH, Fu CH (2002) Survival of four *Ganoderma* species and several wood-inhabiting fungi in different soil matrix potentials. Taiwan J For Sci 17:43–53

Clémençon H (1997) Anatomie der Hymenomyceten. F. Flück-Wirth, Teufen (in German)

Clémençon H (2000) Mycelial morphology, mitospores and primordium formation of *Simocybe sumptosa* in laboratory cultures. Persoonia 17:407–433

Clémençon H (2003) Stellate chlamydospores and thromboplerous hyphae in the mycelium of the agaric *Lepista flaccida*. Mycol Prog 2:69–71

Clémençon H (2004) Cytology and plectology of the Hymenomycetes, vol 199, Bibliotheca mycologica. J. Cramer, Berlin

Clémençon H (2012) Cytology and plectology of the hymenomycetes, 2nd revised edn. J. Cramer in der Gebr. Borntraeger Verlagsbuchhandlung, Stuttgart

Cooke RC, Godfrey BES (1964) A key to the nematode-destroying fungi. Trans Br Mycol Soc 47:61–74

Cooper JA (2005) New Zealand hyphomycete fungi: additional records, new species, and notes on interesting collections. NZ J Bot 43:323–349

Cripps CL, Caesar TJ (1998) A conidia-forming basidiomycete in the Pterulaceae. Mycotaxon 69:153–158

Croan SC, Bursdall HH Jr, Rentmeester RM (1999) Preservation of tropical wood-inhabiting basidiomycetes. Mycologia 91:908–916

Crockatt ME, Campbell A, Allum L, Ainsworth AM, Boddy L (2010) The rare oak polypore *Piptoporus quercinus*: Population structure, spore germination and growth. Fungal Ecol 3:94–106

Dai YC, D'Amico L, Motta E, Annesi T (2010) First report of *Inonotus rickii* causing canker and decay on *Hevea brasiliensis* in China. Plant Pathol 59:806

Danielson RM (1983) Ectomycorrhizal associations in jack pine stands in northeastern Alberta. Can J Bot 62:932–939

Davidson RW, Campbell WA, Weber GF (1942) *Ptychogaster cubensis*, a wood-decaying fungus of southern oaks and waxmyrtle. Mycologia 34:142–153

Day PR (1959) A cytoplasmically controlled abnormality of the tetrads of *Coprinus lagopus*. Heredity 13:81–87

De Fine Licht HH, Boomsma JJ, Tunlid A (2014) Symbiotic adaptations in the fungal cultivar of leaf-cutting ants. Nat Commun 5:5675

de Koker TH, Burdsall H Jr, Janse BJH (2000) A provisional name for a taxon of *Phanerochaete* from South Africa. Sydowia 52:10–15

de Sena-Tomás C, Navarro-González M, Kües U, Pérez-Martín J (2013) A DNA damage checkpoint pathway coordinates the division of dikaryotic cells in the ink cap mushroom *Coprinopsis cinerea*. Genetics 195:47–57

Delgado JC, Cook AA (1976) Arthroconidia formation in cultures of *Marasmius perniciosus*. Phytopathology 66:717–718

Dennis RL (1969) Fossil mycelium with clamp connections from the Middle Pennsylvanian. Science 163:670–671

Descals E (1985) Conidia as modified hyphae. Proc Indiana Acad Sci Plant Sci 94:209–227

Doguet G (1956) Recherces sur le *Rhodotus palmatus* (Fr. Sx Bull.) R. Maire. Bull Soc Mycol France 72:61–87 (in French)

Dörnte B, Kües U (2012) Reliability in transformation of the basidiomycete *Coprinopsis cinerea*. Curr Trends Biotechol Pharm 6:340–355

Douanla-Meli C, Langer E (2009) *Ganoderma carcocalcareus* sp. nov., with crumbly-friable context parasite to saprobe on *Anthocleista nobilis* and its phylogenetic relationship in *G. resinaceum* group. Mycol Prog 8:145–155

Drechsler C (1941) Some hyphomycetes parasitic on free-living terricolous nematodes. Phytopathology 31:773–801

Drechsler C (1946) A clamp-bearing fungus parasitic and predaceous on nematodes. Mycologia 38:1–23

Drechsler C (1949) A nematode-capturing fungus with anastomosing clamp-bearing hyphae. Mycologia 41:369–387

Drechsler C (1960) A clamp-bearing fungus using adhesive young chlamydospores in capturing amoebae. Sydowia 14:246–257

Dumas MT, Laflamme G (2013) Efficacy of two *Phlebiopsis gigantea* formulations in preventing *Heterobasidion irregulare* colonization of red pine stumps in eastern Canada. Phytopathology 93:25–31

Dyko BJ, Sutton BC (1979) Two new and unusual deuteromycetes. Trans Br Mycol Soc 72:411–417

Edman M, Gustafsson M, Stenlid J, Johnsson BG, Ericson L (2004a) Spore deposition of wood-decaying fungi: importance of landscape composition. Ecography 27:103–111

Edman M, Gustafsson M, Stenlid J, Ericson L (2004b) Abundance and viability of fungal spores along a forestry gradient – responses to habitat loss and isolation? Oikos 104:35–42

Esser K (2000) Kryptogamen 1. Cyanobakterien Algen Pilze Flechten. Praktikum und Lehrbuch. Springer, Berlin (in German)

Falck R (1902) Die Cultur der Oidien und ihre Rückführung in die höhere Fruchtform bei den Basidiomyzeten. Cohn's Beitr Biol Pfl 8:308–374 (in German)

Fernando KMEP (2008) The host preference of a *Ganoderma lucidum* strain for three tree species of Fabaceae family; *Cassia nodosa*, *Cassia fistula* and *Delonix regia*. J Nat Sci Found Sri Lanka 36:323–326

Fischer R, Kües U (2006) Asexual sporulation in mycelial fungi. In: Kües U, Fischer R (eds) The mycota, Growth, differentiation and sexuality, vol I, 2nd edn. Springer, Berlin, pp 263–292

Fisher PJ, Webster J, Kane DF (1976) *Peyronelina glomerulata* from submerged substrate in Britain. Trans Br Mycol Soc 67:351–354

Franken P, Varma A (2000) Fungi of the genus *Piriformospora*. German Patent DE69812697D1

Freihorst D, Flowler TJ, Bartholomew K, Raudaskoski M, Horton JS, Kothe E (2016) The mating type genes of the basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 329–349

Furtado JS (1966) Significance of the clamp-connection in the basidiomycetes. Persoonia 4:125–144

Gardiner RB, Day AW (1988) Surface proteinaceous fibrils (fimbriae) on filamentous fungi. Can J Bot 66:2474–2484

Garibova LV, Melik-Khachatrian JH, Badalian SM, Darakov OB (1986) Anamorph in the life history of *Nematoloma fasciculare* (Huds.: Fr.) Karst. Mikol Fitopatol 20:161–166 (in Russian)

Gené J, Guillamon GJ, Guarro J, Pujol I, Ulfig K (1996) Molecular characterization, relatedness and antifungal susceptibility of the basidiomycetous *Hormographiella* species and *Coprinus cinereus* from clinical and environmental sources. Antonie Van Leeuwenhoek 70:49–57

Geng Y, He Z, Lu ZM, Xu HY, Xu GH, Shi JS, Xu ZH (2013) *Antrodia camphorata* ATCC 200183 sporulates asexually in submerged culture. Appl Microbiol Biotechnol 97:2851–2858

Gill WM, Lapeyrie F, Gomi T, Suzuki K (1999) *Tricholoma matsutake*: an assessment of in situ and in vitro infection by observing cleared and stained whole roots. Mycorrhiza 9:227–231

Gilmore KA (1926) Culture studies of *Psilocybe coprophila*. Bot Gaz 81:419–433

Giuma AY, Cooke RC (1972) Some endozoic fungi parasitic on soil nematodes. Trans Br Mycol Soc 59:213–218

Gottlieb AM, Wright JE, Moncalvo J-M (2002) *Inonotus* s.l. in Argentina – morphology, cultural characters and molecular species. Mycol Prog 1:299–313

Granado JD, Kertesz-Chaloupková K, Aebi M, Kües U (1997) Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. Mol Gen Genet 256:28–36

Greig BJW (1998) Field recognition and diagnosis of *Heterobasidium annosum*. In: Woodward S, Stenlid J, Karjalainen R, Hüttermann A (eds) *Heterobasidion annosum:* biology, ecology, impact and control. CAB International, Wellesbourne, pp 35–41

Griffin BR, Wilson CL (1967) Asexual nuclear behaviour and formation of conidia in *Fomes annosum*. Phytopathology 57:1176–1177

Guarro J, Gené J, de Vroey C, Guého E (1992) *Hormographiella*, a new genus of hyphomycetes from clinical sources. Mycotaxon 45:179–190

Gulden G, Stensrud Ø, Shalchian-Tabrizi K, Kauserud H (2005) *Galerina* Earle: a polyphyletic genus in the consortium of dark-spored agarics. Mycologia 97:823–837

Gunulf A, Rönnberg J, Berglund M (2012) Comparison of colonization capacity by asexual spores of *Heterobasidion* species in Norway spruce wood. Forest Pathol 42:338–344

Guzmán G, Bandala VM, Montoya L (1991) A comparative study of teleomorphs and anamorphs of *Pleurotus cystidiosus* and *Pleurotus smithii*. Mycol Res 95:1264–1269

Harmaja H (1979) Studies in the genus *Cystoderma*. Karstenia 19:25–29

Harrach BD, Baltruschat H, Barna B, Fodir J, Kogel K-H (2013) The mutualistic fungus *Piriformospora indica* protects barley roots from a loss of antioxidant capacity caused by the necrotrophic pathogen *Fusarium culmorum*. Mol Plant Microbe Interact 26:599–605

Hattori T, Lee SS (1999) Two new species of *Perenniporia* described from a lowland rainforest of Malaysia. Mycologia 91:525–531

Hattori T, Sotome K, Ota Y, Thi BK, Lee SS, Salleh B (2010) *Postia stellifera* sp. nov., a stipitate and terrestrial polypore from Malaysia. Mycotaxon 114:151–161

Heimel K, Scherer M, Schuler D, Kämper J (2010a) The *Ustilago maydis* Clp1 protein orchestrates pheromone and *b*-dependent signaling pathways to coordinate the cell cycle and pathogenic development. Plant Cell 22:2908–2922

Heimel K, Scherer M, Vranes M, Wahl R, Pothiratana C, Schuler D, Vincon V, Finkernagel F, Flor-Parra I, Kämper J (2010b) The transcription factor Rbf1 is the master regulator for *b*-mating type controlled pathogenic development in *Ustilago maydis*. PLoS Pathog 6, e1001035

Heintz CE, Niederpruem DJ (1970) Ultrastructure and respiration of oidia and basidiospores of *Coprinus lagopus* (*sensu* Buller). Can J Microbiol 16:481–484

Heintz CE, Niederpruem DJ (1971) Ultrastructure of quiescent and germinated basidiospores and oidia of *Coprinus lagopus*. Mycologia 63:745–766

Hibbett DS, Thorn RG (2001) Basidiomycota: Homobasidiomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol VII, Systematics and evolution. Part B. Springer, Heidelberg, pp 121–168

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KS, Ironside JE, Kõljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmesto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüssler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111:509–547

Hollenstein M (1997) Aspekte zur genotypischen Determination der Oidienverteilung in Dikaryen von *Coprinus cinereus*. Master thesis, ETH Zurich, Zurich, Switzerland (in German)

Hong SG, Jung HS (2004) Phylogenetic analysis of *Ganoderma* based on nearly complete mitochondrial small-subunit ribosomal DNA sequences. Mycologia 96:742–755

Hori C, Ishida T, Igarashi K, Samejima M, Suzuki H, Master E, Ferreira P, Ruiz-Duenas FJ, Held B, Canessa P, Larrondo LF, Schmoll M, Druzhinina IS, Kubicek C, Gaskell JA, Kersten P, St John F, Glasner J, Sabat G, BonDurant SS, Syed K, Yadav J, Mgbeahuruike AC, Kovalchuk A, Asiegbu FO, Lackner G, Hoffmeister D, Rencoret J, Gutierrez A, Sun H, Lindquist E, Barry K, Riley R, Grigoriev IV, Henrissat B, Kües U, Berka RM, Martinez AT, Covert SF, Blanchette RA, Cullen D (2014) Analysis of the *Phlebiopsis gigantea* genome, transcriptome and secretome provides insight into its pioneer colonization strategies of wood. PLoS Genet 10:e1004759

Hsiang T, Edmonds RL, Driver CH (1988) Conidia of *Heterobasidion annosum* from *Tsuga heterophylla* forests in western Washington. Can J Bot 67:1262–1266

Hu Y-D, Zhang H, Lu R-Q, Liao X-R, Zhang B-B, Xu G-R (2014) Enabling the biosynthesis of antroquinonol in submerged fermentation of *Antrodia camphorata*. Biochem Eng J 91:157–162

Hübsch P (1978) Nebenfruchtformen bei *Pholiota*-Arten. Česká Mykol 32:82–86 (in German)

Hughes SJ (1953) Conidiophores, conidia, and classification. Can J Bot 31:577–659

Hui C, Tanaka Y, Takeo K, Kitamoto Y (1999a) Morphological and cytological aspects of oidium formation in a basidiomycete, *Pholiota nameko*. Mycoscience 40:95–101

Hui C, Yamamoto H, Ohta T, Takeo K, Kitamoto Y (1999b) Nuclear selection in monokaryotic oidium

formation from dikaryotic mycelia in a basidiomycete, *Pholiota nameko*. Mycoscience 40:199–203
Hutchison LJ (1988) Notes on *Limacella illinita* in pure culture. Mycologia 80:111–114
Hutchison LJ, Madzia SE, Barron GL (1996) The presence and antifeedant function of toxin-producing secretory cells on hyphae of the lawn-inhabiting agaric *Conocybe lactea*. Can J Bot 74:431–434
Hyde KD, Goh T-K (1999) Fungi on submerged wood from the River Coln, England. Mycol Res 103:1561–1574
Hyde KD, McKenzie EHC, Koko TW (2011) Towards incorporating anamorphic fungi in a natural classification – checklist and notes for 2010. Mycosphere 2:1–88
Hyder R, Pennanen T, Hamberg L, Vainio EJ, Piri T, Hantula J (2013) Two viruses of *Heterobasidion* confer beneficial, cryptic or detrimental effects to their hosts in different situations. Fungal Ecol 6:387–396
Ihrmark K, Johannesson H, Stenström E, Stenlid J (2002) Transmission of double-stranded RNA in *Heterobasidion annosum*. Fungal Genet Biol 36:147–154
Ihrmark K, Stenström E, Stenlid J (2004) Double-stranded RNA transmission through basidiospores of *Heterobasidion annosum*. Mycol Res 108:149–153
Inada K, Morimoto Y, Arima T, Murata Y, Kamada T (2001) The *clp1* gene of the mushroom *Coprinus cinereus* is essential for *A*-regulated sexual development. Genetics 157:133–140
Ingold CT (1980) Mycelium, oidia and sporophore initials in *Flammulina velutipes*. Trans Br Mycol Soc 75:107–116
Ingold CT (1983) Homing on basidiospores and production of oidia in *Tubaria furfuracea*. Trans Br Mycol Soc 80:363–364
Ingold CT (1986) Asexual spores of *Polyporus squamosus* and *P. badius*. Trans Br Mycol Soc 87:613–616
Ingold CT (1988) Chlamydospore formation during basidiospore germination in *Marasmius oreades*. Trans Br Mycol Soc 90:495–496
Ingold CT (1991) Conidia of *Polyporus varius*. Mycol Res 95:246–248
Irinyi L, Serena C, Garcia-Hermoso D, Arabatzis M, Desnos-Ollivier M, Vu D, Cardinali G, Arthus I, Normand AC, Giraldo A, da Cunha KC, Sandoval-Denis M, Hendrickx M, Nishikaku AS, de Azevedo Melo AS, Merseguel KB, Khan A, Parente Rocha JA, Sampaio P, da Silva Briones MR, e Ferreira RC, de Medeiros Muniz M, Castañón-Olivares LR, Estrada-Barcenes D, Cassagne C, Mary C, Duan SY, Kong F, Sun AY, Zeng X, Zhao Z, Gantois N, Botterel F, Robbertse B, Schoch C, Gams W, Ellis D, Halliday C, Chen S, Sorrell TC, Piarroux R, Colombo AL, Pais C, de Hoog S, Zancopé-Oliviera RM, Tayler ML, Toriello C, de Almeida Soares CM, Delhaes L, Stubbe D, Dromer F, Ranque S, Guarro J, Cano-Lira JF, Robert V, Velegraki A, Meyer W (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database. The quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol 53:313–337
Iwasa M, Tanabe S, Kamada T (1998) The two nuclei in the dikaryon of the homobasidiomycete *Coprinus cinereus* change position after each conjugate division. Fungal Genet Biol 23:110–116
Jacobsson S (1985) Studies on *Pholiota* in culture. Mycotaxon 36:95–145
James TY, Stenlid J, Olson Å, Johannesson H (2008) Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus *Heterobasidion parviporum*. Evolution 62:2279–2296
James TY, Johansson SBK, Johannesson H (2009) Trikaryon formation and nuclear selection in pairings between heterokaryons and homokaryons of the root pathogen *Heterobasidium parviporum*. Mycol Res 113:583–590
Jurand MK, Kemp RFO (1972) Surface structure of oidia in the basidiomycete *Psathyrella coprophila*. J Gen Microbiol 72:575–579
Kamada T (2002) Molecular genetics of sexual development in the mushroom *Coprinus cinereus*. Bioessays 24:449–459
Kamada T, Sano H, Nakazawa T, Nakahori K (2010) Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. Fungal Genet Biol 47:917–921
Keirle MR, Hemmes DE, Desjardin FE (2004) Agaricales of the Hawaiian Islands. 8. Agaricaceae: *Coprinus* and *Podaxis*; Psathyrellaceae: *Coprinopsis*, *Coprinellus* and *Parasola*. Fungal Divers 15:33–124
Kemp RFO (1975) Breeding biology of *Coprinus* species in section *Lanatuli*. Trans Br Mycol Soc 65:375–388
Kemp RFO (1977) Oidial homing and the taxonomy and speciation of basidiomycetes with special reference to the genus *Coprinus*. In: Clémençon H (ed) The species concept in Hymenomycetes. Cramer, Vaduz, pp 259–276
Kemp RFO (1980) Production of oidia by dikaryons of *Flammulina velutipes*. Trans Br Mycol Soc 74:557–560
Kendrick B, Watling R (1979) Mitospores in basidiomycetes. In: Kendrick B (ed) The whole fungus, vol 2. National Museum of Natural Sciences, Ottawa, pp 473–545
Kernaghan G (2001) Ectomycorrhizal fungi at tree line in the Canadian Rockies. II Identification of ectomycorrhizae by anatomy and PCR. Mycorrhiza 10:217–229
Kertesz-Chaloupková K, Walser PJ, Granado JD, Aebi M, Kües U (1998) Blue light overrides repression of asexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 23:95–109

Kim AY, Kim K-H, Im CH, Ali A, Lee CY, Kong W-S, Ryo J-S (2014) Identification of degenerate nuclei and development of a SCAR marker for *Flammulina velutipes*. PLoS ONE 9, e107207

Kinugawa K (1979) Asymmetric recovery of component nuclei in the oidia from dikaryotic mycelia of *Collybia velutipes*. Mushroom Sci 10:211–217

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi, 10th edn. CAB International, Wallingford

Kirschner R, Chen CJ (2004) *Helicomyxa everhartioides*, a new helicosporous sporodochial hyphomycete from Taiwan with relationships to the Hyaloriaceae (Auriculariales, Basidiomycota). Stud Myol 50:337–342

Kirschner R, Oberwinkler F (2009) Supplementary notes on *Basidiopycnis hyalina* (Basidiomycota, Atractiellales) and its anamorph. Mycotaxon 109:29–38

Kirschner R, Wu S-H (2005) *Echinoporia hydnophora*, a polypore new for Taiwan. Fung Sci 20:99–103

Kirschner R, Lee I-S, Chen C-J (2013) *Ovularia puerariae* Sawada is the hyphomycetous anamorph of a new *Marasmius* species on living leaves of kudzu (*Pueraria montana*, Fabaceae). Mycologia 105:781–792

Kitamoto Y, Shishida M, Yamamoto H, Takeo K, Masuda P (2000) Nuclear selection in oidium formation from dikaryotic mycelia of *Flammulina velutipes*. Mycoscience 41:417–423

Klosterman SJ, Martinez-Espinoza AD, Andrews DL, Seay JR, Gold SE (2008) Ubc2, an ortholog of the yeast Ste50p adaptor, possesses a basidiomycete-specific carboxy terminal extension essential for pathogenicity independent of pheromone response. Mol Plant Microb Interact 21:110–121

Kobayashi H, Yamada A (2003) Chlamydospore formation of *Entoloma clypeatum* f. *hybridum* on mycorrhizas and rhizomorphs associated with *Rosa multiflora*. Mycoscience 44:61–62

Koller B, Jahrmann HJ (1985) Life-cycle and physiological description of the yeast-form of the homobasidiomycete *Asterophora lycoperdoides* (Bull.: Fr.) Ditm. Antonie Van Leeuwenhoek 51:255–261

Koltin Y, Wessels JGH, van der Valk P (1973) Chlamydospores of *Schizophyllum commune*. Arch Mikrobiol 91:179–182

Kooij PW, Rogowska-Wrzesinska A, Hoffmann D, Roepstorff P, Boomsma JJ, Schiøtt M (2014) *Leucoagaricus gongylophorus* uses leaf-cutting ants to vector proteolytic enzymes towards new plant substrate. ISME J 8:1032–1040

Korhonen K, Stenlid J (1998) Biology of *Heterobasidion annosum*. In: Woodward S, Stenlid J, Karjalainen D, Hüttermann A (eds) *Heterobasidion annosum*: biology, ecology, impact and control. CAB International, Wallingford, pp 43–92

Koske RE, Perrin PW (1971) Basidiocarps, annelloconidia, and sclerotia in agar cultures of *Pistillaria* (Clavariadelphaceae). Can J Bot 49:695–697

Kost G, Rexer K-H (2013) Morphology and ultrastructure of *Piriformospora indica*. Soil Biol 33:25–36

Kotiranta H, Ryvarden L (2007) *Botryobasidium baicalinum* sp. *nova* (aphyllophorales, basidiomycetes). Ann Bot Fennici 44:293–297

Koziak ATE, Chavarria Diaz F, Diaz J, Garcia M, Thorn RG (2007a) Costa Rican species of *Nematoctonus* (anamorphic Pleurotaceae). Can J Bot 85:749–761

Koziak ATE, Cheng KC, Thorn RG (2007b) Phylogenetic analyses of *Nematoctonus* and *Hohenbuehelia* (Pleurotaceae). Can J Bot 85:762–773

Kreisel H (1972) Pilze aus Pilzgärten von *Atta insularis* in Kuba. Z Allg Mikrobiol 12:643–654 (in German)

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U (2002) Sexuelle und asexuelle Fortpflanzung bei einem Pilz mit 12000 Geschlechtern. Vierteljschr Naturf Ges Zürich 147:23–34 (in German)

Kües U (2015) From two to many: multiple mating type in basidiomycetes. Fungal Biol Rev 29 (in press)

Kües U, Navarro-Gonzaléz M (2015) How do agaricomycetes shape their fruiting bodies? 1. Morphological aspects of development. Fungal Biol Rev 29 (2):63–97

Kües U, Asante-Owusu RN, Mutasa ES, Tymon AM, Pardo EH, O'Shea SF, Göttgens B, Casselton LA (1994) Two classes of homeodomain proteins specify the multiple *A* mating types of the mushroom *Coprinus cinereus*. Plant Cell 6:1467–1475

Kües U, Granado JD, Hermann R, Boulianne RP, Kertez-Chaloupková K, Aebi M (1998a) The *A* mating type and blue light regulate all known differentiation processes in the basidiomycete *Coprinus cinereus*. Mol Gen Genet 260:81–91

Kües U, Granado JD, Kertesz-Chaloupková K, Walser PJ, Hollenstein M, Polak E, Liu Y, Boulianne RP, Bottoli APF, Aebi M (1998b) Mating types and light are major regulators of development in *Coprinus cinereus*. In: Van Griensven LJLD, Visser J (eds) Proceedings of the fourth meeting on the genetics and cellular biology of basidiomycetes. Mushroom Experimental Station, Horst, pp 113–118

Kües U, Polak E, Bottoli APF, Hollenstein M, Walser PJ, Boulianne RP, Hermann R, Aebi M (2002a) Vegetative development in *Coprinus cinereus*. In: Osiewacz HD (ed) Molecular biology of fungal development. Marcel Dekker, New York, pp 133–163

Kües U, Walser PJ, Klaus MJ, Aebi M (2002b) Influence of the *A* and *B* mating-type pathways on developmental processes in the basidiomycete *Coprinus cinereus*. Mol Gen Genomics 268:262–271

Kües U, Künzler M, Bottoli APF, Walser PJ, Granado JD, Liu Y, Bertossa RC, Ciardo D, Clergeot P-H, Loos S, Ruprich-Robert G, Aebi M (2004) Mushroom development in higher basidiomycetes; implications for human and animal health. In: Kushwaha RKS (ed) Fungi in human and animal health. Scientifc Publishers (India), Jodhpur, pp 431–469

Kües U, Mai C, Militz H (2007) Biological wood protection against decay, microbial staining, fungal moulding and insect pests. In: Kües U (ed) Wood production, wood technology, and biotechnological impacts. Universitätsverlag Göttingen, Göttingen

Kües U, James TY, Heitman J (2011) Mating type in basidiomycetes: unipolar, bipolar, and tetrapolar patterns of sexuality. In: Pöggeler S, Wöstemeyer J (eds) The mycota, vol 14, Evolution of fungi and fungal-like organisms. Springer, Heidelberg, pp 97–160

Kuhlman EG, Hendrix FF (1964) Infection, growth rate and competitive ability of *Fomes annosus* in inoculated *Pinus echinata* stumps. Phytopathology 54:556–561

Kühner R (1978) Les grandes lignes de la classification des Agaricales (suite). Bull Mens Soc Linnéenne de Lyon 47:234–303 (in French)

Kumar V, Sahai V, Bisaria VS (2012) Production of amylase and chlamydospores by *Piriformospora indica*, a root endophytic fungus. Biocatal Agric Biotechnol 1:124–128

Kuratani M, Tanaka K, Terashima K, Muraguchi H, Nakazawa T, Nakahori K, Kamada T (2010) The *dst2* gene essential for photomorphogenesis of *Coprinopsis cinerea* encodes a protein with a putative FAD-binding-4 domain. Fungal Genet Biol 47:152–158

Kurtzman CP (1973) Formation of hyphae and chlamydospores by *Cryptococcus laurentii*. Mycologia 65:388–395

Lange L, Grell MN (2014) The prominent role of fungi and fungal enzymes in the ant-fungus biomass conversion symbiosis. Appl Microbiol Biotechnol 98:4839–4851

Lee J, Huh N, Hong JH, Kim BS, Lim G-H (2012) The antagonistic properties of *Trichoderma* spp. inhabiting woods for potential biological control of wood-damaging fungi. Holzforschung 66:883–887

Lewis D (1961) Genetic analysis of methionine suppressors in *Coprinus*. Genet Res 2:141–155

Lin X, Heitman J (2005) Chlamydospore formation during hyphal growth in *Cryptococcus neoformans*. Eukaryot Cell 4:1746–1754

Linder MB, Szilvay GR, Nakari-Setalä T, Penttilä M (2005) Hydrophobins: the protein-amphiphiles of filamentous fungi. FEMS Microbiol Rev 29:877–896

Lu BC (1974) Meiosis in *Coprinus*. V. The role of light on basidiocarp initiation, meiosis, and hymenium differentiation in *Coprinus lagopus*. Can J Bot 52:299–305

Lu ZM, He Z, Li HX, Gong JS, Geng Y, Xu HY, Xu GH, Shi JS, Xu ZH (2014) Modified arthroconidial inoculation method for the efficient fermentation of *Antrodia camphorata* ATCC 200183. Biochem Eng J 87:41–49

Luo H, Mo M, Huang X, Li X, Zhang K (2004) *Coprinus comatus*: a basidiomycete fungus forms novel spiny structures and infects nematodes. Mycologia 96:1218–1225

Luo H, Liu Y, Fang L, Li X, Tang N, Zhang K (2007) *Coprinus comatus* damages nematode cuticles mechanically with spiny balls and produces potent toxins to immobilize nematodes. Appl Environ Microbiol 73:3916–3923

Madelin MF (1956) The influence of light and temperature on fruiting of *Coprinus lagopus* Fr. in pure culture. Ann Bot 20:794–802

Madelin MF (1960) Visible changes in the vegetative mycelium of *Coprinus lagopus* Fr. at the time of fruiting. Trans Br Mycol Soc 43:105–110

Madrid H, Cano J, Stchigel A, Gené J, Guarro J (2010) *Ramophialophora humicola* and *Fibulochlamys chilensis*, two new microfungi from soil. Mycologia 102:605–612

Malecka M, Sierota Z, Zolciak A (2012) Comparison of efficacy of the biological preparation with some *Phlebiopsis gigantea* isolates used to protect Scots pine stumps against primary infection of *Heterobasidion annosum* on post agricultural lands. Sylwan 156:526–532

Marryat FCR (1908) Chlamydospore-formation in the basidiomycete *Pleurotus subpalmatus*. New Phytol 7:17–22

Martens P, Vandendries R (1933) Le cycle conidien haploïde et diploïde chez *Pholiota aurivella*. Cellule 41:337–388 (in French)

Marvanová L, Bärlocher F (1988) Hyphomycetes from Canadian streams. I. Basidiomycetous anamorphs. Mycotaxon 32:339–351

Masuda PK, Yamanaka K, Sato Y, Kitamoto Y (1995) Nuclear selection in monokaryotization of dikaryotic mycelia of *Pholiota nameko* as described by leading and following nuclei. Mycoscience 36:413–420

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Velinga EC, Vilgalys R, Hibbett DS (2006) Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98:982–995

Mattsson J, Flyen A-C, Nunez M (2010) Wood-decaying fungi in protected buildings and structures on Svalbard. Agarica 29:5–14

Maxwell MB (1954) Studies of Canadian Thelephoraceae: XI. Conidium production in the Thelephoraceae. Can J Bot 32:259–280

Mayorga ME, Gold SE (2001) The *ubc2* gene of *Ustilago maydis* encodes a putative novel adaptor protein required for filamentous growth, pheromone response and virulence. Mol Microbiol 41:1365–1379

Melo I, Ramos P, Frazão Caetano MF (2002) First record of *Inonotus rickii* (basidiomycetes, Hymenochaetaceae) in Portugal. Portugaliae Acta Biol 20:265–269

Metzler B (1987a) The conidia of *Typhula incarnata*. 1. The ultrastructure of conidiogenesis. Can J Bot 66:1316–1320

Metzler B (1987b) The conidia of *Typhula incarnata*. 2. Their function as spermatia. Can J Bot 66: 1321–1324

Miller AN, Metheven AS (2000) Biological species concepts in eastern North American populations of *Lentinellus*. Mycologia 92:792–800

Minnis AM (2015) The shifting sands of fungal naming under the ICN and the one name era for fungi. In: McLaughlin DJ, Spatafora JW (eds) The Mycota, vol VII, Systematics and evolution. Part B. Springer, Heidelberg, pp 179–203

Mohammed C, Rimbawanto A, Page DE (2014) Management of basidiomycete root- and stem-rot diseases in oil palm, rubber and tropical hardwood plantation crops. Forest Pathol 44:428–446

Molitoris PH, Buchalo AS, Grigansky AP (1996) Studies on vegetative mycelium in the genus *Agaricus* L.: Fr. Emend. Karst. In: Wasser SP (ed) Botany and mycology for the next millennium: collection of scientific articles devoted to the 70th anniversary of Academician K. M. Sytnik. N. G. Kolodny. N. G. Kolodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev, pp 316–330

Moore RT (1977) Dolipore disjunction in *Antromycopsis broussonetiae* Pat. Exp Mycol 1:92–101

Moreau P-A, Vila J, Pérez-De-Gregorio MÀ, Llistosella J, Llimona X (2005) *Hemimycena conidiogena*, a new cistophilus basidiomycete. Mycotaxon 91:323–332

Mossebo DC, Akoa A, Eteme A (2001) Ultrastructural studies of the patterns of conidiogenesis in *Dacrymyces stillatus* Nees: Fries (basidiomycete). Cryptog Mycol 22:119–138

Möykklen T (1997) Liberation of *Heterobasidion annosum* conidia by airflow. Eur J Forest Pathol 27:283–289

Mukhin VA, Votintseva AA (2002) Basidiospore germination and conidial stages in the life cycle of *Fomes fomentarius* and *Fomitopsis pinicola* (fungi, polyporales). Pol Bot J 47:265–272

Murata Y, Kamada T (2009) Identification of new mutant alleles of *pcc1* in the homobasidiomycete *Coprinopsis cinerea*. Mycoscience 50:137–139

Murata Y, Fujii M, Zolan ME, Kamada T (1998) Molecular analysis of *pcc1*, a gene that leads to *A*-regulated sexual morphogenesis in *Coprinus cinereus*. Genetics 149:1753–1761

Murmanis L, Highley TL, Ricard J (1988) Hyphal interaction of *Trichoderma harzianum* and *Trichoderma polysporum* with wood decay fungi. Mat Org 23:271–279

Nag Raj RT (1980) Genera coelomycetum. XXIII. *Ellula* anamorph-gen. nov., another coelomycete with basidiomycetous affinities. Can J Bot 56:1484–1491

Nag Raj TR, Holoubová-Jechová V, Castaňeda Ruiz RF (1989) Genera coelomycetum. XXVII. *Pycnocellomyces* redescribed. Can J Bot 67:3386–3390

Nagy LG, Házi J, Vágvölgyi C, Papp T (2012) Phylogeny and species delimitation in the genus *Coprinellus* with special emphasis on the haired species. Mycologia 104:254–275

Nakazawa T, Tatsuta Y, Fujita T, Nakahori K, Kamada T (2010) Mutations in the *Cc.rmt1* gene encoding a putative protein arginine methyltransferase alter developmental programs in the basidiomycete *Coprinopsis cinerea*. Curr Genet 56:361–367

Nakazawa T, Kondo H, Nakahori K, Kamada T (2011) A mutation in the *Cc.ubc2* gene affects clamp cell morphogenesis as well as nuclear migration for dikaryosis in *Coprinopsis cinerea*. Fungal Genet Biol 48:519–525

Naumann A, Navarro-González M, Sánchez-Hernández O, Hoegger PJ, Kües U (2007) Correct identification of wood-inhabiting fungi by ITS analysis. Curr Trends Biotechnol Pharm 1:41–61

Navarro-Gonzaléz M (2008) Growth, fruiting body development and laccase production of selected Coprini. PhD thesis, University of Göttingen, Göttingen, Germany

Nawawi A (1985) Basidiomycetes with branched, waterborne conidia. Bot J Linn Soc 91:51–60

Nichols SP (1904) The nature and origin of the binucleated cells in some basidiomycetes. Trans Wisconsin Acad Sci 15:30–70

Nieuwenhuis BPS, Nieuwhof S, Aanen DK (2013) On the asymmetry of mating in natural populations of the mushroom *Schizophyllum commune*. Fungal Genet Biol 56:25–32

Noble M (1937) The morphology and cytology of *Typhula trifolii* Rostr. Ann Bot 1:67–98

Nobles MK (1935) Conidial formation, mutation and hybridization in *Peniophora allescheri*. Mycologia 27:286–301

Nobles MK (1937) Production of conidia in *Corticum incrustans*. Mycologia 29:557–566

Nobles MK (1942) Secondary spores in *Corticium effuscatum*. Can J Res 20:347–357

Nobles MK (1948) Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can J Res 26:281–431

Nobre T, Koopmanschap B, Baars JJP, Sonnenberg ASM, Aanen DK (2014) The scope for nuclear selection within *Termitomyces* fungi associated with fungus-growing termites is limited. BMC Evol Biol 14:12

Olariaga I, Ryman S, Salcedo I (2008) Lectotypification of *Typhula graminum* and description of *T. berthieri* sp. nov. Cryptog Mycol 29:145–155

Oliva J, Bernat M, Stenlid J (2013) Heartwood stump colonisation by *Heterobasidion parviporum* and *H. annosum s.s.* in Norway spruce (*Picea abies*) stands. Forest Ecol Manage 295:1–10

Olson Å, Aerts A, Asiegbu F, Belbahri L, Bouzid O, Broberg A, Canbäck B, Coutinho PM, Cullen D, Dalman K, Deflorio G, van Diepen LT, Dunand C, Duplessis S, Durling M, Gonthier P, Grimwood J, Fossdal CG, Hansson D, Henrissat B, Hietala A, Himmelstrand K, Hoffmeister D, Högberg N, James TY, Karlsson M, Kohler A, Kües U, Lee YH, Lin YC, Lind M, Lindquist E, Lombard V, Lucas S, Lundén K, Morin E, Murat C, Park J, Raffaello T, Rouzé P, Salamov A, Schmutz J, Solheim H, Ståhlberg J, Vélëz H, de Vries RP, Wiebenga A, Woodward S, Yakovlev I, Garbelotto M, Martin F, Grigoriev IV, Stenlid J

(2012) Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytol 194:1001–1013
Osborn JM, Taylor TN, White JF Jr (1989) *Palaeofibulus* gen. nov., a clamp-bearing fungus from the triassic of Antartica. Mycologia 81:622–626
Pantidou M, Watling R, Gonou Z (1983) Mycelial characters, anamorphs, and teleomorphs in genera and species of various families of Agaricales in culture. Mycotaxon 17:409–432
Pardo EH (1995) Organisation of the *A* mating type locus of *Coprinus cinereus*. PhD thesis, University of Oxford, Oxford, UK
Parihar A, Hembron ME, Das K (2013) New distributional records of *Ganoderma colossus* (Ganodermataceae) from Jharkhand and Rajasthan. Indian J Plant Sci 2:49–53
Paul NC, Deng JX, Shin KS, Yu SH (2012) Molecular and morphological characterization of endophytic *Heterobasidion araucaria* from roots of *Capsium annuum* L. in Korea. Mycobiology 40:85–90
Peddireddi S (2008) Hydrophobins in wood biology and biotechnology. PhD thesis, Georg-August-University, Göttingen, Germany
Pelkmans JF, Lugones LG, Wösten HAB (2016) Fruiting body formation in basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 387–405
Peškan-Berghöfer T, Shahollari B, Going PH, Hehl S, Markert C, Blanke C, Kost G, Oelmüller R (2004) Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant-microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. Phys Plant 122:465–477
Petersen RH (1992) Mating systems of three New Zealand agarics. NZ J Bot 30:189–197
Petersen RH, Hughes KW (2004) A preliminary monograph of *Lentinellus* (Russulales). Bibl Mycol 198:1–180
Peterson RH, Nicholl DBG, Hughes KW (1997) Mating systems of some putative polypore – agaric relatives. Plant Syst Evol 207:135–158
Plątek M, Cabała J (2005) New and noteworthy polypores from Poland with validation of the family *Phaeotrametaceae*. Mycotaxon 91:173–183
Polak E (1999) Asexual sporulation in the basidiomycete *Coprinus cinereus*. PhD thesis, ETH Zurich, Zurich, Switzerland
Polak E, Hermann R, Kües U, Aebi M (1997a) Asexual sporulation in *Coprinus cinereus*: structure and development of oidiophores and oidia in an *AmutBmut* homokaryon. Fungal Genet Biol 22:112–126
Polak E, Kües U, Aebi M (1997b) Replica plating of *Coprinus cinereus* colonies using asexual spores. Fungal Genet Newslett 44:45–46
Polak E, Aebi M, Kües U (2001) Morphological variations in oidium formation in the basidiomycete *Coprinus cinereus*. Mycol Res 105:603–610
Powell MR (2002) A model for probabilistic assessment of phytosanitary risk reduction measures. Plant Dis 86:552–557
Rahman MA, Cowan JW (1972) Effect of light on viability of *Coprinus lagopus*. Heredity 29:125
Rajchenberg M (2011) Nuclear behaviour of the mycelium and the phylogeny of Polypores (Basidiomycota). Mycologia 103:677–702
Rajchenberg M, Buchanan PK (1996) Two newly described polypores from Australasia and southern South America. Aust Syst Biol 9:877–885
Ramos AP, Caetano MF, Melo I (2008) *Inonotus rickii* (Pat.) Reid: an important legnicolous basidiomycete in urban trees. Rev Ciênc Agrárias 31:159–167
Ramsdale M, Rayner ADM (1994) Distribution patterns of number of nuclei in conidia from heterokaryons of *Heterobasidion annosum* (Fr.) Bref. and their interpretation in terms of genomic conflict. New Phytol 128:123–134
Ramsdale M, Rayner ADM (1996) Imbalanced nuclear ratios, post-germination mortality and phenotype-genotype relationships in allopatrically-derived heterokaryons of *Heterobasidion annosum*. New Phytol 133:303–319
Rao PS, Niederpruem DJ (1969) Carbohydrate metabolism during morphogenesis of *Coprinus lagopus* (sensu Buller). J Bacteriol 100:1222–1228
Raper CA, Raper JR (1966) Mutations modifying sexual morphogenesis in *Schizophyllum*. Genetics 54:1151–1168
Raudaskoski M (2015) Mating-type genes and hyphal fusions in filamentous basidiomycetes. Fungal Biol Rev 29 (in press)
Rayner ADM, Boddy L (1988) Fungal communities in the decay of wood. Adv Microbial Ecol 10:115–166
Redfern DB, Stenlid J (1998) Spore dispersal and infection. In: Woodward S, Stenlid J, Karjalainen R, Hüttermann A (eds) *Heterobasidion annosum:* biology, ecology, impact and control. CAB International, Wallingford, pp 105–124
Redhead SA, Ammirati JF, Walker GR, Norvell LL, Puccio MB (1994) *Squamanita contortipes*, the Rosetta Stone of a mycoparasitic agaric genus. Can J Bot 72:1812–1824
Remsberg R (1940) Studies in the genus *Typhula*. Mycologia 32:52–96
Reshetnikov SV (1982) Conidiogenesis of imperfect stage of agaricoid fungus *Pholiota aurivella* (Fr.) Kumm. In: Dorojkin NA, Yurkevich ID, Fedorov NI, Belskaya SI, Gorbach NV, Serjanina GI, Snigirev GS (eds) Ecology and biology of lower plants. Abstracts of IX all union symposium of mycologists and lichenologists. Nauka i Technika, Minsk, pp 144–145 (in Russian)
Reshetnikov SV (1991) Evolution of asexual reproduction in higher basidiomycetes. Naukova Dumka, Kiev (in Russian)
Risbeth L (1959) Dispersal of *Fomes annosus* Fr. and *Peniophora gigantea* (Fr.). Trans Br Mycol Soc 42:243–260

Romero AI, Cabral D, Lopez SE (1989) Studies on xylophilous fungi from Argentina. IV. Anamorphs of basidiomycetes on *Eucalyptus viminalis* (Myrtaceae). Mycotaxon 34:429–440

Ryvarden L, Gilbertson RL (1994) European polypores, part 2. Syn Fung 7:394–743

Saar I (2003) The genera *Cystoderma* and *Cystodermella* (Tricholomataceae) in temperate Eurasia. Mycotaxon 86:455–473

Saar I, Põldmaa K, Kõljag U (2009) The phylogeny and taxonomy of genera *Cystoderma* and *Cystodermella* (Agaricales) based on nuclear ITS and LSU sequences. Mycol Prog 8:59–73

Šašek V, Buchalo AS, Zakordonec OA (1986) Confirmation of identity of *Pleurotus abalone* with *Pleurotus cystidiosus* by scanning electron microscopy of anamorphs. Folia Microbiol 31:309–311

Saxena RK, Sangeetha L, Vohra A, Gupta R, Gulati R (2001) Induction and mass sporulation in lignin degrading fungus *Ceriporiopsis subvermispora* for its potential usage in pulp and paper industry. Curr Sci 81:591–594

Schubert D, Raudaskoski M, Knabe N, Kothe E (2006) Ras GTPase-activating protein Gap1 of the homobasidiomycete *Schizophyllum commune* regulates hyphal growth orientation and sexual development. Eukaryot Cell 5:683–695

Schuurs TA, Dalstra HJ, Scheer JM, Wessels JG (1998) Positioning of nuclei in the secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. Fungal Genet Biol 23:150–161

Schwarze FWMR (2007) Wood decay under the microscope. Fungal Biol Rev 21:133–170

Schwarze FWMR, Mattheck C, Engels J (2004) Fungal strategies of wood decay in trees. Springer, Heidelberg

Sede SM, Lopéz SE (1999) Cultural studies of *Agrocybe cylindrica*, *Gymnopilus pampeanus* and *Oudemansiella canarii* (Agaricales) isolated from urban trees. Mycotaxon 70:377–386

Segedin BP (1996) A new species of *Lentinellus* (Hericiales, Lentinellaceae) and a revision of taxa attributed to *Lentinellus* in New Zealand. NZ J Bot 34:249–261

Segedin BP, Buchanan PK, Wilkie JP (1995) Studies in the Agaricales of New Zealand: new species, new records and renamed species of *Pleurotus* (Pleurotaceae). Aust Syst Bot 8:453–482

Seifert KA, Gams W (2001) The taxonomy of anamorphic fungi. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol VII, Systematics and evolution. Part A. Springer, Heidelberg, pp 307–347

Shimazono H (1979) Comparative studies on morphological characteristics of the colonies of *Tricholoma matsutake*, *T. fulvocastaneum* and *T. bakamatsutake* on agar medium. Trans Mycol Soc Jpn 20:176–184 (in Japanese)

Singer R (1986) The Agaricales in modern taxonomy, 4th edn. Koeltz Scientific Books, Koenigstein

Sivanesan A, Watling R (1980) A new mitosporic basidiomycete. Trans Br Mycol Soc 74:424–431

Slippers B, Coutinho TA, Wingfield BD, Wingfield MJ (2003) A review of the genus *Amylostereum* and its association with woodwasps. S Afr J Sci 99:70–74

Spirin V, Miettinen O, Pennanen J, Kotiranta H, Niemelä T (2013) *Antrodia hyalina*, a new polypore from Russia, and *A. leucaena*, new to Europe. Mycol Prog 12:53–61

Srivilai P, Loutchanwoot P, Sukha J (2009) Blue light signalling inactivates the mating type genes-mediated repression of asexual spore production in the higher basidiomycete *Coprinopsis cinerea*. Pak J Biol Sci 12:110–118

Stalpers JA (1974) *Spiniger*, a new genus for imperfect states of basidiomycetes. Proc Koninkl Nederl Akad Weten Ser C Biol Med Sci 77:402–407

Stalpers JA (1978) Identification of wood-inhabiting aphyllophorales in pure culture. Stud Mycol 16:1–248

Stalpers JA (1984) Revision of the genus *Sporotrichum*. Stud Mycol 24:1–105

Stalpers JA (1988) Teleomorph-anamorph relationships in the aphyllophorales. In: Wolkinger F (ed) Aphyllophorales-Symposium, Eisenstadt 1982. Austrian Academy of Sciences, Graz, pp 13–15

Stalpers JA (2000) The genus *Ptychogaster*. Karstenia 40:167–180

Stalpers JA, Vlug I (1983) *Confistulina*, the anamorph of *Fistulina hepatica*. Can J Bot 61:1660–1666

Stalpers JA, Seifert K, Samson RA (1991) A revision of the genera *Antromycopsis*, *Sclerostilbum*, and *Tilachlidiopsis* (Hyphomycetes). Can J Bot 69:6–15

Stenlid J (1985) Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility, and isozyme patterns. Can J Bot 63:2268–2273

Stenlid J, Rayner ADM (1989) Environmental and endogenous controls of developmental pathways: variation and its significance in the forest pathogen, *Heterobasidion annosum*. New Phytol 113:245–258

Stenlid J, Rayner ADM (1991) Patterns of nuclear migration and heterokaryosis in pairings between sibling homokaryons of *Heterobasidion annosum*. Mycol Res 95:1275–1283

Steyaert RL (1972) Species of *Ganoderma* and related genera mainly of the Bogor and Leiden Herbaria. Persoonia 7:55–118

Steyaert RL (1980) Study of some *Ganoderma* species. Bull Jard Bot Nat Belg/Bull Nat Plantentiun Belg 50:135–186

Sun C, Oelmüller R (2010) *Piriformospora indica* hyphae and chlamydospores by scanning electron microscopy. J Endocyt Cell Res 20:34–37

Swamy S, Uno I, Ishikawa T (1984) Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 130:3219–3224

Swamy S, Uno I, Ishikawa T (1985a) Regulation of cyclic AMP metabolism by the incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 131:3211–3217

Swamy S, Uno I, Ishikawa T (1985b) Regulation of cyclic AMP-dependent phosphorylation of cellular pro-

teins by the incompatibility factors in *Coprinus cinereus*. J Gen Appl Microbiol 31:339–346

Takamaru T (1954) Genetics of *Collybia velutipes*. II. Dediploidization and its genetical implication. Jpn J Genet 29:1–7

Talbot PHB (1977) The *Sirex-Amylostereum-Pinus* association. Ann Rev Phytopathol 15:41–54

Tanesaka E, Honda R, Sasaki S, Yoshisa M (2012) Assignment of RAPD marker probes designed from 12 linkage groups of *Flammulina velutipes* to CHEF-separated chromosomal DNAs. Mycoscience 53:238–243

Tangthirasunum N, Solar P, Bhat DJ, Chukeatirote E, Wikee S, Maharachchikumburu SSN, Hyde KD, Wang Y (2014) Morphology and phylogeny of *Chaetospermum* (asexual coelomycetous Basidiomycota). Phytopathology 175:61–72

Tanney JB, Hutchison LJ (2012) The production of nematode-immobilizing secretory cells by *Climacodon septentrionalis*. Mycoscience 53:31–35

Terashima Y, Tomiya K, Takahashi M, Iwai H (1993) Distribution and characteristics of shiros of *Tricholoma bakamatsutake* in a mixed forest of *Pasania edulis* and *Castanopsis cuspidata* var. *sieboldii*. Trans Mycol Soc Japan 34:229–238

Terashima K, Yuki K, Muraguchi H, Akiyama M, Kamada T (2005) The *dst1* gene involved in mushroom photomorphogenesis of *Coprinus cinereus* encodes a putative photoreceptor for blue light. Genetics 171:101–108

Thielke C (1984) Wirtelschnallen bei homo- und heterothallischen *Coprinus*-Arten (Agaricales). Plant Syst Evol 148:35–49 (in German)

Thompson GE (1936) *Nyctalis parasitica* and *N. asterophora* in culture. Mycologia 28:222–227

Thomsen IM (1998) Characters of fruitbodies, basidiospores and cultures useful for recognizing *Amylostereum areolatum* and *A. chailletii*. Mycotaxon 69:419–428

Thor M, Bendz-Hellgren M, Stenlid J (1997) Sensitivity of root rot antagonist *Phlebiopsis gigantea* spores to high temperature or pressure. Scan J For Res 12:356–361

Thorn RG, Barron GL (1986) *Nematoctonus* and the tribe Resupinateae in Ontario, Canada. Mycotaxon 25:321–453

Tokuda S, Hattori T, Dai Y-C, Ota Y, Buchanan PK (2009) Three species of *Heterobasidion* (Basidiomycota, Hericales), *H. parviporum*, *H. orientale* sp. nov. and *H. ecrustosum* sp. nov. from East Asia. Mycoscience 50:190–202

Tóth A, Hausknecht A, Krisai-Greilhuber I, Papp T, Vágvölgyi C, Nagy LG (2013) Iteratively refined guide trees help improving alignment and phylogenetic inference in the mushroom family Bolbitiaceae. PLoS ONE 8, e56143

Tripathi S, Das A, Chandra A, Varma A (2015) Development of carrier-based formulations of root endophyte *Piriformospora indica* and its evaluation on *Phaseolus vulgaris* L. World J Microbiol Biotechnol 31:337–344

Truong B-N, Okazaki K, Suzuki H, Neda H, Fukiharu T, Le Y-T, Suzuki A (2006) Dikaryotic arthroconidiation of *Pleurotus* subgenus *Coremiopleurotus*. Mycoscience 47:84–90

Tsukamoto A, Kojima Y, Kita Y, Sugiura J (2003) Transformation of the white-rot basidiomycete *Coriolus hirsutus* using the ornithine carbamoyltransferase gene. Biosci Biotechnol Biochem 67:2075–2082

Tsusué YM (1969) Experimental control of fruit-body formation in *Coprinus macrorhizus*. Dev Growth Differ 11:164–178

Tymon AM, Kües U, Richardson WVJ, Casselton LA (1992) A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain. EMBO J 5:1805–1813

Ujor VC, Monti M, Peiris DG, Clements MO, Hegder JN (2012) The mycelial response of the white-rot fungus, *Schizophyllum commune* to the biocontrol agent, *Trichoderma viride*. Fungal Biol 116:332–341

Uno I, Ishikawa T (1971) Chemical and genetical control of induction of monokaryotic fruiting bodies in *Coprinus macrorhizus*. Mol Gen Genet 113:228–239

Uno I, Ishikawa T (1973a) Purification and identification of the fruiting-inducing substances in *Coprinus cinereus*. J Bacteriol 113:1240–1248

Uno I, Ishikawa T (1973b) Metabolism of adenosine 3',5'-cyclic monophosphate and induction of fruiting bodies in *Coprinus macrorhizus*. J Bacteriol 113:1249–1255

Uno I, Ishikawa T (1974) Effect of glucose on the fruiting body formation and adenosine 3',5'-cyclic monophosphate levels in *Coprinus macrorhizus*. J Bacteriol 120:96–100

Uno I, Ishikawa T (1976) Effect of cyclic AMP on glycogen phosphorylase in *Coprinus macrorhizus*. Biochim Biophys Acta 452:112–120

Uno I, Ishikawa T (1978) Effect of cyclic AMP on glycogen synthetase in *Coprinus macrorhizus*. J Gen Appl Microbiol 24:193–197

Uno I, Ishikawa T (1981) Adenosine 3',5'-monophosphate-receptor protein and protein kinase in *Coprinus macrorhizus*. J Biochem 89:1275–1281

Uno I, Yamaguchi M, Ishikawa T (1974) The effect of light on fruiting body formation and adenosine 3',5'-cyclic monophosphate metabolism in *Coprinus macrorhizus*. Proc Natl Acad Sci USA 71:479–483

Vainio EJ, Hakanpaa J, Dai YC, Hansen E, Korhonen K, Hantula J (2011) Species of *Heterobasidion* host a diverse pool of partitiviruses with global distribution and interspecies transmission. Fungal Biol 115:1234–1243

Vainio EJ, Müller MM, Korhonen K, Piri T, Hantula J (2015) Viruses accumulate in aging infection centers of a fungal forest pathogen. ISME J 9:497–507

Valenzuela E, Garnica S (2000) *Pseudohelicomyces*, a new anamorph of *Psilocybe*. Mycol Res 104:738–741

Valenzuela E, Ramirez C, Garnica S (1997) Macro-microscopic and qualitative enzymatic characterization of mycelial strains obtained from basidiocarps of *Mycena* species (Agaricales) in Chile. Rev Chil Hist Nat 70:521–530

van der Westhuizen GCA (1958) Studies on wood-rotting fungi: A. Cultural characteristics of some common species. Bothalia 7:83–99

Verma S, Varma A, Rexer K-H, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia 90:896–903

Verweij PE, van Kasteren M, van de Nes J, de Hoog GS, de Pauw BE, Meis JFGM (1997) Fatal pulmonary infection caused by the basidiomycete *Hormographiella aspergillata*. J Clin Microbiol 35:2675–2678

Vizzini A, Antonín V, Noordeloos ME (2007) *Crinipellis pedemontana* sp. nov. (agaricomycetes), a new basidiomycete from Italy. Mycologia 99:786–791

Volz PA, Niederpruem JL (1970) The sclerotia of *Coprinus lagopus*. Arch Mikrobiol 70:369–377

von Arx JA (1971) Über die Typusart, zwei neue und einige weitere Arten der Gattung *Sporotrichium*. Persoonia 6:179–184 (in German)

Waller F, Achatz B, Baltruschat H, Fofor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, von Wettstein D, Franken P, Kogel K-H (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci USA 102:13386–13391

Walser PJ (1997a) The influence of environmental factors on asexual sporulation of the basidioymcete *Coprinus cinereus*. Semester thesis, ETH Zurich, Zurich, Switzerland

Walser PJ (1997b) Environmental regulation of asexual sporulation and fruit body formation in the basidiomycete *Coprinus cinereus*. Master thesis, ETH Zurich, Zurich, Switzerland

Walser PJ, Hollenstein M, Klaus MJ, Kües U (2001) Genetic analysis of basidiomycete fungi. In: Talbot NJ (ed) Molecular and cell biology of filamentous fungi: a practical approach, Practical approach series. IRL Press, Oxford, pp 59–90

Walser PJ, Velagapudi R, Aebi M, Kües U (2003) Extracellular matrix proteins in mushroom development. Recent Res Dev Microbiol 7:381–415

Walther G, Weiß M (2006) Anamorphs of the Bolbitiaceae (Basidiomycota, Agaricales). Mycologia 98:792–800

Walther G, Weiß M (2008) Anamorphs in the Strophariaceae (Basidiomycota, Agaricales). Botany 86:551–566

Walther G, Garnica S, Weiß M (2005) The systematic relevance of conidiogenesis modes in the gilled Agaricales. Mycol Res 109:525–544

Waters H, Moore D, Butler RD (1972) Thick-walled sclerotial medullary cells in *Coprinus lagopus*. Trans Br Mycol Soc 59:167–169

Waters H, Moore D, Butler RD (1975) Structure of aerial and submerged sclerotia of *Coprinus lagopus*. New Phytol 174:199–205

Watling R (1971) Polymorphism in *Psilocybe merdaria*. New Phytol 70:307–326

Watling R (1979) The morphology, variation and ecological significance of anamorphs in the Agaricales. In: Kendrick B (ed) The whole fungus, vol 2. National Museum of Natural Sciences, Ottawa, pp 453–472

Wermelinger B, Thomsen IM (2012) The woodwasp *Sirex noctilio* and its associated fungus *Amylostereum areolatum* in Europe. In: Slippers B, de Groot P, Wingfield MJ (eds) The Sirex woodwasp and its fungal symbiont: research and management of a worldwide invasive pest. Springer, Netherlands, pp 65–80

Wessels JGH (1965) Morphogenesis and biochemical processes in *Schizophyllum commune* Fr. Wentia 13:1–113

Whitney RD, Bohaychuk WP (1975) Growth of *Polyporus tomentosus* cultures derived from basidiospores, sporophore tissue, and decayed wood. Can J Bot 54:2597–2602

Wongsawas M, Wanh H-K, Hyde KD, Lin F-C (2009) Freshwater fungi in Zhejiang Province I: four newly recorded hyphomycetes for the mainland of China. Mycostema 28:683–688

Wösten HAB (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55:625–646

Xing X-K, Guo S-X (2008) Electron microscopic study of conidia produced by the mycelium of *Polyporus umbellatus*. Mycosystema 27:554–558

Xu JW, Zhao W, Xu YN, Zhong JJ (2012) Isolation and analysis of differentially expressed gene during asexual sporulation in liquid static culture of *Ganoderma lucidum* by suppression subtractive hybridization. Mol Biol Rep 39:3603–3610

Yamada A, Meada K, Ohmasa M (1999) Ectomycorrhiza formation of *Tricholoma matsutake* isolates on seedlings of *Pinus densiflora* in vitro. Mycoscience 40:455–463

Yamaguchi K, Degawa Y, Nakagiri A (2009) An aero-aquatic fungus, *Peyronelina glomerulata*, is shown to have teleomorphic affinities with cyphelloid basidiomycetes. Mycoscience 50:156–164

Yoon KS (2004) Electron and light microscopic studies on the development of oidia from somatic mycelium of *Coprinus cinereus*. Mycobiology 32:164–169

Yun W, Hall IR, Evans LA (1997) Ectomycorrhizal fungi with edible fruiting bodies. 1. *Tricholoma matsutake* and related fungi. Econ Bot 51:311–327

Zervakis G (1998) Mating competence and biological species within the subgenus *Coremiopleurotus*. Mycologia 90:1063–1074

13 The Mating-Type Genes of the Basidiomycetes

Daniela Freihorst[1], Thomas J. Fowler[2], Kirk Bartholomew[3], Marjatta Raudaskoski[4], J. Stephen Horton[5], Erika Kothe[1]

CONTENTS

I. Introduction

The process of fungal mating needs to be controlled with respect to timing, mate choice and subsequent developmental processes. Since the 1920s (Kniep 1920, 1922), basidiomycete mating behaviour has been known to be regulated by two independent mating factors, *A* and *B*. The four potential outcomes of a mating interaction define what is termed the tetrapolar mating system. For the mushroom-forming basidiomycetes, hyphae from different strains fuse irrespective of mating types, and as a result, the mating-type genes do not determine the outcome of a cross before hyphal anastomosis. In case of partners of identical mating type, the resulting incompatible interaction lacks any fertile stages. Each monokaryotic hypha containing a single haploid nucleus per cell will continue to grow as before. There are two types of interaction termed semi-compatible matings. If the two mates differ in their *B* mating types, a reciprocal nuclear exchange takes place, while a difference solely at *A* mating types leads to a barrage reaction separating the two mycelia on solid media. It is only if there is a difference in both *A* and *B* that a fully compatible mating will ensue, with the growth of dikaryotic hyphae containing the two different nuclei derived from each mating partner present in every cell. This fertile dikaryon is able to develop fruiting bodies (reviewed in Pelkmans et al. 2016). In the developing basidia within the hymenium of fruiting bodies, karyogamy will take place, resulting in a very short diploid stage, quickly followed by meiosis and sexual spore production. In many basidiomycetes including *Schizophyllum commune* and *Coprinopsis cinerea*, clamp connections will develop in the dikaryotic mycelia subsequent to a compatible mating. These cellular structures help to ensure the proper distribution of the two nuclei during mitosis. However, in many other basidiomycetes, one example being the ectomycorrhizal fungus *Tricholoma vaccinum*, there is no formation of clamp connections, yet these fungi still form a true dikaryon.

From classical genetic analysis of the semi-compatible interactions in *S. commune* (Raper 1966), it had been deduced that *A* controls clamp cell development, while *B* exerts control over the final step of clamp fusion in addition to nuclear migration (Casselton 2008;

[1]Institute for Microbiology, Microbial Communication, Friedrich Schiller University Jena, Neugasse 25, 07743 Jena, Germany; e-mail: erika.kothe@uni-jena.de
[2]Department of Biological Sciences, Southern Illinois University Edwardsville, Box 1651, Edwardsville, IL, 62026, USA
[3]Department of Biology, Sacred Heart University, 5151 Park Avenue, Fairfield, CT 06825, USA
[4]Department of Biochemistry, Plant Molecular Biology, University of Turku, Biocity A, Tykistök. 6A 20520, Turku, Finland
[5]Department of Biological Sciences, Union College, Peter Irving Wold Center, Schenectady, NY 12308, USA

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

Raudaskoski and Kothe 2010; Kües et al. 2011; Heitman et al. 2013).

The homeodomain (HD) transcription factors encoded within *A* regulate the initial steps of clamp connection formation as well as the pairing of the two haploid nuclei (Kües and Casselton 1992b; Specht et al. 1992b; Stankis et al. 1992). This developmental programme is found in all the phylogenetic lineages of the basidiomycetes, with prominent examples being the agaricomycete *C. cinerea* (Kües et al. 1994; O'Shea et al. 1998), the human pathogenic yeast *Cryptococcus neoformans* (Kwon-Chung et al. 1982; Hull and Heitman 2002) and the maize pathogen *Ustilago maydis* (Schulz et al. 1990; Bölker et al. 1992), among others discussed below. On a molecular level, the reciprocal nuclear exchange and nuclear migration are controlled by a pheromone/receptor system (P/R) encoded within *B* (Specht 1995; Wendland et al. 1995; Vaillancourt et al. 1997).

An interesting twist in the tale is that both *A* and *B* complexes may consist of several repeated units, each encoding mating-type genes of the respective type. These independent subloci act autonomously, with differences in at least one subset of *A* and also in at least one subset of *B* being sufficient to result in a fully compatible mating. Since any subset may exist in multiple allelic specificities, a tremendously high number of variants may therefore exist in nature. This ensures a very high chance to randomly encounter a compatible partner with a different genetic background, while within the progeny of the same fruiting body, compatible mates are encountered only 25 % of the time (Badalyan et al. 2004). It thus seems interesting to compare different basidiomycetes with respect to numbers of subsets and numbers of allelic specificities in order to evaluate both the essential functions in mating and of the molecular evolution of its control. Starting from the *S. commune* H4-8 genome (Ohm et al. 2010) and strains being publically available, we will discuss the current knowledge on *HD* and *P/R* mating-type loci across the basidiomycetes which expanded substantially. Predictions on the interaction of proteins with different specificity are now available to be tested experimentally. For the *B* or *P/R* subloci in *S. commune*, the distance between the *Bα* and *Bβ* loci was found to be close to 7 kb, whereas the entire *B* locus consists of approximately 32 kb. However, the entire *A* or *HD* locus of this species consists of over 580 kb from border to border. A summarized view of the *HD* and *P/R* loci of some selected basidiomycetes is given in Fig. 13.1 (Table 13.1).

As a guide through the evolutionary maze of basidiomycete mating-type genes, we will use the model *S. commune*, to which other systems will be compared. This tetrapolar species is a wood-decaying fungus which, aside from a plenitude of experimental data on mating-type genes, is also exploited for its biotechnological relevance, for example, in producing the glucan schizophyllan used for third-generation enhanced oil recovery (Kothe and Freihorst 2012) or the production of secondary metabolites (Scholtmeijer et al. 2014). Its lifestyle is thought to resemble the ancestor of *Agaricomycetes* which, like *S. commune*, is predicted to have been a tetrapolar white-rot fungus (Hibbett and Donoghue 2001). The mating-type genes regulate all of the following processes: recognition of a compatible mate, formation of a dikaryotic mycelium and the developmental switching to fruiting body and sexual spore formation (for detailed review of the latter, see Pelkmans et al. 2016). In *S. commune*, the mating-type genes are encoded in two linked *A* or *HD* subloci, while another chromosome encodes the two linked *B* or *P/R* subloci (Ohm et al. 2010). These subloci were initially detected by genetic recombination analysis: *Aα* with 9 specificities, *Aβ* with an estimated 32, *Bα* with 9 and *Bβ* with 9. These subspecificities multiply to the 23,328 different mating types calculated to potentially exist in nature (Raper et al. 1960; Raper 1966; Koltin et al. 1967). However, the *S. commune* genome sequence requires us to redraw the initial picture somewhat with yet more subsets of *HD* genes, while for *B* the two hypothesized subloci were indeed confirmed. The numbers of subloci vary between different species (compare Kües et al. 2011; James et al. 2013) with the overall principle of subloci being followed throughout the phylum of basidiomycetes. Anywhere between two (for *Postia placenta*) and eight

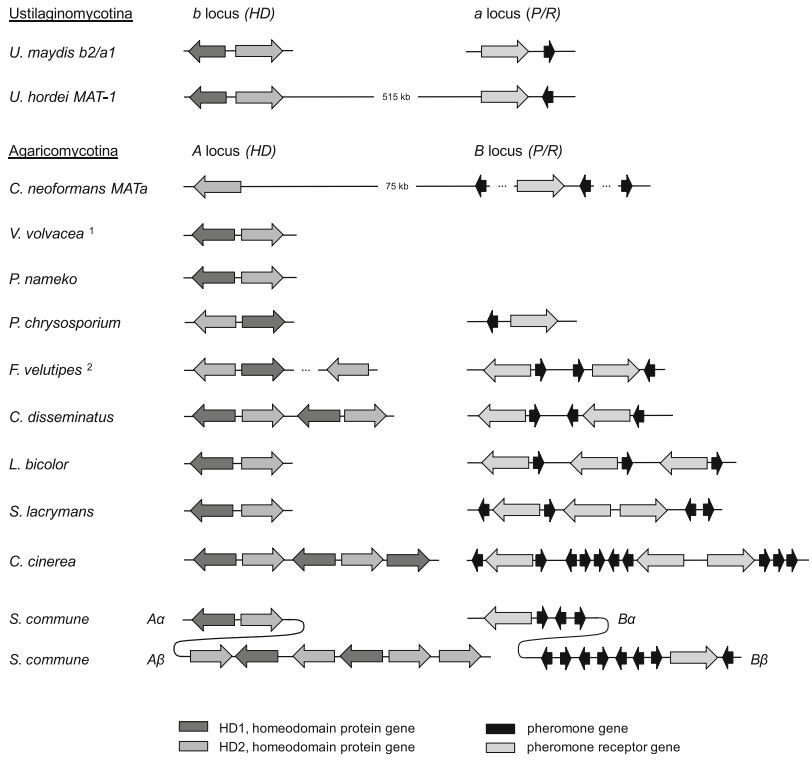


Fig. 13.1 Organization of mating-type loci (*HD* and *P/R* genes). For citations, see Table 13.1 except for 1, Bao et al. (2013), and 2, van Peer et al. (2011)

(for *S. commune*) *HD* representatives were identified, while an even broader spectrum ranging from one pheromone receptor gene (for *Tremella mesenterica*) to up to 26 (for *Serpula lacrymans*) have been identified to date. The latter case of P/R systems is additionally complicated by multiple pheromone genes associated with one receptor gene (compare below and Kües et al., 2011). This complexity is explored in the following paragraphs.

II. Homeodomain Transcription Factor Gene Sets

The paradigm for function of the homeodomain (HD) proteins has been well established, primarily using *C. cinerea*, *S. commune* and *U. maydis* (Feldbrügge et al. 2004; Casselton and Kües 2007; Fraser et al. 2007; Stankis and Specht 2007; Stanton and Hull 2007). It has been shown that interaction of non-self HD proteins of two different classes initiates gene expression in a cascade of developmental events essential to successfully establish and maintain a dikaryon. With large-scale genomic sequencing, it is now feasible to investigate the diversity and evolution of the proteins of the *HD* mating-type locus. The archetype *HD* locus in basidiomycetes contains two divergently transcribed genes (see Fig. 13.1), each encoding a protein with an embedded HD motif. The proteins cluster into two evolutionarily related groups based on the molecular characteristics of their homeodomains: HD1 and HD2. The proteins of the HD1 group are considered 'atypical' or 'TALE' (TALE = **t**hree-**a**mino-acid-**l**oop **e**xtension) HD proteins, in that they contain three extra amino acids located between helices one and two of the three helices that comprise the homeodomain motif. The 'typical' homeodomain is characteristic of the HD2 class of homeodomain proteins, which include a highly

Table 13.1 Mating-type systems of basidiomycetes (an updated selection, based on Kües et al. 2011)

	Species	Mating system	Mating types	*HD* alleles	Receptors and NMR alleles
Microbotryomycetes	*Rhodosporidium toruloides*[a]	Bipolar	2 (*A1*/*A* and *A2*/*a*)	2	2
Pucciniomycetes	*Puccinia graminis*[b]	Bipolar	2 (+ and −)		3
Ustilaginomycetes	*Ustilago hordei*[c]	Bipolar	2 (*MAT-1* and *MAT-2*)	2*	2
	Ustilago maydis[d]	Tetrapolar	≥38	≥19	2
Tremellomycetes	*Cryptococcus gattii*[e]	Bipolar	2 (*a* and α)	2	2
	Cryptococcus neoformans[f]	Bipolar	2 (*a* and α)	2	2
	Kwoniella heveanensis[g]	Tetrapolar	Multiple	2	≥6
	Tremella mesenterica[h]	Tetrapolar	Multiple (68–146)	2	
Agaricomycetes	*Auricularia auricula judae*[i]	Bipolar	Multiple (20 *A*, 14 *B*)		
	Coprinellus disseminatus[j]	Bipolar	Multiple (estimated 123)		4
	Coprinopsis cinerea[k]	Tetrapolar	>12,000 (108–160 *A*, 79 *B*)	4–7*	14 (4 + 2 NMRs in *B43**)
	Coprinopsis scobiola[l]	Tetrapolar	Multiple (7 *A*, 7 *B*)		
	Cyathus stercoreus[m]	Tetrapolar	Multiple (estimated 39 *A*, 24 *B*)		
	Ganoderma lucidum[n]	Tetrapolar	Multiple	2*	8*
	Gloeophyllum trabeum[n]	Bipolar	Multiple	2*	8*
	Heterobasidion irregulare[n]	Bipolar	Multiple	4*	4*
	Laccaria bicolor[o]	Tetrapolar	Multiple (45 *A*, 24 *B*)	2*	8*
	Lentinula edodes[p]	Tetrapolar	Multiple (40–65 *A*, 63–100 *B*)		
	Moniliophthora perniciosa[q]	Homothallic	0		
	Phanerochaete chrysosporium[n]	Bipolar	Multiple	2*	4*
	Pholiota nameko[r]	Bipolar	Multiple (≥6)		
	Pleurotus djamor[s]	Tetrapolar	Multiple (ca. 58 *A*, 231 *B*)		
	Postia placenta[n]	Bipolar	Multiple	2*	5*
	Pycnoporus cinnabarinus[t]	Tetrapolar	Multiple	2*	5*
	Schizophyllum commune[u]	Tetrapolar	23,328 (9 *Aα*, 32 *Aβ*; 9 *Bα*, 9 *Bβ*)	41 (6*)	18 (2 + 4 NMRs*)

Serpula lacrymans[v]	Tetrapolar	Multiple	2*	26*
Stereum hirsutum[n]	Bipolar?		4*	4*
Trametes versicolor[n]	Tetrapolar	Multiple	2*	6*

Model organisms are highlighted in grey. Asterisk indicates abundance of genes in one genome
[a]Banno (1967), Fell and Statzell-Tallman (1998) and Coelho et al. (2008)
[b]Craigie (1927, 1931)
[c]Groth (1975), Martinez-Espinoza et al. (1993), Lee et al. (1999) and Bakkeren et al. (2008)
[d]Bakkeren et al. (2008)
[e]Fraser et al. (2003)
[f]Kwon-Chung (1976) and Kwon-Chung and Bennett (1978)
[g]Metin et al. (2010)
[h]Bandoni (1965) and Wong and Wells (1985)
[i]Whitehouse (1949)
[j]James et al. (2006)
[k]Raper (1966)
[l]Kemp (1974)
[m]Malloure and James (2013)
[n]James et al. (2013)
[o]Raffle et al. (1995) and Niculita-Hirzel et al. (2008)
[p]Fox et al. (1994)
[q]Griffith and Hedger (1994)
[r]Aimi et al. (2005)
[s]James et al. (2004)
[t]Levasseur et al. (2014)
[u]Raper (1966) and Ohm et al. (2010)
[v]James et al. (2013) and Skrede et al. (2013)

conserved DNA-binding region and homology across the entire 60 amino acids of the homeodomain motif (Gillissen et al. 1992; Kües and Casselton 1992a; Stankis et al. 1992).

A model for how the HD1 and HD2 proteins interact is well supported by substantial experimentation. Following hyphal fusion of two potential monokaryotic mates, development controlled by the *HD* locus is initiated if a non-self interaction occurs between HD1 and HD2 proteins of differing mating type. Generally, this complex is thought to form at the level of a dimer and only between non-self HD1/HD2 protein pairs (Kües et al. 1994; Kämper et al. 1995; Asante-Owusu et al. 1996; Schlesinger et al. 1997). However, evidence from experimental work with *S. commune* has indicated that both HD1 and HD2 proteins may also form homodimers and that the active complex may be of a higher order than a dimer in this species (Asada et al. 1997; Robertson et al. 2002). Following formation of the now active hetero-protein complex, it is transported into the nucleus, where it can bind to regulatory sequences found in target genes and initiate the changes in gene expression required for sexual development. In this way, *HD* mating-type proteins initiate events that culminate in the formation of a stable dikaryon.

Aside from the homeodomain region itself, several characteristic features are commonly found in HD proteins across the basidiomycetes. Typically, a nuclear localization signal can be found C-terminal to the homeodomain in either the HD1 or HD2 protein of an interacting pair but not with both. Since this protein sequence is essential for translocation of the active heterodimer into the nucleus (Spit et al. 1998; Robertson et al. 2002), it is imperative that at least one of the proteins should carry this nuclear localization domain. However, the dimer likely would not be transported properly if both HD proteins carried the signal. Regions that have high concentrations of negative charge localized C-terminal to the homeodomain region have been shown to activate transcription both in vivo and in heterologous systems. One important difference between the HD1 proteins may lie in the function of the HD1 atypical homeodomain, which has been shown to be tolerant of significant deletion and mutations in vivo both in *C. cinerea* and *S. commune* but not in *U. maydis* (Luo et al. 1994; Asante-Owusu et al. 1996; Schlesinger et al. 1997). The primary structure of the N-terminal region is highly divergent between alleles derived from different mating types and contains regions imparting mating-type specificity and also mediating dimerization (Kämper et al. 1995; Yue et al. 1997).

The *HD* locus of *S. commune* covers more than 580 kb sequence making it the largest discovered so far and substantially different in its chromosomal organization than other basidiomycete fungi investigated to date (James et al. 2013). Within different strains, the number of HD1 and HD2 proteins encoded varies. Within the sequenced *S. commune* strain H4-8, six predicted homeodomain genes were found (compare also Fig. 13.1). One of these, *abv6*, showed mating-specific function (Shen et al. 1996) while the others still await functional analysis. Recent investigations led us to conclude that the *Aβ* locus with its *HD* genes is derived from the *Aα* locus after speciation. By duplication, additional gene pairs diverged to become the more complex sublocus with additional specificities (KAB unpublished).

III. Pheromone and Receptor Gene Sets

As a group, the *B* genes encode pheromones and their respective receptors. The lipopeptide pheromones act as ligands only to the G-protein-coupled receptors (GPCRs) derived from a different specificity. Thus, any given monokaryotic, haploid individual cannot induce its own pheromone response by stimulating its own receptor. The signal transduction pathway activated upon recognition of a pheromone by an appropriate GPCR involves Ras, a MAP kinase cascade, and Cdc42, as well as cAMP-dependent signalling (Feldbrügge et al. 2004; Weber et al. 2005; Palmer and Horton 2006; Raudaskoski and Kothe 2010; Raudaskoski et al. 2012; Knabe et al. 2013).

One of the least complicated but best understood basidiomycete P/R systems discovered to date is in the corn smut *Ustilago maydis* (Banuett and Herskowitz 1989; Gillissen et al. 1992). In this species, the P/R system is encoded

by one of either two allelic *a* loci, *a1* or *a2*, corresponding to the two specificities. Each *a* locus, named long before any molecular homologies to agaricomycete *B* genes were discovered, codes for one pheromone gene, either *mfa1* or *mfa2*, and one receptor gene, either *pra1* or *pra2*. The lipopeptide pheromones are similar to the yeast **a**-factor, as they are short peptides modified by both carboxymethylation and farnesylation. The receptor proteins are homologous to the yeast Ste3 receptor. While Pra1 can only recognize Mfa2, Pra2 only responds to Mfa1.

In *Agaricomycetes*, the redundant *B* subloci encode one Ste3-type receptor and several pheromone proteins each (Raudaskoski and Kothe 2010). The most complex *B* locus examined so far is the *Bβ2* locus of *S. commune* (Ohm et al. 2010) with one receptor gene *bbr2*, eight functionally characterized pheromone genes (*bbp* genes) and, in addition, four putative pheromone genes or pheromone-like genes (*bpl* genes).

A. Receptors

Basidiomycete pheromone receptors are homologous to the *S. cerevisiae* pheromone receptor Ste3. The protein function of Ste3 (Hagen et al. 1986) is associated with its localization in the membrane enabled by seven hydrophobic transmembrane domains, and certain mutations in this gene can lead to insensitivity towards the **a**-factor pheromone. The receptors are coupled via heterotrimeric G proteins to a MAP kinase cascade, resulting in mating-dependent gene regulation (Nakayama et al. 1987; Feng and Davis 2000).

Analysis of the predicted protein products encoded by the *S. commune* receptor genes revealed the typical seven-transmembrane domain structure of G-protein-coupled receptors (GPCRs). Compared to *S. cerevisiae* Ste3, there were rather extended C-termini. The functional analysis of receptor genes in *S. commune* was initially performed utilizing transformation assays, in which a putative *B* receptor gene was introduced into recipient strains of different *B* specificity (containing pheromones of different specificities). The resulting transformants displayed a phenotype of *B*-regulated development. When crossed with a strain differing only in the *A* mating specificity, a fully compatible development could be seen (Wendland et al. 1995; Vaillancourt et al. 1997). A *B* locus deletion mutant, B_{null}, has been identified that carries no pheromone or receptor genes and thus was used for more straightforward analysis of *B* genes after transformation (Raper and Raper 1973; Fowler et al. 2001, 2004; Gola et al. 2002; Gola and Kothe 2003). In order to identify the domains responsible for the different specificities in allelic receptors, chimeric receptors derived from orthologous receptor genes of two different specificities were constructed. These experiments revealed that the specificity domains are situated in extracellular loops along the entire length of the receptor, for example, the specificity for *Bα1* resides in the last extracellular loop (Hegner et al. 1999; Gola et al. 2002; Gola and Kothe 2003; Kothe et al. 2003). With respect to *Bα2*, other sites in at least two distinct extracellular locations along the receptor molecule were necessary to prevent binding of 'self' *Bα2* specificity pheromones. Chimeric receptors were also created that were constitutive, i.e. active in turning on *B*-regulated development without the need for any pheromone to bind. Even more interestingly, two other phenotypes were detected: promiscuous receptors (any pheromone can induce subsequent development) and highly selective receptors (with only a small subset of pheromones of all non-self specificities able to activate development). These mutants allowed for characterization of receptor activation in an induced state following the mode of action that had been described for pharmaceutically important G-protein-coupled receptors (Fowler et al. 2001; Gola et al. 2002; Erdmann et al. 2012).

The high redundancy of pheromones recognized as non-self poses the question of how does one receptor molecule discriminate between the few self and more than 20 different non-self pheromones? The activation of a receptor by a non-self pheromone can be visualized as the interaction between the ligand with receptor domains presented at the outer surface of the membrane. By binding to the receptor, the pheromone stabilizes an activated state in a multistate model for receptor structure in which a specific conformation is favoured that allows for transduction of the signal. This transduction is initiated by dissociation of the heterotrimeric G protein from its C-terminal binding site. The stabilizing effect of the bound ligand is the result of

interaction of certain amino acids of the peptide backbone of the pheromone with specific amino acids of the receptor. Only if these interacting amino acids are spaced in a way that permits the adoption of the active state conformation will the signal be transduced and *B*-regulated development subsequently ensue. If this model is correct, then a single point mutation could alter the array of pheromones inducing signal transduction. This idea was confirmed using the chimeric receptors described above, where one promiscuous receptor was changed to a super-specific one by one single point mutation in an extracellular domain of the receptor (Bar2-1-1Q61M, Gola et al. 2002).

The molecular explanation of induction of a pheromone receptor by different ligands, however, is only half the story. A key remaining question is, in which developmental stage of the mycelium is the receptor required to be activated? The localization of pheromone receptor protein in wild-type monokaryotic cells has proven to be problematic, likely because of the rather low level of expression of this protein. However, receptor expression was induced in a specific mutant in which the signal transduction network was disrupted by inactivation of a Ras-Gap. The latter is involved in the regulation of Ras, a small G protein (Raudaskoski et al. 2012) and activator of the intrinsic GTPase activity of Ras, with the effect of deactivating Ras. In deletion mutants lacking this Gap function, Ras is therefore activated (Schubert et al. 2006; Knabe et al. 2013). Dikaryotic hyphae homozygous for the Ras-Gap deletion were observed to produce unfused clamps, consistent with *B*-regulated development being necessary for clamp fusion. These unfused clamps showed abundance of receptor proteins, essential for the receptor localization (Erdmann et al. 2012). This allowed us to describe the role of the pheromone response pathway in mate recognition, with a specifically defined impact in clamp fusion.

One remaining question concerns the evolution of the multitude of receptors in *S. commune*. As discussed above, both super-selective and promiscuous receptors can be constructed in vitro. These experiments support the idea that gene duplication along with recombination events could lead to new types of receptors. Indeed, in the *S. commune* strain collection from the Raper laboratory at Harvard, two strains are known with promiscuous mating behaviour for *Bα*, which now can easily be explained on a molecular level. These isolates from nature, as well as the synthetically constructed promiscuous chimeric receptors, show a phenotype that resembles semi-compatible mating interactions in which *B*-regulated development is turned on. In addition, selection for reversion to a new state of *B-off* receptor has also been achieved (Gola and Kothe 2003). The result of mutation then would be a new *B* mating type originating from an initial gene duplication event. Especially with *S. commune*, the selection pressure against the B-on phenotype is easily seen. Here, *B*-regulated development is associated with a phenotype lacking aerial mycelium formation ('flat') which, in dead wood, would lead to drying out of the hyphae and hence immediately presenting a strong selective pressure for reversion to *B-off*. However, this 'flat' phenotype is not phenotypically true for all B-on basidiomycetes (e.g. *C. cinereus*), and therefore an alternate selection scheme must be postulated in those species. A potential solution to this conundrum is that semi-compatible mating interactions mimicked by promiscuous receptor molecules are in fact detrimental when it comes to achieving compatible matings in encounters with other mycelia.

The evolution of tetrapolar receptor systems in other agaricomycetes reveals an essentially common picture with some specifics to each fungus. Both *C. cinerea* and *S. lacrymans*, the latter a prominent destroyer of building timber, contain three receptor genes (*rcb1*, *rcb2*, *rcb3*) associated with the respective pheromone genes (Skrede et al. 2013),each of which fails to cross-hybridize to the others (Riquelme et al. 2005). For *C. cinerea*, two alleles of group 1 type, five of group 2 type and seven of group 3 type have been identified, which totals to 70 *B* mating-type specificities, close to the 79 specificities estimated previously by population analysis (Raper 1966). In *S. lacrymans*, there seems to be less richness in allele diversity (three HD, four P/R specificities), to which the authors ascribe a genetic bottleneck in the investigated European strains. As a result, only two dikaryons would have been sufficient to found the entire European *S. lacrymans* population (Skrede et al. 2013). In the ectomycor-

rhizal fungus *Laccaria bicolor*, in addition to three receptor genes (Riquelme et al. 2005; Niculita-Hirzel et al. 2008), five partial, dysfunctional genes were identified, and yet another five genes with a cryptic function may in fact be non-mating-type receptors (see Section 15.3 C, this chapter). With *Flammulina velutipes*, an edible mushroom (enokitake) from East Asia, two receptor genes surrounded by pheromone genes and three putative non-mating-type receptors were found (van Peer et al. 2011). In the basal clades of basidiomycetes with the comparatively simple smut *P/R* mating-type loci described above, the anther smut of several Caryophyllaceae *Microbotryum violaceum* has linked mating-type loci characteristic for bipolar species such as *U. hordei*. However, homothallic development has been described as well for this fungus (Giraud et al. 2008). In the rust *Puccinia graminis*, three hypothetical Ste3-like proteins have been identified which await further analysis (Devier et al. 2009). Another group of rust fungi, the red yeasts, is considered to be bipolar heterothallic with two true alleles, *A1* and *A2*, such as *Rhodosporidium toruloides* (Coelho et al. 2008).

B. Pheromones

Whether biochemically isolated, genetically confirmed or merely predicted from DNA sequences, basidiomycete mating pheromones have one common feature: a farnesylated cysteine at the carboxyl-terminus of the mature pheromone. The first examples were isolated from *R. toruloides* (rhodotorucine A) and from basidiomycete jelly fungi in the genus *Tremella* (Kamiya et al. 1978; Sakagami et al. 1981; Ishibashi et al. 1984). The *S. cerevisiae* **a**-factor pheromone has been the target for intense genetic and biochemical studies, and it now serves as the paradigm for the fungal kingdom (Michaelis and Barrowman 2012). Since these initial studies, farnesylated lipopeptide pheromones have been investigated from *U. maydis* (Bölker et al. 1992; Spellig et al. 1994), *U. hordei* (Anderson et al. 1999), the human pathogen *C. neoformans* (Moore and Edman 1993; McClelland et al. 2002) and also from the mushroom fungi *S. commune* (Wendland et al. 1995; Vaillancourt et al. 1997) and *C. cinerea* (O'Shea et al. 1998). Several research groups have produced synthetic pheromones based on predicted or known processing of the precursors for use in assays and structural studies (Anderson et al. 1999; Hegner et al. 1999; Olesnicky et al. 1999; Kosted et al. 2000; Davidson et al. 2000; Szabo et al. 2002). This flurry of basidiomycete pheromone research was built on two common themes that were recognized and reconfirmed with each additional study, namely that: (1) mating pheromone genes are located in the *MAT* loci as determinants of mating-type identity and (2) basidiomycete mating pheromones are all lipopeptides. Both of these themes differed from the pattern established at the time in the ascomycete yeast models in which pheromones came in prenylated *and* unmodified peptide pairs and that the *MAT* loci only harboured transcriptional regulators (Herskowitz 1988; Kelly et al. 1988; Davey 1998).

Not surprisingly, the total number of predicted basidiomycete pheromones has increased in parallel with the number of sequenced genomes (Niculita-Hirzel et al. 2008; Kües et al. 2011; van Peer et al. 2011; Wu et al. 2013; Kim et al. 2014). In predicting pheromone genes, a C-terminal prenylation signalling motif has been the most readily identifiable feature in an in silico translation. This CAAX motif is composed of C for cysteine, A for an aliphatic residue and X as one of several residues, with leucine and few other amino acids at the X position acting as a signal for geranylgeranylation rather than farnesylation (Clarke 1992; Michaelis and Barrowman 2012). Exceptional gene structures are found within *R. toruloides*, *Melampsora larici-populina* and two additional red yeast species, for which pheromone genes each encode tandem copies of a CAAX-containing pheromone precursors (Akada et al. 1989) (Coelho et al. 2008; Duplessis et al. 2011; Kües et al. 2011). A recent report on *F. velutipes* suggests that a predicted mating pheromone gene encodes CAAXW at its prenylation motif, rather than CAAX(STOP)

(van Peer et al. 2011). Functional testing is needed to determine if this variation on a CAAX motif is actually recognized and modified biochemically.

Genes for mating pheromones and their receptors are linked: this makes genetic sense because pheromones and receptors are mating-type determinants. However, some deviations to this pattern are found. The bipolar fungus *Pholiota nameko* does not include pheromone genes in its *MAT* locus but instead is able to promote full sexual development solely through its homeodomain proteins (Aimi et al. 2005; Yi et al. 2010). James and colleagues (2006) showed that pheromones and receptors are not mating-type determinants of the bipolar fungus *Coprinellus disseminatus*, yet can substitute in that role in heterologous expression of mating assays in *C. cinerea*, a host species that has the pheromones and receptors as determinants of mating-type identity (O'Shea et al. 1998; Halsall et al. 2000). Most recently, for the king oyster mushroom *Pleurotus eryngii*, four genes for mating pheromones were identified, two of which have been confirmed to produce active pheromones by their expression in transformed strains followed by mating assays (Kim et al. 2014).

Basidiomycete mating pheromone precursors are presumed to be processed in a manner that parallels maturation of **a**-factor of *S. cerevisiae* (Fig. 13.2). The multistep process for **a**-factor was recently reviewed by Michaelis and Barrowman (2012). Currently, basidiomycete pheromone processing is only understood by analogy to the yeast paradigm. Active pheromones that have been isolated and characterized indicate that the precursors predicted from gene sequence data have been modified by farnesylation, N- and C-terminal proteolytic cleavages and, typically, carboxymethylation (Fig. 13.2). Rhodotorucine A and tremerogen a-13 are exceptions that do not have a carboxymethylated C-terminal cysteine residue (Kamiya et al. 1978; Sakagami et al. 1981; Ishibashi et al. 1984; Anderegg et al. 1988; Davey 1992; Spellig et al. 1994). In efforts towards understanding pheromone precursor processing, an essential farnesyltransferase β subunit and an ABC transporter were explored in *C. neoformans* (Vallim et al. 2004; Hsueh and Shen 2005). Basidiomycete pheromone precursor processing has been assessed indirectly through heterologous expression of basidiomycete pheromone genes in *S. cerevisiae*. Fowler et al. (1999) expressed *S. commune* mating pheromone and receptor genes as substitutes for the **a**-factor and its receptor (Ste3p) in *S. cerevisiae*. Two different *S. commune* pheromones, Bbp2(4) and Bbp1(1), were properly exported and detected in yeast pheromone response assays. The response to Bbp2(4) was abolished in a farnesyltransferase-deficient yeast strain (Δ*ram1*), severely reduced in the absence of the AAX-removing proteases (Δ*rce1* Δ*ste24*) and carboxymethylase (Δ*ste14*), but was near wild-type response levels in a yeast host that is deficient for one of the N-terminal protease activities (Δ*axl1* Δ*ste23*). Thus, for at least this one *S. commune* pheromone, C-

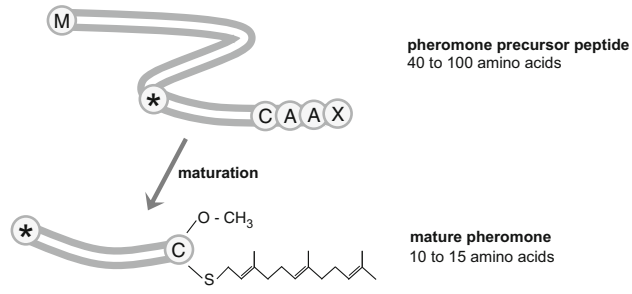


Fig. 13.2 Pheromone maturation process. Before N-terminal processing, farnesylation followed by removal of AAX motif and carboxymethylation are accomplished. *M* methionine, *C* cysteine, *A* aliphatic residue, *X* one of several residues that cue farnesyl isoprenylation illustrated by the structural formula; *asterisk* stands for one to two acidic or basic residues

terminal processing of the pheromone precursor in yeast required the same enzymes that *S. cerevisiae* uses to process its own **a**-factor. The status of N-terminal processing remains unclear. Export of the *S. commune* pheromone was reduced in the absence of the **a**-factor transporter Ste6p but only by about 50 % in a Δ*ste6* background in the **a**-cell type. This result indicates that export of the *S. commune* pheromone Bbp2(4) from yeast cells involves an alternative pathway in addition to facilitation by the **a**-factor transporter (Fowler et al. 1999). *S. cerevisiae* has also successfully been used for heterologous expression of *C. cinerea* pheromones and receptors (Olesnicky et al. 1999). In contrast to *S. commune* pheromone Bbp2 (4), *C. cinerea* pheromone Phb2.2^{42} absolutely required the **a**-factor transporter for export of the pheromone to elicit mating.

Structure-function studies of pheromones using synthesized, unmodified peptides with amino acid sequences corresponding to the natural pheromones from *U. maydis* (Spellig et al 1994; Szabo et al. 2002), *U. hordei*, *C. neoformans* and *C. cinerea* have shown that both C-terminal modification and increased hydrophobicity of the peptides were necessary for near wild-type pheromone activity (Spellig et al. 1994; Kosted et al. 2000; Davidson et al. 2000; Olesnicky et al. 2000). Synthetic analogues of the *C. cinerea* pheromone Phb2.2^{42}, however, were only about half as effective when only carboxymethylated rather than both carboxymethylated and farnesylated (Olesnicky et al. 1999). Synthetic *C. neoformans* MFα derivatives required both farnesylation and carboxymethylation to induce a response in a filamentation assay (Davidson et al. 2000). Synthetic analogues with variations at the N-termini were produced to test the effectiveness of peptides of different lengths. *C. cinerea* Phb2.2^{42} had the least tolerance for N-terminal truncation (Olesnicky et al. 1999). *U. hordei* tridecapeptide mfa1 and decapeptide mfa2 remained fully active with a single N-terminal amino acid truncation from the predicted mature pheromones, with some activity being retained even in a tetrapeptide for Uhmfa2 if the peptide was farnesylated and carboxymethylated (Kosted et al. 2000). Synthetic analogues of *U. maydis* tridecapeptide pheromone mfa1 were strongly active even with the loss of two N-terminal residues, and a version missing five N-terminal residues had some residual activity in a live cell assay (Szabó et al. 2002). Notably, a single amino acid truncation of mfa1 increased the effectiveness compared to the known pheromone sequence, suggesting that at least in these assays, the potency of a pheromone may not always be maximized in vivo. The *U. maydis* nonapeptide mfa2 could be reduced by three N-terminal residues before its activity was undetectable. In addition to truncations at the N-terminus, Szabó and colleagues (2002) examined alanine and D-amino acid replacements to identify residues critical for pheromone function. Generally, the N-terminal half of the peptide was less sensitive to substitutions, similar to the conclusion reached for comparable modifications to the M-factor lipopeptide pheromone of *S. pombe* (Seike et al. 2012).

Alignments of the predicted pheromone precursors from their C-terminal CAAX sequence hinted at a potential consensus protease recognition site for N-terminal cleavage to achieve the mature peptide size. The consensus appears to be a pair of charged amino acids, such as glutamate-arginine (ER) in *C. cinerea* precursors (Riquelme et al. 2005). Some *S. commune* precursors have a charged dipeptide, while others have a single charged residue at about the same relative position from the CAAX motif (Fowler et al. 2004; Riquelme et al. 2005). If the N-terminal cleavage prediction is correct, then the mature pheromones are about 10–15 amino acids in length. Levels of amino acid similarity on each side of the putative cleavage sites also hint that these proposed recognition sites may be used (Fowler et al. 2004; Riquelme et al. 2005). On the C-terminal side where the mature pheromone sequences reside, there are several examples of near identity, and the genes for these pheromones are presumed to have arisen by duplication. However, on the N-terminal side of the putative cleavage site of the same predicted pheromone precursor peptides, the sequences are often too different to align with any confidence and can vary widely in length [see Kües et al. (2011) for several examples]. This suggests that selection pressure is different for the two ends of these precursors and with the cleavage position being the boundary.

In addition to investigating the pheromone receptors for specificity determinants (see above), mutations in basidiomycete pheromone genes have yielded some understanding of amino acid residues that are important for recognition in specific pheromone/receptor pairs. Examples from the mushroom fungi illustrate that the difference between two pheromones that activate different receptors can be as little as a single amino acid (Olesnicky et al. 2000; Fowler et al. 2001).

The *S. commune* pheromone Bbp2(1) has a predicted mature sequence of EHGYGGSNVHGWC and is presumed to be C-terminally farnesylated and carboxymethylated. Bbp2(1) activates three mating receptors: Bbr4, Bbr6 and Bbr7 (Fowler et al. 2001). A change in a single amino acid (V to A) converts Bbp2(1) to EHGYGGSNAHGWC (= Bbp2(1-1)), which has an altered activity spectrum of Bbr4, Bbr6 and Bbr7 (same as wild-type) *plus* the additional ability to activate receptor Bbr2 (Fowler et al. 2001). This expanded range of pheromone activity coincidentally led to self-activation of pheromone response in the strain and a mutant 'B-on' phenotype (Parag 1962). Another example comes from *C. cinerea* pheromone Phb3.2^6 with the sequence ERRTHGGNGLTFWC (Olesnicky et al. 2000). This pheromone naturally activates a receptor produced by strains with the B42 version of *matB* but not any of the receptors in the B6 version. The two aromatic residues, FW, were reversed in their order to WF with sequential site-directed mutations. Strain B42 did not respond to the 'WF' mutant pheromone but instead strain B6 responded to the mutant pheromone. A second mutant pheromone derived from Phb3.2^6 with WW in the aromatic positions activated pheromone response in both B42 and B6 strains (Olesnicky et al. 2000; Brown and Casselton 2001) which is similar to functions identified with *S. commune* (Fowler et al. 2001, 2004; Fowler 2010).

Finally, the large numbers of mating pheromones expressed by *S. commune* and *C. cinerea* are the product of co-evolution and extensive gene duplication. These are in accordance with the above discussion of duplications of receptor genes. Accumulated sequence information and predicted protein products have distinguished five pheromone groups in *S. commune* (Fowler et al. 2004) and three pheromone groups in *C. cinerea* (Riquelme et al. 2005). While different criteria were used in these classifications, it appears that the pheromones group with particular receptors and co-evolved with the receptors as the number of mating receptors expanded by gene duplication and divergence. Pheromone genes also experienced duplications and divergence that led to specificity for one or a few receptors and some incomplete overlap of receptor recognition spectra. By accumulating a well-homed repertoire of pheromone genes, each different version of the *P/R* genes codes for pheromones that activate all possible mating receptors in the species, except for the receptors expressed from its own self-specificity. Thus, at the molecular level, mating pheromones and their receptors reinforce the obligate outcrossing and heterothallic behaviours of these species.

C. Non-mating Type Receptors

When annotating genes derived from the genome sequence of *S. commune*, homologues to the Ste3-type receptor gene were discovered which had not been identified by extensive functional transformation assays or by hybridization (Ohm et al. 2010). These so-called non-mating-type receptors (NMRs) belong to the class of fungal pheromone receptors and are either linked to the *B* locus or are unlinked in other genomic locations. Two striking differences are seen in comparison to the mating receptors: (1) there are no classical pheromone genes associated to these new receptor genes, and (2) they possess even longer C-termini than the mating receptors, suggesting an additional function of this region in cytoplasmic interactions. NMRs can also be found in the genomes of other basidiomycetes (James et al. 2006; Niculita-Hirzel et al. 2008; Hsueh et al. 2009; van Peer et al. 2011; Bao et al. 2013). The function of NMRs remains elusive, but several interesting possibilities have been proposed, such as self-recognition or foreign-species recognition (Kües et al. 2011). Fungi with putative non-mating receptors are *L. bicolor* (Niculita-Hirzel et al. 2008), *C. cinerea* and *T. mesenterica* (Kües et al. 2011), *Phanerochaete chrysosporium* (Martinez et al. 2004), *Postia placenta* (Martinez et al. 2009) and *C. disseminatus* (James et al. 2006). Potential NMRs have also been detected in several *Polyporales* species (Kües and Navarro-Gonzalez 2010; Kües et al. 2011; James et al. 2013).

Experimental evidence available to date for *S. commune* suggests that NMRs are not sufficient to induce mating-specific development. The NMRs are not activated through lipopeptide pheromones of a compatible mate, since the B_{null} strain still harbours and expresses the four NMRs (DF unpublished) but lacks sexual competence (Raper and Raper 1973; Fowler et al. 2004). Another characteristic of NMRs in *S. commune* is their low degree of polymorphism, which has also been shown with other species (van Peer et al. 2011). This would suggest that they are unlikely to function as specificity determinants. The amino acid sequence sim-

ilarity of all mating-type pheromone receptors of *S. commune* is around 20 %; only among allelic groups (only *Bα* or only *Bβ* receptors) it is higher, around 50 %. In contrast, the non-mating receptors (called *B*-like receptors, *brl*, in *S. commune*) of different strains share overall amino acid similarities between 72 and 96 %. Phylogenetic reconstruction of *S. commune* receptor proteins shows a clear separation of mating receptors (clustering in *Bα* and *Bβ* receptors) from non-mating receptors (Fig. 13.3).

In the phylogenetic tree combining *S. commune* receptors with Ste3 homologues of other fungi (Fig. 13.3), the receptors of the *Ustilaginomycetes*, *Pucciniomycotina* and *Saccharomycetes* clustered as expected, similar to the speciation of these fungi (Niculita-Hirzel et al. 2008; Kües et al. 2011) and not with the *Agaricomycetes*. The *Bα* receptors of *S. commune* cluster with the receptors Rcb2B44 and Rcb2B1 of *C. cinerea* and several proteins from *Lentinula edodes* and *Postia placenta*, which might be indicative that they have the same function as pheromone receptors during mating. The sister group contains some *Bβ* receptors and, interestingly, Brl1 of *S. commune* (which is an NMR) but with no close relatives except the *Bβ* receptors of its own species. Noteworthy, another clade contains most of the Rcb3 proteins of *C. cinerea*. Several proteins seem to have an unresolved phylogeny, e.g. the Brl3 and Brl4 NMRs of *S. commune*, proteins of *T. vaccinum*, *F. velutipes* and *P. placenta*. Another well-resolved clade contains *Bβ* receptors of *S. commune*, several *C. cinerea* proteins (all classes of Rcb1, Rcb2 and Rcb3) and receptors of all other investigated agaricomycetes. Hinting at the possibility of a common origin, a sister group to this clade contains *S. commune* NMR Brl2, *V. volvacea* Ste3.3 and four other proteins, two of which are from *P. placenta*. The pseudo-homothallic straw mushroom *V. volvacea* carries both *HD1*- and *HD2*-encoding genes, but no *P/R* locus was detected (Bao et al. 2013). Nevertheless, three clustered *Ste3*-like genes were identified in a chromosomal region, showing no synteny when compared to the *P/R* loci of other fungi. In addition, no corresponding pheromone genes were found, arguing for an NMR nature of these genes. A compatible mate to the sequenced *V. volvacea* strain again carried only the same alleles with low polymorphism. Thus, like with *S. commune*, the respective genes likely are not functional pheromone receptors but NMRs.

Some promising efforts have been made to reveal the function of a putative NMR, Crp2, of *C. neoformans* (Hsueh et al. 2009). While this gene acts independently of a ligand and is as a result constitutively active, it has important functions during morphogenesis of this basal, bipolar basidiomycete. Abnormal hyphal structures and fusion defects were detected in *crp2* deletion mutants. Heterologous expression in *S. cerevisiae* revealed that Crp2 activated the pheromone response signalling cascade in yeast. Thus, a clear indication as to whether or not this particular gene is evolutionarily derived from a formerly pheromone-dependent, true receptor is not possible.

One working hypothesis concerning the function of NMRs is that heterodimer formation might increase the amino acids available for binding of different pheromone ligands. Both ways are possible: a heterodimerization among the NMRs or even with the true mating receptors. Thus, the NMRs would contribute to the high redundancy of pheromones and the discrimination of multitudes of self versus non-self pheromones. An example of such a heterodimerization of G-protein-coupled receptors from the animal kingdom is found in the D2 domain receptor (Lee et al. 2000).

D. Basidiomycete Mating Type Gene Radiation: Evolutionary Models

James and colleagues investigated the genomes of *Polyporales* focusing on genes associated with tetrapolar and bipolar mating: they summarized general information available for all *Agaricomycetes* and concluded that bipolarity has evolved independently and with multiple origins within the *Agaricomycetes*. All the genomes investigated contained *HD* and *P/R* genes, based on synteny and sequence similarities (James et al. 2013). An unusual situation was seen with the straw mushroom *Volvariella volvacea*, which seems to contain only NMRs and no *P/R* genes; meanwhile *HD* genes show a normal arrangement (Bao et al. 2013).

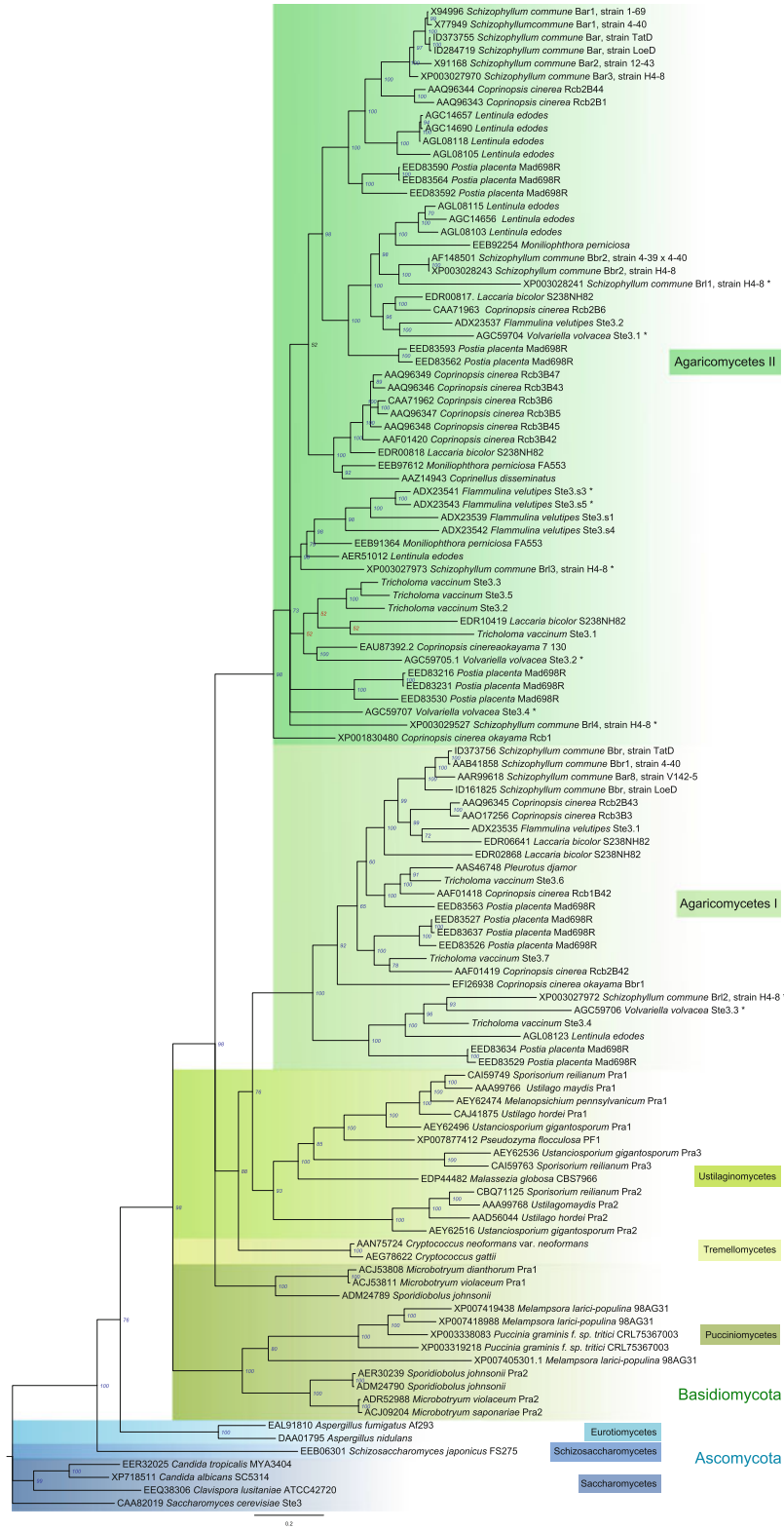


Fig. 13.3 Phylogeny of pheromone receptors and NMRs. Proteins were aligned using Mafft (Katoh et al. 2005), phylogeny calculated with MrBayes (Huelsenbeck and Ronquist 2001) and neighbour joining

In tetrapolar, heterothallic species, *P*/*R* genes are unlinked to *HD* genes. Homothallism (self-compatibility) might arise from a lack of mating-type genes, which is true for 5 % of *Polyporales* species (James et al. 2013). Possible reasons might be that there are mutations rendering the receptors either constitutive or promiscuous (Gola et al. 2000; Gola and Kothe 2003), HD1/2 fusions which are constitutively active, or they might have an aberrant mechanism of spore production containing already mated, potentially even diploid nuclei (e.g. *Agaricus bisporus*). Bipolar heterothallism with a single functional mating-type locus and obligate outcrossing is observed in 18 % of the *Polyporales* species, while true tetrapolar species are making up the majority with 77 % (Rajchenberg 2011). These numbers corroborate the findings of Raper almost 50 years ago (Raper 1966).

Over 100 years ago, Albert Blakeslee defined heterothallic species to be self-sterile, requiring a mating partner in order to undergo reproduction in contrast to homothallic species (Blakeslee 1904). The genetics of heterothallism requires mating-type genes to discriminate self from non-self.

The evolution of mating types was nicely discussed by Nicholas Perrin (2012) not only for fungi but also for algae and ciliates. He defines two hypotheses for their evolution:

1. Mating-type genes evolved to control organelle transmission during sexual reproduction or to prevent inbreeding or same-clone mating.
2. Mating-type genes evolved primarily to switch on the correct developmental/genetic programme (e.g. meiosis, gametogenesis, mating) at the right stage of life cycle (for plants: gametophytes or sporophytes, for fungi: monokaryon, dikaryon, fruiting body).

Two mating types would lead to 50 % inbreeding and 50 % outcrossing in random matings but at the same time would be less highly selected if, in the case of fungi, the dikaryotic lifestyle is advantageous. A large number of mating types simply maximizes the chance to meet a compatible partner. Basidiomycetes have increased the number of mating types by functional redundancy, i.e. gene duplication and development of new specificities (Casselton and Olesnicky 1998). This is seen with putatively 160 different mating types with *HD* loci in *C. cinereus* and 288 types in *S. commune* and holds true for the *P*/*R* genes as well.

In order to explore the potential increase in fitness by both mates contributing organelles, a potential conflict based on competition between organelles needs to be considered. In *S. cerevisiae*, mitochondria are transmitted by both partners and mitochondrial fusion and recombination of mtDNA will occur (Takano et al. 2010). This maximizes fitness of the resulting organelle. Within basidiomycetes like *S. commune* (Specht et al. 1992a), a uniparental inheritance of organelles has evolved, which prevents such costs of competition. Thus, the first hypothesis proposed by Perrin (2012) seems unlikely for tetrapolar basidiomycetes. As to Perrin's second hypothesis, although it is clear that mating-type genes evolved to switch on sexual development in basidiomycetes, it is hard to imagine this being the sole cause to explain the impressive radiation of mating-type genes in these fungi.

An alternate theory was introduced by Billiard and colleagues: the 'sex-advantage enhancer' model (Czaran and Hoekstra 2004; Billiard et al. 2011). The model states that mating maximizes recombination, DNA repair and breakdown of negative epistasis (recessive and dominant alleles, epigenetics) and generally increases genetic variability. In order to avoid mating and recombination between genetic

Fig. 13.3 (continued) method (Dayhoff model, 3×10^6 generations) and verified using Tracer (Rambaut and Drummond 2003). NCBI accession numbers or JGI protein identifiers are given followed by species name, protein name and strain if available. Bootstraps are indicated and branches with less than 50 % support have been collapsed. Putative NMRs are labelled with an *asterisk*

clones, mating types evolved. While this might be true, this hypothesis also does not explain the high radiation of the mating-type genes. An interesting idea revolves around the fact that pheromones are secreted factors and might therefore have the additional function of allowing quorum sensing. This notion might go some way towards explaining the high variability of pheromones and their receptors. Indeed, close examination of opposed hyphae of *S. commune* has revealed some attraction of hyphae at short distance (Raudaskoski 1998). Thus the attraction towards a potential mate, which is in concurrence with exploitation of the habitat for new resources in the filamentous growth state, would be limited to those cases where mating will be—at least for the *P*/*R* genes—fruitful.

For the *HD* genes' radiation, another line of thought may be exploited. In order to assess the state of ploidy, since meiosis and sexual spore production should not happen when in the haploid state, the *HD* transcription factors serve as a developmental switch ensuring that polyploidy is avoided. At the same time, sexual development in haploids is prevented (Perrin 2012). Maximizing control over assessment of a potential mating partner is realized in the *P*/*R* genes by the high number of allelic versions. As in ciliates and algae and the ascomycete heterokaryon incompatibility system, the basidiomycetes have evolved a developmental switch using a multiallelic and multipolar system of the highest complexity.

Acknowledgements The authors very much appreciate the comments and help regarding MrBayes/phylogenetic analysis of Dr. M. Gube and D. Sammer, and we also thank the latter for providing protein data on *T. vaccinum*. We also would like to gratefully acknowledge the Graduate School JSMC and the Deutsche Forschungsgemeinschaft for funding.

References

Aimi T, Yoshida R, Ishikawa M, Bao D, Kitamoto Y (2005) Identification and linkage mapping of the genes for the putative homeodomain protein (*hox1*) and the putative pheromone receptor protein homologue (*rcb1*) in a bipolar basidiomycete, *Pholiota nameko*. Curr Genet 48(3):184–194. doi:10.1007/s00294-005-0012-7

Akada R, Minomi K, Kai J, Yamashita I, Miyakawa T, Fukui S (1989) Multiple genes coding for precursors of rhodotorucine A, a farnesyl peptide mating pheromone of the basidiomycetous yeast *Rhodosporidium toruloides*. Mol Cell Biol 9(8):3491–3498

Anderegg RJ, Betz R, Carr SA, Crabb JW, Duntze W (1988) Structure of *Saccharomyces cerevisiae* mating hormone a-factor. Identification of S-farnesyl cysteine as a structural component. J Biol Chem 263(34):18236–18240

Anderson CM, Willits DA, Kosted PJ, Ford EJ, Martinez-Espinoza AD, Sherwood JE (1999) Molecular analysis of the pheromone and pheromone receptor genes of *Ustilago hordei*. Gene 240 (1):89–97

Asada Y, Yue CL, Wu J, Shen GP, Novotny CP, Ullrich RC (1997) *Schizophyllum commune Aα* mating-type proteins, Y and Z, form complexes in all combinations in vitro. Genetics 147(1):117–123

Asante-Owusu RN, Banham AH, Böhnert HU, Mellor EJC, Casselton LA (1996) Heterodimerization between two classes of homeodomain proteins in the mushroom *Coprinus cinereus* brings together potential DNA-binding and activation domains. Gene 172(1):25–31

Badalyan SM, Polak E, Hermann R, Aebi M, Kües U (2004) Role of peg formation in clamp cell fusion of homobasidiomycete fungi. J Basic Microbiol 44 (3):167–177. doi:10.1002/jobm.200310361

Bakkeren G, Kämper J, Schirawski J (2008) Sex in smut fungi: structure, function and evolution of mating-type complexes. Fungal Genet Biol 45:S15–S21. doi:10.1016/j.fgb.2008.04.005

Bandoni RJ (1965) Secondary control of conjugation in *Tremella mesenterica*. Can J Bot 43(6):627–630

Banno I (1967) Studies on the sexuality of *Rhodotorula*. J Gen Appl Microbiol 13(16):196

Banuett F, Herskowitz I (1989) Different a alleles of *Ustilago maydis* are necessary for maintenance of filamentous growth but not for meiosis. Proc Natl Acad Sci USA 86(15):5878–5882

Bao D, Gong M, Zheng H, Chen M, Zhang L, Wang H, Jiang J, Wu L, Zhu Y, Zhu G, Zhou Y, Li C, Wang S, Zhao Y, Zhao G, Tan Q (2013) Sequencing and comparative analysis of the straw mushroom (*Volvariella volvacea*) genome. PLoS One 8(3), e58294. doi:10.1371/journal.pone.0058294

Billiard S, Lopez-Villavicencio M, Devier B, Hood ME, Fairhead C, Giraud T (2011) Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. Biol Rev 86 (2):421–442. doi:10.1111/j.1469-185X.2010.00153.x

Blakeslee AF (1904) Zygospore formation a sexual process. Science 19(492):864–866. doi:10.1126/science.19.492.864

Bölker M, Urban M, Kahmann R (1992) The *a* mating type locus of *U. maydis* specifies cell signaling components. Cell 68(3):441–450

Brown AJ, Casselton LA (2001) Mating in mushrooms: increasing the chances but prolonging the affair. Trends Genet 17(7):393–400

Casselton LA (2008) Fungal sex genes—searching for the ancestors. Bioessays 30(8):711–714. doi:10.1002/Bies.20782

Casselton LA, Kües U (2007) The origin of multiple mating types in the model mushrooms *Coprinopsis cinerea* and *Schizophyllum commune*. In: Heitman J, Kronstad JW, Taylor JW, Casseleton LA (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 283–300

Casselton LA, Olesnicky NS (1998) Molecular genetics of mating recognition in basidiomycete fungi. Microbiol Mol Biol Rev 62(1):55–70

Clarke S (1992) Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. Annu Rev Biochem 61:355–386. doi:10.1146/annurev.bi.61.070192.002035

Coelho MA, Rosa A, Rodrigues N, Fonseca A, Goncalves P (2008) Identification of mating type genes in the bipolar basidiomycetous yeast *Rhodosporidium toruloides*: first insight into the *MAT* locus structure of the Sporidiobolales. Eukaryot Cell 7(6):1053–1061. doi:10.1128/EC.00025-08

Craigie JH (1927) Discovery of the function of the pycnia of the rust fungi. Nature 120:765–767

Craigie JH (1931) An experimental investigation of sex in the rust fungi. Phytopathology 21:1001–1040

Czaran TL, Hoekstra RF (2004) Evolution of sexual asymmetry. BMC Evol Biol 4:34. doi:10.1186/1471-2148-4-34

Davey J (1992) Mating pheromones of the fission yeast *Schizosaccharomyces pombe*: purification and structural characterization of *M*-factor and isolation and analysis of two genes encoding the pheromone. EMBO J 11(3):951

Davey J (1998) Fusion of a fission yeast. Yeast 14 (16):1529–1566, Doi: 10.1002/(SICI)1097-0061 (199812)14:16<1529::AID-YEA357>3.0.CO;2-0

Davidson RC, Moore TD, Odom AR, Heitman J (2000) Characterization of the *MFα* pheromone of the human fungal pathogen *Cryptococcus neoformans*. Mol Microbiol 38(5):1017–1026

Devier B, Aguileta G, Hood ME, Giraud T (2009) Ancient trans-specific polymorphism at pheromone receptor genes in basidiomycetes. Genetics 181(1):209–223. doi:10.1534/genetics.108.093708

Duplessis S, Cuomo CA, Lin YC, Aerts A, Tisserant E, Veneault-Fourrey C, Joly DL, Hacquard S, Amselem J, Cantarel BL, Chiu R, Coutinho PM, Feau N, Field M, Frey P, Gelhaye E, Goldberg J, Grabherr MG, Kodira CD, Kohler A, Kües U, Lindquist EA, Lucas SM, Mago R, Mauceli E, Morin E, Murat C, Pangilinan JL, Park R, Pearson M, Quesneville H, Rouhier N, Sakthikumar S, Salamov AA, Schmutz J, Selles B, Shapiro H, Tanguay P, Tuskan GA, Henrissat B, Van de Peer Y, Rouze P, Ellis JG, Dodds PN, Schein JE, Zhong S, Hamelin RC, Grigoriev IV, Szabo LJ, Martin F (2011) Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proc Natl Acad Sci USA 108 (22):9166–9171. doi:10.1073/pnas.1019315108

Erdmann S, Freihorst D, Raudaskoski M, Schmidt-Heck W, Jung EM, Senftleben D, Kothe E (2012) Transcriptome and functional analysis of mating in the basidiomycete *Schizophyllum commune*. Eukaryot Cell 11(5):571–589. doi:10.1128/Ec.05214-11

Feldbrügge M, Kämper J, Steinberg G, Kahmann R (2004) Regulation of mating and pathogenic development in *Ustilago maydis*. Curr Opin Microbiol 7 (6):666–672. doi:10.1016/j.mib.2004.10.006

Fell J, Statzell-Tallman A (1998) *Rhodosporidium* Banno. The yeasts, a taxonomic study, 4th edn. Elsevier, Amsterdam, The Netherlands, pp 678–692

Feng Y, Davis NG (2000) Feedback phosphorylation of the yeast **a**-factor receptor requires activation of the downstream signaling pathway from G protein through mitogen-activated protein kinase. Mol Cell Biol 20(2):563–574

Fowler TJ (2010) A carboxy-subterminal aromatic residue in *Schizophyllum commune* mating pheromones controls specific recognition by Bar4 receptor. Fungal Genet Reports 57:4–6

Fowler TJ, DeSimone SM, Mitton MF, Kurjan J, Raper CA (1999) Multiple sex pheromones and receptors of a mushroom-producing fungus elicit mating in yeast. Mol Biol Cell 10(8):2559–2572

Fowler TJ, Mitton MF, Vaillancourt LJ, Raper CA (2001) Changes in mate recognition through alterations of pheromones and receptors in the multisexual mushroom fungus *Schizophyllum commune*. Genetics 158(4):1491–1503

Fowler TJ, Mitton MF, Rees EI, Raper CA (2004) Crossing the boundary between the *Bα* and *Bβ* mating-type loci in *Schizophyllum commune*. Fungal Genet Biol 41(1):89–101

Fox HM, Burden J, Chang S-T, Peberdy JF (1994) Mating-type incompatibility between commercial strains of *Lentinula edodes*. Exp Mycol 18(2):95–102

Fraser JA, Subaran RL, Nichols CB, Heitman J (2003) Recapitulation of the sexual cycle of the primary fungal pathogen *Cryptococcus neoformans* var. *gattii*: implications for an outbreak on Vancouver Island, Canada. Eukaryot Cell 2(5):1036–1045

Fraser JA, Hsueh Y-P, Findley KM, Heitman J (2007) Evolution of the mating-type locus: the basidiomycetes. In: Heitman J, Kronstad J, Taylor J, Casselton L (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 19–34

Gillissen B, Bergemann J, Sandmann C, Schroeer B, Bölker M, Kahmann R (1992) A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell 68(4):647–657

Giraud T, Yockteng R, Lopez-Villavicencio M, Refregier G, Hood ME (2008) Mating system of the anther smut fungus *Microbotryum violaceum*: selfing under heterothallism. Eukaryot Cell 7(5):765–775. doi:10.1128/EC.00440-07

Gola S, Kothe E (2003) The little difference: in vivo analysis of pheromone discrimination in *Schizophyllum commune*. Curr Genet 42(5):276–283. doi:10.1007/s00294-002-0353-4

Gola S, Hegner J, Kothe E (2000) Chimeric pheromone receptors in the basidiomycete *Schizophyllum commune*. Fungal Genet Biol 30(3):191–196. doi:10.1006/fgbi.2000.1222

Gola S, Hegner J, Kothe E (2002) Chimeric pheromone receptors in the basidiomycete *Schizophyllum commune* (vol 30, pg 191, 2000). Fungal Genet Biol 36(3):255–255. doi:10.1016/S1087-1845(02)00045-2

Griffith G, Hedger J (1994) The breeding biology of biotypes of the witches' broom pathogen of cocoa, *Crinipellis perniciosa*. Heredity 72(3):278–289

Groth J (1975) Two additive, independent genes for mycelial growth versus sporidial growth of haploid cultures of *Ustilago hordei*. Can J Bot 53(19):2233–2239

Hagen DC, McCaffrey G, Sprague GF Jr (1986) Evidence the yeast *STE3* gene encodes a receptor for the peptide pheromone a factor: gene sequence and implications for the structure of the presumed receptor. Proc Natl Acad Sci USA 83(5):1418–1422

Halsall JR, Milner MJ, Casselton LA (2000) Three subfamilies of pheromone and receptor genes generate multiple *B* mating specificities in the mushroom *Coprinus cinereus*. Genetics 154(3):1115–1123

Hegner J, Siebert-Bartholmei C, Kothe E (1999) Ligand recognition in multiallelic pheromone receptors from the basidiomycete *Schizophyllum commune* studied in yeast. Fungal Genet Biol 26(3):190–197. doi:10.1006/fgbi.1999.1119

Heitman J, Sun S, James TY (2013) Evolution of fungal sexual reproduction. Mycologia 105(1):1–27. doi:10.3852/12-253

Herskowitz I (1988) Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol Rev 52(4):536–553

Hibbett DS, Donoghue MJ (2001) Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. Syst Biol 50(2):215–242

Hsueh YP, Shen WC (2005) A homolog of Ste6, the **a**-factor transporter in *Saccharomyces cerevisiae*, is required for mating but not for monokaryotic fruiting in *Cryptococcus neoformans*. Eukaryot Cell 4(1):147–155. doi:10.1128/EC.4.1.147-155.2005

Hsueh YP, Xue C, Heitman J (2009) A constitutively active GPCR governs morphogenic transitions in *Cryptococcus neoformans*. EMBO J 28(9):1220–1233. doi:10.1038/emboj.2009.68

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8):754–755

Hull CM, Heitman J (2002) Genetics of *Cryptococcus neoformans*. Annu Rev Genet 36:557–615. doi:10.1146/annurev.genet.36.052402.152652

Ishibashi Y, Sakagami Y, Isogai A, Suzuki A (1984) Structures of tremerogens A-9291-I and A-9291-VIII: peptidal sex hormones of *Tremella brasiliensis*. Biochemistry 23(7):1399–1404

James TY, Liou SR, Vilgalys R (2004) The genetic structure and diversity of the *A* and *B* mating-type genes from the tropical oyster mushroom, *Pleurotus djamor*. Fungal Genet Biol 41(8):813–825. doi:10.1016/j.fgb.2004.04.005

James TY, Srivilai P, Kües U, Vilgalys R (2006) Evolution of the bipolar mating system of the mushroom *Coprinellus disseminatus* from its tetrapolar ancestors involves loss of mating-type-specific pheromone receptor function. Genetics 172(3):1877–1891. doi:10.1534/genetics.105.051128

James TY, Sun S, Li W, Heitman J, Kuo HC, Lee YH, Asiegbu FO, Olson A (2013) Polyporales genomes reveal the genetic architecture underlying tetrapolar and bipolar mating systems. Mycologia 105(6):1374–1390. doi:10.3852/13-162

Kamiya Y, Sakurai A, Tamura S, Takahashi N (1978) Structure of rhodotorucine A, a novel lipopeptide, inducing mating tube formation in *Rhodosporidium toruloides*. Biochem Biophys Res Commun 83(3):1077–1083

Kämper J, Reichmann M, Romeis T, Bölker M, Kahmann R (1995) Multiallelic recognition—nonself-dependent dimerization of the bE and bW homeodomain proteins in *Ustilago maydis*. Cell 81(1):73–83. doi:10.1016/0092-8674(95)90372-0

Katoh K, Kuma K, Miyata T, Toh H (2005) Improvement in the accuracy of multiple sequence alignment program MAFFT. Genome Inform 16(1):22–33

Kelly M, Burke J, Smith M, Klar A, Beach D (1988) Four mating-type genes control sexual differentiation in the fission yeast. EMBO J 7(5):1537–1547

Kemp R (1974) Bifactorial incompatibility in the two-spored basidiomycetes *Coprinus sassii* and *C. bilanatus*. Trans Br Mycol Soc 62(3):547–555

Kim K-H, Kang YM, Im CH, Ali A, Kim SY, Je H-J, Kim M-K, Rho HS, Lee HS, Kong W-S (2014) Identification and functional analysis of pheromone and receptor genes in the *B3* mating locus of *Pleurotus eryngii*. PLoS One 9(8), e104693

Knabe N, Jung EM, Freihorst D, Hennicke F, Horton JS, Kothe E (2013) A central role for Ras1 in morphogenesis of the basidiomycete *Schizophyllum commune*. Eukaryot Cell 12(6):941–952. doi:10.1128/Ec.00355-12

Kniep H (1920) Über morphologische und physiologische Geschlechtsdifferenzierung: (Untersuchungen an Basidiomyzeten). Physikal.-med Gesellschaft, Würzburg

Kniep H (1922) Über Geschlechtsbestimmung und Reduktionsteilung: (Untersuchungen an Basidiomyzeten). Verlag der Physik.-Med. Ges, Würzburg

Koltin Y, Raper JR, Simchen G (1967) The genetic structure of the incompatibility factors of *Schizophyllum commune*: the *B* factor. Proc Natl Acad Sci USA 57(1):55–62

Kosted PJ, Gerhardt SA, Anderson CM, Stierle A, Sherwood JE (2000) Structural requirements for activity of the pheromones of *Ustilago hordei*. Fungal Genet Biol 29(2):107–117. doi:10.1006/fgbi.2000.1191

Kothe E, Freihorst D (2012) Schleim aus Pilzen zur verbesserten Erdölförderung. BIOspektrum 18(7-12):1

Kothe E, Gola S, Wendland J (2003) Evolution of multispecific mating-type alleles for pheromone perception in the homobasidiomycete fungi. Curr Genet 42(5):268–275

Kües U, Casselton LA (1992a) Homeodomains and regulation of sexual development in basidiomycetes. Trends Genet 8(5):154–155

Kües U, Casselton LA (1992b) Molecular and functional analysis of the *A* mating type genes of *Coprinus cinereus*. Genet Eng 14:251–268

Kües U, Navarro-Gonzalez M (2010) Mating-type orthologous genes in the primarily homothallic *Moniliophthora perniciosa*, the causal agent of witches' broom disease in cacao. J Basic Microbiol 50(5):442–451. doi:10.1002/jobm.201000013

Kües U, Asante-Owusu RN, Mutasa ES, Tymon AM, Pardo EH, O'Shea SF, Gottgens B, Casselton LA (1994) Two classes of homeodomain proteins specify the multiple a mating types of the mushroom *Coprinus cinereus*. Plant Cell 6(10):1467–1475. doi:10.1105/tpc.6.10.1467

Kües U, James T, Heitman J (2011) Mating type in basidiomycetes: Unipolar, bipolar, and tetrapolar patterns of sexuality. In: Pöggeler S, Wöstemeyer J (eds) The Mycota XIV: Evolution of fungi and fungal-like organisms. Springer, Berlin, Germany, pp 97–160

Kwon-Chung K (1976) Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. Mycologia 821–833

Kwon-Chung K, Bennett JE (1978) Distribution of *a* and α mating types of *Cryptococcus neoformans* among natural and clinical isolates. Am J Epidemiol 108 (4):337–340

Kwon-Chung KJ, Bennett JE, Rhodes JC (1982) Taxonomic studies on *Filobasidiella* species and their anamorphs. Antonie van Leeuwenhoek 48(1):25–38

Lee N, Bakkeren G, Wong K, Sherwood JE, Kronstad JW (1999) The mating-type and pathogenicity locus of the fungus *Ustilago hordei* spans a 500-kb region. Proc Natl Acad Sci USA 96(26):15026–15031

Lee SP, O'Dowd BF, Ng GY, Varghese G, Akil H, Mansour A, Nguyen T, George SR (2000) Inhibition of cell surface expression by mutant receptors demonstrates that D2 dopamine receptors exist as oligomers in the cell. Mol Pharmacol 58(1):120–128

Levasseur A, Lomascolo A, Chabrol O, Ruiz-Duenas FJ, Boukhris-Uzan E, Piumi F, Kües U, Ram AF, Murat C, Haon M, Benoit I, Arfi Y, Chevret D, Drula E, Kwon MJ, Gouret P, Lesage-Meessen L, Lombard V, Mariette J, Noirot C, Park J, Patyshakuliyeva A, Sigoillot JC, Wiebenga A, Wosten HA, Martin F, Coutinho PM, de Vries RP, Martinez AT, Klopp C, Pontarotti P, Henrissat B, Record E (2014) The genome of the white-rot fungus *Pycnoporus cinnabarinus*: a basidiomycete model with a versatile arsenal for lignocellulosic biomass breakdown. BMC Genomics 15:486. doi:10.1186/1471-2164-15-486

Luo YH, Ullrich RC, Novotny CP (1994) Only one of the paired *Schizophyllum commune Aα* mating-type, putative homeobox genes encodes a homeodomain essential for *Aα*-regulated development. Mol Gen Genet 244(3):318–324

Malloure BD, James TY (2013) Inbreeding depression in urban environments of the bird's nest fungus *Cyathus stercoreus* (Nidulariaceae: Basidiomycota). Heredity 110(4):355–362. doi:10.1038/hdy.2012.95

Martinez D, Larrondo LF, Putnam N, Gelpke MD, Huang K, Chapman J, Helfenbein KG, Ramaiya P, Detter JC, Larimer F, Coutinho PM, Henrissat B, Berka R, Cullen D, Rokhsar D (2004) Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. Nat Biotechnol 22(6):695–700. doi:10.1038/nbt967

Martinez D, Challacombe J, Morgenstern I, Hibbett D, Schmoll M, Kubicek CP, Ferreira P, Ruiz-Duenas FJ, Martinez AT, Kersten P, Hammel KE, Vanden Wymelenberg A, Gaskell J, Lindquist E, Sabat G, Bondurant SS, Larrondo LF, Canessa P, Vicuna R, Yadav J, Doddapaneni H, Subramanian V, Pisabarro AG, Lavin JL, Oguiza JA, Master E, Henrissat B, Coutinho PM, Harris P, Magnuson JK, Baker SE, Bruno K, Kenealy W, Hoegger PJ, Kües U, Ramaiya P, Lucas S, Salamov A, Shapiro H, Tu H, Chee CL, Misra M, Xie G, Teter S, Yaver D, James T, Mokrejs M, Pospisek M, Grigoriev IV, Brettin T, Rokhsar D, Berka R, Cullen D (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. Proc Natl Acad Sci USA 106 (6):1954–1959. doi:10.1073/pnas.0809575106

Martinez-Espinoza AD, Gerhardt SA, Sherwood JE (1993) Morphological and mutational analysis of mating in *Ustilago hordei*. Exp Mycol 17(3):200–214

McClelland CM, Fu J, Woodlee GL, Seymour TS, Wickes BL (2002) Isolation and characterization of the *Cryptococcus neoformans MATa* pheromone gene. Genetics 160(3):935–947

Metin B, Findley K, Heitman J (2010) The mating type locus (*MAT*) and sexual reproduction of *Cryptococcus heveanensis*: insights into the evolution of sex and sex-determining chromosomal regions in fungi. PLoS Genet 6(5), e1000961

Michaelis S, Barrowman J (2012) Biogenesis of the *Saccharomyces cerevisiae* pheromone **a**-factor, from yeast mating to human disease. Microbiol Mol Biol Rev 76(3):626–651. doi:10.1128/MMBR.00010-12

Moore T, Edman JC (1993) The α-mating type locus of *Cryptococcus neoformans* contains a peptide pheromone gene. Mol Cell Biol 13(3):1962–1970

Nakayama N, Miyajima A, Arai K (1987) Common signal transduction system shared by *STE2* and *STE3* in haploid cells of *Saccharomyces cerevisiae*: autocrine cell-cycle arrest results from forced expression of *STE2*. EMBO J 6(1):249–254

Niculita-Hirzel H, Labbe J, Kohler A, le Tacon F, Martin F, Sanders IR, Kües U (2008) Gene organization of the mating type regions in the ectomycorrhizal fungus *Laccaria bicolor* reveals distinct evolution between the two mating type loci. New Phytol 180 (2):329–342. doi:10.1111/j.1469-8137.2008.02525.x

Ohm RA, de Jong JF, Lugones LG, Aerts A, Kothe E, Stajich JE, de Vries RP, Record E, Levasseur A, Baker SE, Bartholomew KA, Coutinho PM, Erdmann S, Fowler TJ, Gathman AC, Lombard V, Henrissat B, Knabe N, Kües U, Lilly WW, Lindquist E, Lucas S, Magnuson JK, Piumi F, Raudaskoski M, Salamov A, Schmutz J, Schwarze FWMR, vanKuyk PA, Horton JS, Grigoriev IV, Wosten HAB (2010) Genome sequence of the model mushroom *Schizophyllum commune*. Nat Biotechnol 28 (9):957–U910. doi:10.1038/Nbt.1643

Olesnicky NS, Brown AJ, Dowell SJ, Casselton LA (1999) A constitutively active G-protein-coupled receptor causes mating self-compatibility in the mushroom *Coprinus*. EMBO J 18(10):2756–2763. doi:10.1093/emboj/18.10.2756

Olesnicky NS, Brown AJ, Honda Y, Dyos SL, Dowell SJ, Casselton LA (2000) Self-compatible *B* mutants in *Coprinus* with altered pheromone-receptor specificities. Genetics 156(3):1025–1033

O'Shea SF, Chaure PT, Halsall JR, Olesnicky NS, Leibbrandt A, Connerton IF, Casselton LA (1998) A large pheromone and receptor gene complex determines multiple *B* mating type specificities in *Coprinus cinereus*. Genetics 148(3):1081–1090

Palmer GE, Horton JS (2006) Mushrooms by magic: making connections between signal transduction and fruiting body development in the basidiomycete fungus *Schizophyllum commune*. FEMS Microbiol Lett 262(1):1–8. doi:10.1111/j.1574-6968.2006.00341.x

Parag Y (1962) Mutations in the *B* incompatibility factor of *Schizophyllum commune*. Proc Natl Acad Sci USA 48:743–750

Pelkmans JF, Lugones LG, Wösten HAB (2016) Fruiting body formation in basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 387–405

Perrin N (2012) What uses are mating types? The "developmental switch" model. Evolution 66 (4):947–956. doi:10.1111/j.1558-5646.2011.01562.x

Raffle V, Anderson N, Furnier G, Doudrick R (1995) Variation in mating competence and random amplified polymorphic DNA in *Laccaria bicolor* (Agaricales) associated with three tree host species. Can J Bot 73(6):884–890

Rajchenberg M (2011) Nuclear behavior of the mycelium and the phylogeny of polypores (Basidiomycota). Mycologia 103(4):677–702. doi:10.3852/10-310

Rambaut A, Drummond A (2003) Tracer: a program for analysing results from Bayesian MCMC programs such as BEAST & MrBayes. Oxford, UK. http://evolve.zoo.ox.ac.uk/software.html

Raper JR (1966) Genetics of sexuality in higher fungi. Ronald Press Company, New York

Raper CA, Raper JR (1973) Mutational analysis of a regulatory gene for morphogenesis in *Schizophyllum*. Proc Natl Acad Sci USA 70(5):1427–1431

Raper JR, Baxter MG, Ellingboe AH (1960) The genetic structure of the incompatibility factors of *Schizophyllum commune*: The *A*-factor. Proc Natl Acad Sci USA 46(6):833–842

Raudaskoski M (1998) The relationship between *B*-mating-type genes and nuclear migration in *Schizophyllum commune*. Fungal Genet Biol 24(1-2):207–227. doi:10.1006/fgbi.1998.1069

Raudaskoski M, Kothe E (2010) Basidiomycete mating type genes and pheromone signaling. Eukaryot Cell 9(6):847–859. doi:10.1128/Ec.00319-09

Raudaskoski M, Kothe E, Fowler TJ, Jung EM, Horton JS (2012) Ras and Rho small G proteins: insights from the *Schizophyllum commune* genome sequence and comparisons to other fungi. Biotechnol Genet Eng Rev 28:61–100

Riquelme M, Challen MP, Casselton LA, Brown AJ (2005) The origin of multiple *B* mating specificities in *Coprinus cinereus*. Genetics 170(3):1105–1119. doi:10.1534/genetics.105.040774

Robertson CI, Kende AM, Toenjes K, Novotny CP, Ullrich RC (2002) Evidence for interaction of *Schizophyllum commune* Y mating-type proteins in vivo. Genetics 160(4):1461–1467

Sakagami Y, Yoshida M, Isogai A, Suzuki A (1981) Peptidal sex hormones inducing conjugation tube formation in compatible mating-type cells of *Tremella mesenterica*. Science 212(4502):1525–1527. doi:10.1126/science.212.4502.1525

Schlesinger R, Kahmann R, Kämper J (1997) The homeodomains of the heterodimeric bE and bW proteins of *Ustilago maydis* are both critical for function. Mol Gen Genet 254(5):514–519

Scholtmeijer K, Cankar K, Beekwilder J, Wösten HA, Lugones LG, Bosch D (2014) Production of (+)-

valencene in the mushroom-forming fungus *S. commune*. Appl Microbiol Biotechnol 98 (11):5059–5068. doi:10.1007/s00253-014-5581-2

Schubert D, Raudaskoski M, Knabe N, Kothe E (2006) Ras GTPase-activating protein Gap1 of the homobasidiomycete *Schizophyllum commune* regulates hyphal growth orientation and sexual development. Eukaryot Cell 5(4):683–695. doi:10.1128/Ec.5.4.683-695.2006

Schulz B, Banuett F, Dahl M, Schlesinger R, Schafer W, Martin T, Herskowitz I, Kahmann R (1990) The *b* alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell 60 (2):295–306

Seike T, Yamagishi Y, Iio H, Nakamura T, Shimoda C (2012) Remarkably simple sequence requirement of the *M*-factor pheromone of *Schizosaccharomyces pombe*. Genetics 191(3):815–825. doi:10.1534/genetics.112.140483

Shen GP, Park DC, Ullrich RC, Novotny CP (1996) Cloning and characterization of a *Schizophyllum* gene with *Aβ6* mating-type activity. Curr Genet 29 (2):136–142

Skrede I, Maurice S, Kauserud H (2013) Molecular characterization of sexual diversity in a population of *Serpula lacrymans*, a tetrapolar basidiomycete. G3 3(2):145–152. doi:10.1534/g3.112.003731

Specht CA (1995) Isolation of the *Bα* and *Bβ* mating-type loci of *Schizophyllum commune*. Curr Genet 28(4):374–379

Specht CA, Novotny CP, Ullrich RC (1992a) Mitochondrial DNA of *Schizophyllum commune*: restriction map, genetic map, and mode of inheritance. Curr Genet 22(2):129–134

Specht CA, Stankis MM, Giasson L, Novotny CP, Ullrich RC (1992b) Functional analysis of the homeodomain-related proteins of the *Aα* locus of *Schizophyllum commune*. Proc Natl Acad Sci USA 89(15):7174–7178

Spellig T, Bölker M, Lottspeich F, Frank RW, Kahmann R (1994) Pheromones trigger filamentous growth in *Ustilago maydis*. EMBO J 13(7):1620–1627

Spit A, Hyland RH, Mellor EJC, Casselton LA (1998) A role for heterodimerization in nuclear localization of a homeodomain protein. Proc Natl Acad Sci USA 95(11):6228–6233. doi:10.1073/pnas.95.11.6228

Stankis MM, Specht CA (2007) Cloning the mating-type genes of *Schizophyllum commune*: a historical perspective. In: Heitman J, Kronstad JW, Taylor JW, Casselton LA (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, USA, pp 267–282

Stankis MM, Specht CA, Yang H, Giasson L, Ullrich RC, Novotny CP (1992) The *Aα* mating locus of *Schizophyllum commune* encodes two dissimilar multiallelic homeodomain proteins. Proc Natl Acad Sci USA 89(15):7169–7173

Stanton BC, Hull CM (2007) Mating-type locus control of cell identity. In: Heitman J, Kronstad JW, Taylor JW, Casselton LA (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 59–73

Szabo Z, Tonnis M, Kessler H, Feldbrügge M (2002) Structure-function analysis of lipopeptide pheromones from the plant pathogen *Ustilago maydis*. Mol Genet Genomics 268(3):362–370. doi:10.1007/s00438-002-0756-4

Takano H, Onoue K, Kawano S (2010) Mitochondrial fusion and inheritance of the mitochondrial genome. J Plant Res 123(2):131–138. doi:10.1007/s10265-009-0268-y

Vaillancourt LJ, Raudaskoski M, Specht CA, Raper CA (1997) Multiple genes encoding pheromones and a pheromone receptor define the *Bβ1* mating-type specificity in *Schizophyllum commune*. Genetics 146(2):541–551

Vallim MA, Fernandes L, Alspaugh JA (2004) The *RAM1* gene encoding a protein-farnesyltransferase β-subunit homologue is essential in *Cryptococcus neoformans*. Microbiology 150(Pt 6):1925–1935. doi:10.1099/mic.0.27030-0

van Peer AF, Park SY, Shin PG, Jang KY, Yoo YB, Park YJ, Lee BM, Sung GH, James TY, Kong WS (2011) Comparative genomics of the mating-type loci of the mushroom *Flammulina velutipes* reveals widespread synteny and recent inversions. PLoS One 6 (7), e22249. doi:10.1371/journal.pone.0022249

Weber M, Salo V, Uuskallio M, Raudaskoski M (2005) Ectopic expression of a constitutively active Cdc42 small GTPase alters the morphology of haploid and dikaryotic hyphae in the filamentous homobasidiomycete *Schizophyllum commune*. Fungal Genet Biol 42(7):624–637. doi:10.1016/j.fgb.2005.03.012

Wendland J, Vaillancourt LJ, Hegner J, Lengeler KB, Laddison KJ, Specht CA, Raper CA, Kothe E (1995) The mating-type locus *Bα1* of *Schizophyllum commune* contains a pheromone receptor gene and putative pheromone genes. EMBO J 14(21):5271–5278

Whitehouse H (1949) Heterothallism and sex in the fungi. Biol Rev 24(4):411–447

Wong GJ, Wells K (1985) Modified bifactorial incompatibility in *Tremella mesenterica*. Trans Br Mycol Soc 84(1):95–109

Wu L, van Peer A, Song W, Wang H, Chen M, Tan Q, Song C, Zhang M, Bao D (2013) Cloning of the *Lentinula edodes B* mating-type locus and identification of the genetic structure controlling *B* mating. Gene 531(2):270–278. doi:10.1016/j.gene.2013.08.090

Yi R, Mukaiyama H, Tachikawa T, Shimomura N, Aimi T (2010) *A*-mating-type gene expression can drive clamp formation in the bipolar mushroom *Pholiota microspora (Pholiota nameko)*. Eukaryot Cell 9(7):1109–1119. doi:10.1128/EC.00374-09

Yue CL, Osier M, Novotny CP, Ullrich RC (1997) The specificity determinant of the Y mating-type proteins of *Schizophyllum commune* is also essential for Y-Z protein binding. Genetics 145(2):253–260

14 Mating-Type Structure, Function, Regulation and Evolution in the Pezizomycotina

P.S. Dyer[1], P. Inderbitzin[2], R. Debuchy[3]

CONTENTS

I. Introduction

The ability to undergo sexual reproduction, involving the fusion of haploid gametes to form a diploid zygote followed by meiosis, is a key feature of many fungal species as with eukaryotes in general. Indeed, fungi have evolved unusually diverse and fascinating methods to achieve such sexual union (Dyer 2008). **In the case of the Pezizomycotina (also termed euascomycete fungi or filamentous ascomycetes), species can exhibit heterothallic (self-incompatible) or homothallic (self-compatible) lifestyles, and some species are even able to switch from one reproductive lifestyle to another. Thus, by definition, individuals of homothallic species can complete the sexual cycle without the need for a mating partner, whereas individuals of heterothallic species are obligately outcrossing and require a compatible partner for sexual reproduction to occur. In some circumstances, one of the two compatible partners of a heterothallic species can engage in sexual reproduction on its own, revealing a potential for a unisexual lifestyle.** However, unlike higher eukaryotes which show clear morphological differences between the outcrossing sexes, in the case of heterothallic Pezizomycotina, the two mating partners are morphologically indistinguishable for most of their life cycles (Debuchy et al. 2010). **Thus, rather than being referred to as male or female, based on morphological differences, the term 'mating type' is instead used to define the sexual identity of a fungal individual with respect to the ability to recognise and mate with different sexual cell types. In heterothallic Pezizomycotina, there are normally only two mating types present, which by convention are termed MAT1-1 and MAT1-2, although for some species alternative established terminologies such as mat A and mat a (e.g. in *Neurospora*) or mat+ and mat− (e.g. *Podospora*) are used (Turgeon and Yoder 2000).** The mating type of a fungal cell is itself determined by genes present at a

[1]School of Life Sciences, University of Nottingham, Nottingham NG7 2RD, United Kingdom; e-mail: paul.dyer@nottingham.ac.uk
[2]Department of Plant Pathology, University of California, Davis, CA, 95616, USA; e-mail: prin@ucdavis.edu
[3]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France; e-mail: robert.debuchy@i2bc.paris-saclay.fr

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

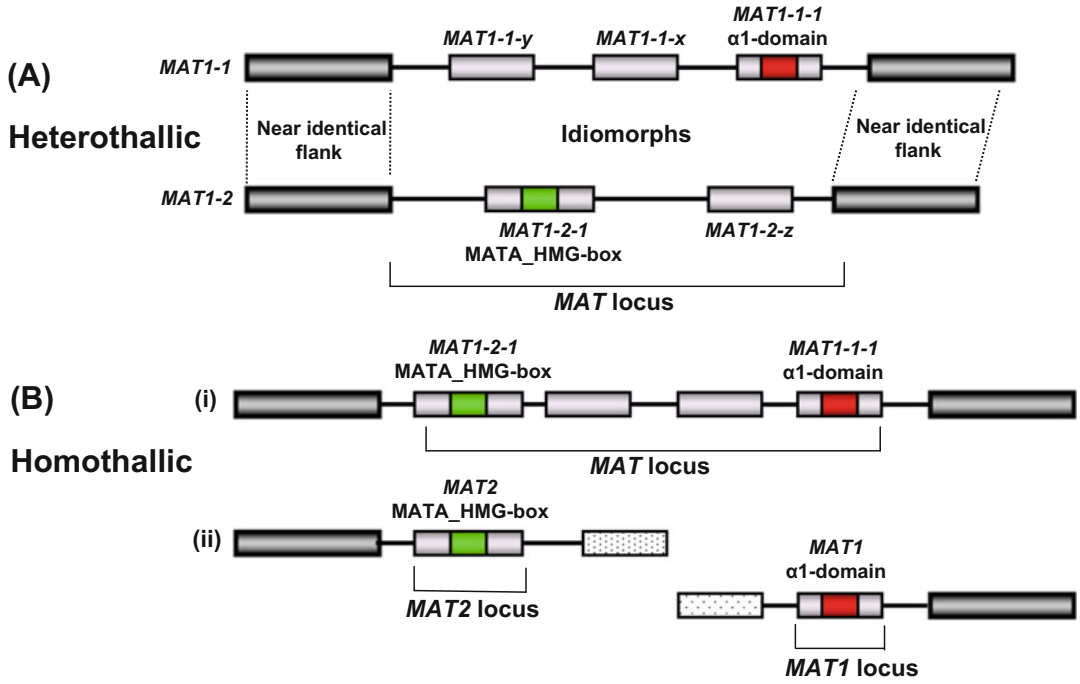


Fig. 14.1 Typical structural organisation of *MAT* regions in heterothallic and homothallic Pezizomycotina. (**a**) In heterothallic species, the *MAT* locus is flanked by DNA sequence (shown as *dark grey rectangles*) both upstream and downstream which is conserved between isolates of complementary mating type. At the *MAT* locus, either a *MAT1-1* or *MAT1-2* idiomorph is present, containing at least a *MAT1-1-1* α-domain gene (shown as *red* and *grey rectangle*) or a *MAT1-2-1* MATA_HMG-box gene (shown as *green* and *grey rectangle*), respectively. In addition, further genes may be present at the *MAT* locus, numbered in succession *MAT1-1-x*, *MAT1-1-y*, etc. (shown as *light grey rectangles*). The genes flanking the idiomorph are fairly well conserved within the Pezizomycotina, for example, often including *SLA2* and *APN2* genes among others. See Fig. 15.3 of chapter 'Fruiting Body Formation in Basidiomycetes' of *The Mycota I* for more details (Debuchy and Turgeon 2006). (**b**) In homothallic species, a single *MAT* locus may be present containing both *MAT1-1* α-domain and *MAT1-2* MATA_HMG-box genes [shown as arrangement (i)], or the *MAT1* α-domain and *MAT2* MATA_HMG-box genes may be unlinked, for example, being present on different chromosomes [shown as arrangement (ii)]. *Stippled rectangles* represent unrelated sequences; other *rectangles coloured* as per (**a**) above

particular region of the genome. The term mating type has also been extended to refer to this region of the genome and the genes contained therein, which are therefore termed the mating-type (or *MAT*) region or locus and mating-type (or *MAT*) genes, respectively. In heterothallic filamentous ascomycetes, the *MAT* regions have been shown to exhibit high sequence divergence between isolates of different mating type (Fig. 14.1). **These regions have therefore been termed 'idiomorphs', in accordance with a suggestion by Professor J. Wyatt, and popularised by a seminal paper by Metzenberg and Glass (1990).** This term denotes DNA sequences that occupy the same locus in individuals of different mating type and was preferred to the standard use of the term alleles to emphasise the apparent absence of similarity between different mating-type regions, an unusual occurrence as alleles normally have fairly strong sequence similarity. Mating-type regions are fascinating parts of the genome, due to their diverse structures and functions. The structure determines whether the species is heterothallic, is homothallic or is able to switch from one lifestyle to another. Furthermore, there can be dramatic fluidity of mating-type structures, even within a genus, implying lifestyle change and regulatory circuit rewiring within a short evolutionary time frame. Thus, *MAT* regions provide an intriguing exam-

ple of how dissimilar structures can evolve at the same locus. Mating-type genes are involved in the control of fertilisation and can also have critical functions during fruit body development (see Peraza-Reyes and Malagnac 2016). The relationship between mating-type structure and function has already revealed remarkable findings when the evolution of this developmental pathway is considered in Saccharomycetales (Scannell and Wolfe 2004; Tsong et al. 2003, 2006, 2007; Tuch et al. 2008).

In this review, we summarise and discuss recent advances that raise critical issues or represent significant progress in our understanding of mating-type function and evolution specifically in the Pezizomycotina. In some sections, we have also included reference to yeasts where a comparison with this group was considered highly pertinent. This chapter follows on from several previous reviews concerning mating-type structure and features of *MAT* genes (e.g. Debuchy et al. 2010; Debuchy and Turgeon 2006; Coppin et al. 1997) and various chapters in the *Sex in Fungi* book by Heitman et al. (2007), to which the reader is referred for additional detail. Other earlier reviews on mating types are listed in the second edition of *The Mycota I* (Debuchy and Turgeon 2006). We do not deal with several fields that have recently been reviewed elsewhere, for example, a general presentation of sex in fungi (Ni et al. 2011), the evolution of sex in the fungal kingdom (Lee et al. 2010; Whittle et al. 2011) and sexual reproduction specifically in fungal pathogens (Butler 2010; Heitman 2010; Heitman et al. 2014).

II. Mating-Type Structure and Evolution

A. Mating-Type Genes and Evolution

1. Core HMG-Box Genes

The core fungal mating-type genes are derived from ancestral high-mobility group box (HMG-box) alleles found throughout the Eukaryota. These have evolved to form fungal-specific phylogenetic groupings of HMG-box genes, which in one case has diverged so much that it was not even recognised as a member of the HMG-box superfamily for a long time. Two main categories of mating-type genes are present in the Pezizomycotina, which are referred to by standardised mating-type nomenclature according to differences in their sequence and specific features (Turgeon and Yoder 2000). **The core *MAT1-1* gene family (also called *MAT1-1-1*) is characterised by the presence of a region encoding an α1 domain (Fig. 14.1). The core *MAT1-2* gene family (also called *MAT1-2-1*) is characterised by the presence of a MATA_HMG-box (Fig. 14.1).** The MATA_HMG-box binds to DNA-specific sequences and is present in fungal transcription factors, most of which are involved in sexual development. The MATA_HMG-box motif defines a HMG-box subfamily that was identified by Soullier et al. (1999), a few years after the discovery of the first *MAT1-2-1* gene (Staben and Yanofsky 1990). By contrast, the evolutionary origin and relationship of the α1 domain with the MATA_HMG-box and other transcription factors were questioned for nearly 30 years (Souza et al. 2003). The α1 domain was first identified based on sequence conservation between the mating-type proteins MAT A-1 from *Neurospora crassa* and MATα1p from *Saccharomyces cerevisiae* (Glass et al. 1990a). The molecular function of the α1 domain is not known, but several lines of evidence indicate that MATα1p of *S. cerevisiae* is a transcriptional co-activator (Hagen et al. 1993; Yuan et al. 1993). A first hint as to the origin of the α1 domain was the report of weak sequence identity between MAT1-1-1 and the HMG-box (Idnurm et al. 2008). A consensus sequence representation of α1 and MATA_HMG-box indeed revealed conserved features (Fig. 14.2). **Analyses of the 3-D structures of MATα1p from *S. cerevisiae* and MAT1-1-1 proteins of the Pezizomycotina revealed that their best-fitting model is the HMG-box structure (Fig. 14.3) (Jackson et al. 2013; Martin et al. 2010).** Most significantly, the identification of the binding motif of MAT1-1-1 from *Penicillium chrysogenum* revealed strong similarities to the binding site of Sox9, a SRY-related HMG-box protein (Becker et al. 2015). Numerous HMG proteins have been crystallised and analysed to determine their 3-D structure (Remenyi et al. 2003; Werner et al. 1995; van Houte et al. 1995), but up to this

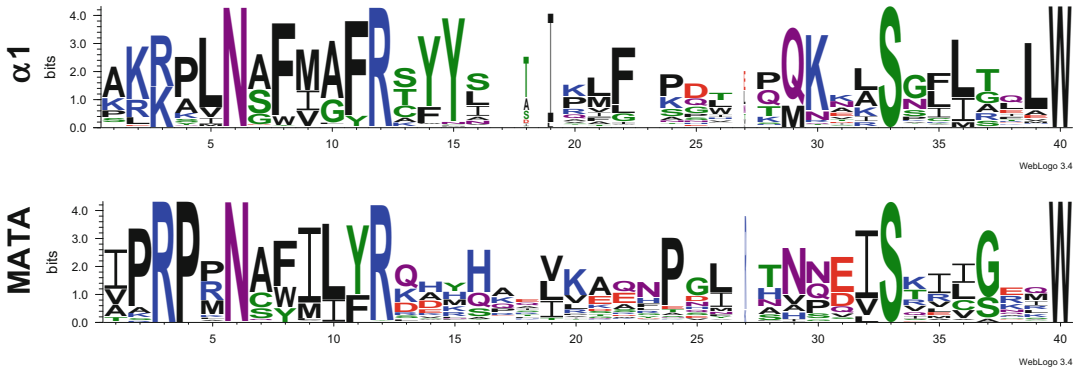


Fig. 14.2 Conserved sequence of the α1 domain and MATA_HMG-box. The *x*-axis represents the amino acid positions from the N to C termini. Logos represent an ~40 residue core sequence from 300 α1 domains and 257 MATA_HMG-box. WebLogo (Crooks et al. 2004; Schneider and Stephens 1990) representation with the *chemistry colour scheme*. Adapted from Martin et al. (2010)

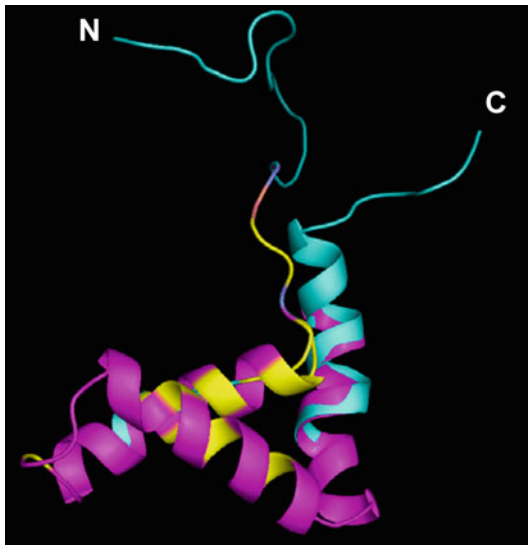


Fig. 14.3 Superposition of the α1 domain and MATA_HMG-box. The α1 domain of *Fusarium sacchari* (accession number 97974007) is shown in *magenta*. The MATA_HMG-box of *Aspergillus flavus* (accession number XP_002374195) is shown in *cyan*. Regions with conserved residues are indicated in *yellow*. The superposition of the structures predicted by PHYRE (Kelley and Sternberg 2009) shows considerable *overlap*. N and C terminal ends are labelled. Images were made using PyMOL. Adapted from Martin et al. (2010)

point, there are no reports of the crystallisation of any protein from the MATA_HMG-box subfamily. The expression of MATα1p or MAT1-1-1 proteins of *Podospora anserina* and *Cochliobolus heterostrophus*, as well as of truncated versions reduced to the α1 domain, in *Escherichia coli* or in *Pichia pastoris* was unsuccessful (N. Lazar, K. Blondeau, B.G. Turgeon, R. Debuchy and H. van Tilbeurgh; unpublished results) with other laboratories encountering similar difficulties (Jackson et al. 2013). Further attempts will be necessary to analyse the 3-D structure of the α1 domain and its interactions with DNA.

The placement of the α1 domain in the HMG-box superfamily reveals that opposite idiomorphs are related by common descent, in contrast to the definition proposed by Metzenberg and Glass (1990). Phylogenetic analyses indicate that the α1 domains form a subgroup within the MATA_HMG-box proteins (Martin et al. 2010). It is, therefore, likely that the α1 domain diverged from this subfamily, which contains the MAT1-2-1 proteins. These data provide insight regarding the evolutionary trajectory of *MAT* in fungi. Idnurm et al. (2008) found that the sex-determining alleles in the Zygomycota, a basal lineage in fungi, consist of single genes encoding an HMG-box protein and proposed that the HMG-box proteins are an earlier form of fungal *MAT* locus (Idnurm et al. 2008). **The presence of the HMG-box in *MAT1-1* is in agreement with models proposed by Idnurm et al. (2008) and Dyer (2008) concerning the evolution of *MAT* genes within the fungal kingdom.** However, these models do not explain why home-

odomain transcription factors have evicted and replaced HMG-box transcription factors in the *MAT* loci of the Basidiomycota (see Freihorst et al. 2016). It is noteworthy that MATA_HMG-box genes still play a critical role in mating in Basidiomycota, such as in *Ustilago maydis* (Brefort et al. 2005; Hartmann et al. 1996; Urban et al. 1996), *Coprinopsis cinerea* (Murata et al. 1998) and *Cryptococcus neoformans* (Kruzel et al. 2012). Any model should also explain why yeasts (Taphrinomycotina and Saccharomycotina) harbour *MAT* loci with HMG- and homeobox genes. Further analyses will be necessary to understand why HMG-box genes are not the core mating-type genes in all fungi. Strikingly, the binding site of the MAT1-1-1 protein has also some similarities with the binding site from homeodomains (Becker et al. 2015), suggesting that alpha1 and homeodomains may have some evolutionary or functionnal connections.

2. Acquired Genes

The typical structure for *MAT* regions in heterothallic Pezizomycotina is that the flanking DNA sequences, and gene synteny, both upstream and downstream of the *MAT* locus are highly conserved in isolates of complementary mating type. It is only specifically in the idiomorph region that isolates of different mating type show very strong sequence divergence (Debuchy et al. 2010). Within the idiomorphs, *MAT1-1* isolates by definition harbour at least a *MAT1-1* gene, whereas *MAT1-2* isolates by definition harbour at least a *MAT1-2* gene (Fig. 14.1). The smallest idiomorphs in the Pezizomycotina contain only a single *MAT1-1-1* or *MAT1-2-1* gene and are approximately 1 kb in size (Debuchy et al. 2010). **However, aside from the core mating-type genes that are derived from ancestral HMG-box alleles, the *MAT* locus in certain taxonomic groupings of the Pezizomycotina can also include additional genes, resulting in the presence of larger idiomorphs, which can be up to 6 kb or larger in size (Debuchy et al. 2010)**. A list of these acquired genes is shown in Table 14.1. Unfortunately, authors quite frequently use the same nomenclature for genes that are undoubtedly unrelated. For instance, *MAT1-1-5* designates *COX13* in *Coccidioides* spp. and *COX13*-unrelated mating-type genes in Leotiomycetes. *MAT1-2-4* in *Botrytis cinerea* and *MAT1-2-4* in *Aspergillus* spp. represent another instance of unfortunate generic designations applied to unrelated genes. BLAST analysis of these two genes failed to reveal any similarity. By contrast, weak sequence conservation was reported for MAT1-2-4 among *Aspergillus* spp. (Eurotiales) and *Coccidioides* spp. (Onygenales) (Swilaiman et al. 2013), warranting the use of the same designation for these genes.

Capture of adjacent genes into the *MAT* locus has occurred in *Coccidioides immitis* and *Uncinocarpus reesii* (Fraser et al. 2007; Mandel et al. 2007). In these heterothallic species, both idiomorphs have captured the genes encoding the DNA lyase (*APN2*) and the cytochrome c oxidase subunit VIa (*COX13*). Both of these genes are adjacent to the *MAT* locus in the closely related species *Microsporum gypseum* (Li et al. 2010) and in other more distantly related fungi. **The capture process involves more than just the incorporation of adjacent sequences. It is accompanied by changes in gene order, gene orientation and also gene loss.** This process illustrates the dynamic nature of *MAT* loci, but the influence of *MAT* remodelling on gene function and mating ability is unknown.

In many cases, the evolutionary origin of the additional genes at the *MAT* locus remains obscure because they are specific to a particular idiomorph and taxonomic grouping. The most studied examples are *MAT1-1-2* and *MAT1-1-3*, which are both present in sordariomycete genomes. *MAT1-1-2* encodes a protein with a domain of unknown function. This domain was previously termed HPG (Debuchy and Turgeon 2006) and later PPF (Kanematsu et al. 2007), based on conserved residues identified in an alignment. A family for MAT1-1-2 was built in Pfam and will appear in release 28.0 with the reference PF17043. The deletion of this gene in *Podospora anserina* (Arnaise et al. 2001), *Sordaria macrospora* (Klix et al. 2010) and *Gibberella zeae* (Zheng et al. 2013) results in arrest at an early stage of fruit body development following fertilisation. These results emphasise the critical role of *MAT1-1-2* in heterothallic and homothallic Sordariomycetes,

Table 14.1 Mating-type genes adjacent to the core genes *MAT1-1-1* and *MAT1-2-1*

Gene nomenclature	Fungal group	Species[a]	Conserved domain and function[a]
MAT1-1-2	Sordariomycetes	Ubiquitous in Sordariomycetes	PF17043 domain, required for fruit body development[b]
MAT1-1-3	Sordariomycetes	Ubiquitous in Sordariomycetes except in a group of *Clavicipitaceae*[c]	HMG-box, required for fruit body development[b]
MAT1-1-4	Leotiomycetes	*Pyrenopeziza brassicae*[d]	Unknown function
	Eurotiomycetes	*Coccidioides immitis*[e], *C. posadasii*[f], *Trichophyton verrucosum*[g], *Arthroderma benhamiae*[h]	Unknown function
	Dothideomycetes	*Diplodia pinea*[i]	Unknown function
MAT1-1-5	Eurotiomycetes	*Coccidioides* spp.[j,k]	COX13
	Leotiomycetes	*Sclerotinia sclerotiorum*[l], *S. homoeocarpa*[m], *Botrytis cinerea*[l]	Required for disc development[n]
MAT1-1-6	Eurotiomycetes	*Coccidioides* spp.[j,k]	APN2
	Leotiomycetes	*Pseudogymnoascus destructans*[o]	Unknown function
MAT1-1-7	Eurotiomycetes	*Coccidioides* spp.[j,k]	Unknown function
MAT1-2-2 (*mat a2*)	Sordariomycetes	*Neurospora crassa*[p]	Unknown function
MAT1-2-3	Eurotiomycetes	*Coccidioides* spp.[j,k]	Mannosyl transferase
	Hypocreales	*Fusarium* spp.[q]	Unknown function
MAT1-2-4	Eurotiomycetes	*Coccidioides* spp.[j,k]	Unknown function
		Aspergillus fumigatus[r], *A. lentulus*[s], *Neosartorya fischeri*[s], *Talaromyces marneffei*[t]	Unknown function
	Leotiomycetes	*S. sclerotiorum*[l], *S. homoeocarpa*[m], *B. cinerea*[l]	Required for disc development[n]
MAT1-2-5	Eurotiomycetes	*Coccidioides* spp.[j,k]	COX13
	Leotiomycetes	*P. destructans*[o]	Unknown function
	Dothideomycetes	*D. pinea*[i]	Unknown function
MAT1-2-7	Eurotiomycetes	*Coccidioides* spp.[j,k]	APN2
	Sordariomycetes	*Huntiella omanensis*[u], *H. moniliformis*[u]	Unknown function
MAT1-2-8	Hypocreales	*Villosiclava virens*[v], *Metarhizium acridum*[v], *Ophiocordyceps sinensis*[v], *Trichoderma* spp.[v]	Unknown function

[a]References
[b]Reviewed in Debuchy et al. (2010)
[c]Yokoyama et al. (2006)
[d]Singh et al. (1999)
[e]Accession number ABS19617
[f]Accession number ABS19621
[g]Accession number XP_003023854
[h]Accession number XP_003014758
[i]Bihon et al. (2014)
[j]Fraser et al. (2007)
[k]Mandel et al. (2007)
[l]Amselem et al. (2011)
[m]Liberti et al. (2012)
[n]Terhem et al. (2013)
[o]Palmer et al. (2014)
[p]Pöggeler and Kück (2000)
[q]Martin et al. (2011)
[r]Fedorova et al. (2008)
[s]Swilaiman et al. (2013)
[t]Woo et al. (2006)
[u]Wilson et al. (2015)
[v]Yu et al. (2015)

although it is non-functional in some homothallic species of the *Sordariaceae* (Wik et al. 2008) and absent in *Metarhizium robertsii*, a putatively heterothallic species (Pattemore et al. 2014). *MAT1-1-3* encodes HMG-box transcription factors that form a clade in a phylogenetic tree based on HMG-box sequences (Ait Benkhali et al. 2013). *MAT1-1-3* has a critical role during fruit body development in *P. anserina* (Arnaise et al. 2001) but is absent in a group of *Clavicipitaceae* species (Yokoyama et al. 2006) and *Huntiella omanensis* (Wilson et al. 2015). MAT1-1-3 is non-functional or not required for the sexual cycle in some homothallic *Sordariaceae* species (Klix et al. 2010; Wik et al. 2008). *MAT1-1-4* is present in Eurotiomycetes and *Diplodia pinea* (Dothideomycetes) (Bihon et al. 2014). It displays weak sequence conservation across species but was identified by reciprocal BLAST searches (our unpublished results), suggesting an orthologous relationship. The function of *MAT1-1-5* was functionally investigated in *B. cinerea*. The deletion of this gene prevents the development of the stipes into apothecial discs (Terhem et al. 2013). Interestingly, the deletion of *MAT1-2-4* in the opposite mating type of *B. cinerea* results in an identical phenotype, suggesting that these two genes jointly control the transition from stipes to disc development (Terhem et al. 2013).

B. Reproductive Lifestyles and Evolution

1. Pseudo-homothallic Lifestyle

a) Definition of Pseudo-homothallism

Pseudo-homothallism (also called secondary homothallism) is a reproductive strategy adopted by some heterothallic fungi involving the production of ascospores that contain sexually compatible nuclei within the same single ascospore envelope. This typical packaging is achieved via strict regulation of recombination between the mating-type locus and the centromere and by programmed nuclear choreography during ascospore delineation. The regulation of recombination raises fascinating questions in the field of meiosis, which was widely explored in fungi (Zickler 2006). The mycelium resulting from the germination of these ascospores displays heterokaryosis, namely, the association of nuclei of opposite mating type in vegetative hyphae. This type of self-fertile mycelium could be confused with homothallism but can be distinguished when it is resolved into homokaryotic self-sterile cultures, thereby revealing its heterothallic nature. The resolution of heterokaryons into homokaryons requires drastic treatments, such as shearing the mycelium into uninucleate fragments, indicating the presence of mechanisms that maintain a strong association between the complementary nucleotypes in the mycelium. The use of the term heterokaryosis was expanded to cover all conditions in which two or more genetically distinct nuclei are associated in a common cytoplasmic system, a feature commonly observed in many fungi. This type of heterokaryosis exists in homothallic species, some of which display a strong bias in favour of mating between genetically distinct nuclei, even though they are self-compatible. This phenomenon is termed relative heterothallism (Hoffmann et al. 2001; Pontecorvo et al. 1953) and must not be confused with pseudo-homothallism.

b) *MAT* Chromosome Structure in Pseudo-homothallic Species

Neurospora tetrasperma and *P. anserina* serve as model systems for the analysis of pseudo-homothallism. Idiomorph analysis indicates unambiguously that the two species have a heterothallic structure (Ellison et al. 2011; Grognet et al. 2014). Genome sequencing of *N. tetrasperma MAT1-1* (also called *mat A*) and *MAT1-2* (also called *mat a*) isolates and comparison with the close relative *N. crassa* revealed massive differences in the genome architecture of *N. tetrasperma* (Ellison et al. 2011). Whereas the two mating-type chromosomes of *N. crassa* and the *mat a* chromosome of *N. tetrasperma* are colinear, a series of three inversions affect the central region of the chromosome that contains the *mat A* idiomorph in *N. tetrasperma*. None of the rearrangement events include the mating-type locus itself, but the region of suppressed recombination encompasses this locus and the centromere. **The region of suppressed recombination includes ~2000 genes and spans a distance of ~7.8 Mb, representing 80 % of the *mat A* chromosome of *N. tetrasperma* (Ellison**

***et al.* 2011**). As a consequence of suppressed recombination, mating types segregate at the first meiotic division, and two non-sister nuclei resulting from post-meiotic mitosis are included in ascospores. This results in the correct packaging of nuclei of opposite mating types (Raju 1992). **Two observations indicate that suppressed recombination on *mat* chromosome is regulated by yet unknown trans-acting factors in *N. tetrasperma*.** First, reciprocal introgression of the mating-type chromosomes between *N. tetrasperma* and *N. crassa* reveals that the inversions are not essential for suppressed recombination (Jacobson 2005). The *N. crassa mat A* and *N. tetrasperma mat a* chromosomes, which are colinear, recombine in *N. crassa* but not in *N. tetrasperma*. Second, pericentric inversion has been observed on the mating-type chromosome of *N. crassa* and results in the formation of typical inversion loops during meiosis, allowing both pairing and crossing of the inverted region as well as the formation of inviable and unstable progeny (Newmeyer and Taylor 1967). Such inversion loops or crossovers do not occur in *N. tetrasperma*. As suppressed recombination is independent of chromosome inversion, it may predate these genomic rearrangements, but the timeline of these events is not yet established. (Sun et al. 2012). Pseudo-homothallism in *P. anserina* is achieved by a mechanism that is different from the suppressed recombination observed in *N. tetrasperma*. One obligate crossover event occurs between the mating-type locus and the centromere, and additional crossovers are suppressed around the mating-type locus (Marcou et al. 1979). A specific nuclear choreography after post-meiotic mitosis ensures nuclei of opposite mating types are delivered to each ascospore (Raju and Perkins 1994). **In *P. anserina*, the region of suppressed recombination includes ~229 genes and spans ~0.8 Mb (Grognet et al. 2014). Interestingly, the *MAT1-1* (also called *mat*−) and *MAT1-2* (also called *mat*+) chromosomes are colinear. This indicates that suppressed recombination is controlled by as yet unknown trans-acting elements in *P. anserina*, as well as in *N. tetrasperma*.** In many plants and animals, it is unclear whether suppressed recombination is created by chromosome rearrangements, as initially suggested (Lahn and Page 1999), or by recombination modification without rearrangements (with rearrangements occurring after recombination suppression) (Bergero and Charlesworth 2009; Marais and Galtier 2003). Suppressed recombination in colinear regions in *P. anserina* and *N. tetrasperma* is an interesting model for decreased recombination by the spread of recombination modifiers. Further analyses will be necessary to elucidate the mechanism of suppressed crossover in these fungi and to identify commonalities with suppressed recombination in centromeric regions (Talbert and Henikoff 2010) and in sex chromosomes of plants and animals (Charlesworth et al. 2005).

c) Heterokaryosis in Pseudo-homothallic Species

Factors that may affect heterokaryosis were described by Pontecorvo (1946) and include (i) drift (i.e. random variation in the proportion of each nucleotype in a population), (ii) migration (influx and outflow of nuclei following hyphal anastomoses), (iii) variation in nucleus number, (iv) selection and (v) mutation (Pontecorvo 1946). Genetic drift models predict that heterozygosity at the moving colonisation front decays exponentially over time owing to stochastic fluctuation (Hallatschek and Nelson 2008). This process eventually creates homokaryotic hyphae. **Although this diversity of factors suggests that the maintenance of heterokaryosis may be challenging, heterokaryons are remarkably stable.** Genetic drift is counteracted by the nuclear dynamics within the hyphal network (Roper et al. 2011, 2013). Nuclei undergo intense and complex mixing flows that are assumed to maintain the genetic diversity throughout the mycelium. The impact of the other factors on heterokaryosis is discussed below.

Selection and variation in nucleus number add another layer of complexity to nuclear dynamics. **Our comparison of the variation in nucleus number reported in *N. crassa* and *N. tetrasperma* suggests that the ratio of *mat A*/*mat a* nuclei may be regulated in *N. tetrasperma*.** The analysis in *N. crassa*, conducted with biochemical mutants, indicated that the nuclear ratio changes very little during heterokaryotic growth, and, surprisingly, the growth rate is maximal over a wide range of nuclear ratio (Pittenger and Atwood 1956). In *N. tetra-*

sperma, an analysis of the ratio of *mat A*/*mat a* nuclei indicated that the nucleotype distribution is stable in the mycelium from the inoculation point to the growth edge of plates (Samils et al. 2014), which is in agreement with the observations in *N. crassa*. However, the *mat A*/*mat a* ratio was biased for *mat A* nuclei in three isolates of *N. tetrasperma* (mean of 87%; range, 67–99 %). This conserved bias in independent experiments disagrees with the various nuclear ratios that can be accommodated by heterokaryons of biochemical mutants in *N. crassa* (Pittenger and Atwood 1956) and suggests that the *mat A*/*mat a* ratio is controlled by an as yet unknown mechanism. The switch from mycelial growth to sexual reproduction in *N. tetrasperma* is associated with a significant change in the *mat A*/*mat a* ratio that evens out and does not display any bias for *mat A* nuclei (range, 40–57 %) (Samils et al. 2014). This result also supports the regulation of the nucleotype ratio in pseudo-homothallic species. However, the data may be misleading because the experiments did not dissociate the mycelium from fruit bodies, and this may introduce a bias in nucleotype counting. Various heterokaryons in *P. anserina* also show a remarkable stability (Grognet et al. 2014). Further investigations are necessary to determine whether independent *mat*+/*mat*− heterokaryons display a biased nucleotype ratio, which would point to a conserved mechanism for nucleotype maintenance in pseudo-homothallic species.

Once recombination between a pair of homologous chromosomes has ceased, they are free to follow separate evolutionary trajectories, independently accumulating mutations. Accordingly, the non-recombining regions of mating-type chromosomes in *N. tetrasperma* and *P. anserina* contain various polymorphisms that affect gene expression (Grognet et al. 2014; Samils et al. 2014) and possibly protein activity (Contamine et al. 2004; Ellison et al. 2011). **Fixation and accumulation of deleterious mutations are expected to affect the fitness of homokaryons, while the heterokaryon benefits from the presence of a functional gene in either *MAT1-1* or *MAT1-2* nuclei. This model predicts that heterokaryons grow better than homokaryons. However, neither *P. anserina* nor *N. tetrasperma* behaves as predicted by this model.** In *P. anserina*, homokaryons grow as well as heterokaryons (Grognet et al. 2014), suggesting that the differences between the nucleotypes are not important for maintaining heterokaryosis. In *N. tetrasperma*, *mat A* homokaryotic isolates display greater radial growth than *mat a* or heterokaryotic isolates (Samils et al. 2014). This result is in agreement with the *mat A*-biased nuclear ratio during vegetative growth (see above) and may suggest that the *mat A* nucleotype has a greater fitness than the *mat a* nucleotype for vegetative growth. As *mat a*/*mat A* heterokaryons are able to engage in sexual reproduction, it is not yet clear whether the greater fitness of the *mat A* nucleotype reflects differences in vigour or differences in developmental programmes between heterokaryons and homokaryons. Moreover, the authors emphasised that their finding contradicts previous reports of reduced vigour of homokaryotic nucleotype of *N. tetrasperma* relative to heterokaryons during vegetative (Dodge 1942) and reproductive stages (Raju 1992). Microarray experiments were performed in *N. tetrasperma* (Samils et al. 2013) and *P. anserina* (Grognet et al. 2014) to identify gene families preferentially expressed in each nucleotype. The numbers of differentially transcribed genes were 196 and 1054 in the comparison between *N. tetrasperma mat a* and *mat A* and between *P. anserina mat*+ and *mat*−, respectively. It is likely that the gene number was underestimated for *N. tetrasperma*, owing to the use of *N. crassa* microarrays (Kasuga et al. 2005). A high proportion of the 196 *N. tetrasperma* genes locate in the region of suppressed recombination, while the 1054 *P. anserina* genes are not clustered on any particular chromosome. There is an excess of *mat A*-biased genes when *N. tetrasperma* is grown on crossing medium that induces fruit body formation. By contrast, the majority of genes expressed when grown on vegetative medium, inducing formation of the mycelium and macroconidia, are *mat a* biased. Samils et al. (2013) proposed that sex-specific selection resulted in the feminisation of the *mat A* chromosome and masculinisation of the *mat a* chromosome. However, the *mat a* strain produces more fruit bodies than the *mat A* strain (Howe 1964). Moreover, macroconidia are primarily vegetative propagules that give nuclei to female organs and must not be con-

fused with microconidia, which are true male cells with specific features (Maheshwari 1999). Therefore, the production of macroconidia is not conclusive evidence for masculinisation. Taken together, these observations counter the hypothesis of mating-type dimorphism proposed by Samils et al. for *N. tetrasperma* (Samils et al. 2013). In *P. anserina*, the 1054 mating-type differentially expressed genes were further divided in 860 genes controlled by the mating-type genes, while the remaining 194 genes were differentially expressed owing to polymorphisms that differentiate the *mat*+ and *mat*− strains (Grognet et al. 2014). Among these 194 genes, all of those encoding glycoside hydrolases ($n = 21$) had higher expression in *mat*− than in *mat*+. The genes with higher expression in *mat*+ than in *mat*− showed a significant enrichment in products with predicted mitochondrial localisation ($n = 13$). This analysis revealed that the non-recombining regions determine the physiological state, but their significance in heterokaryosis needs additional investigation.

d) Mating-Type Chromosomes as Models for Sexual Chromosomes in Animals and Plants

The spread of the non-recombining regions on sex chromosomes is attributed to (i) selection against recombination between linked genes with a positive interaction effect upon fitness, (ii) genetic drift of mutations causing suppression of recombination and (iii) selection of recombination suppression because it prevents homozygosity of deleterious recessive genes (Ironside 2010). However, there is little evidence so far to support each of these hypotheses in *N. tetrasperma* and *P. anserina*. The first point was addressed in the above discussion about the validity of the suggested masculinisation of *mat a* chromosome and feminisation of the *mat A* chromosome in *N. tetrasperma* (Samils et al. 2013). Second, the hypothesis that a mutation could invade through genetic drift is challenging to model beyond a single isolated population (Ironside 2010). And third, the presence of deleterious recessive genes is questionable, as *N. tetrasperma* and *P. anserina* individuals can grow as haploid homokaryons that are capable of outcrossing and acting as maternal and paternal parents. It is also noteworthy that normal recombination along the *N. crassa mat* chromosome indicates that suppression of recombination is not necessarily associated with the *mat* locus. The driving force for the spread of non-recombining regions in pseudo-homothallic may be the preservation of heterozygosity in fungi with obligate intratetrad mating (Zakharov 2005). Alternatively, non-recombining regions may be selected to favor the packaging of nuclei with opposite mating types in the same ascospore. **These hypotheses question the use of mating-type chromosomes as models for the evolution of animal and plant sexual chromosomes (e.g. Menkis et al. 2008), because the evolutionary forces applying on sex chromosomes in fungi, and in plants and animals, may be different.**

2. Homothallic Lifestyle

Heterothallism is the most likely ancestral mating state of most extant homothallic species. This is illustrated by detailed studies of genera that contain both homothallics and heterothallics, including *Cochliobolus* spp. (Yun et al. 1999) and its relative *Stemphylium* spp. (Inderbitzin et al. 2005) and the *Aspergillus* section *Fumigati* (Rydholm et al. 2007). **Generally, in those examples, homothallism evolved by fusion of the two idiomorphs, with the bringing together in the same genome of *MAT1-1* and *MAT1-2* genes thought to be a critical factor enabling self-fertility.** But idiomorphs in homothallic species are not always tightly linked in the genome as exemplified by *Cochliobolus cymbopogonis* in which the locations of *MAT1-1* with respect to *MAT1-2* are unknown (Yun et al. 1999) and *Aspergillus nidulans* in which the two opposite mating-type loci *MAT1* and *MAT2* exist in the same haploid nucleus but on different chromosomes 6 and 3, respectively (Dyer et al. 2003; Paoletti et al. 2007). The evolutionary mechanism leading to unlinked idiomorphs being in the same nucleus of a homothallic species is unknown, but a chromosomal breakage between initially fused idiomorphs followed by a translocation could distribute opposite mating-type genes onto different chromosomes. **The shift from heterothallism to homothallism is fairly common, resulting in the frequent coexistence**

of heterothallic and homothallic species within a genus. For instance, homothallism evolved independently at least six times in the evolutionary history of the genus *Neurospora* (Nygren et al. 2011). There are thought to be various ecological factors favouring the evolution of homothallism, such as the ability to produce environmentally resistant sexual propagules without the need for a mating partner or metabolic investment in finding a mate and the retention of favourable gene combinations which might be broken up during outcrossing (Billiard et al. 2012; Murtagh et al. 2000). **Furthermore, most homothallic species are not restricted to self-fertility; instead, isolates retain the ability to outcross if opportunities arise, and in *A. nidulans*, there is evidence that outcrossing is favoured, as exemplified by relative heterothallism (Hoffmann et al. 2001).**

The role of expression of mating-type genes in determining reproductive lifestyle has been investigated by four different experimental approaches: (i) exchange of *MAT* loci between homothallic and heterothallic species; (ii) deletion of one mating-type gene in a homothallic species, to see if this results in a heterothallic species; (iii) introduction of the complementary *MAT* idiomorph in a heterothallic strain; and (iv) overexpression of mating-type genes in homothallic species.

The exchange of *MAT* loci was thoroughly investigated by using the homothallic *Cochliobolus luttrellii*, which contains fused *MAT1-1-1* and *MAT1-2-1* mating type genes (Yun et al. 1999), and its heterothallic relative, *C. heterostrophus* (Turgeon et al. 1993). The fused (*MAT1-1-1*)-(*MAT1-2-1*) gene was introduced into a *mat*-deleted strain of *C. heterostrophus* (Yun et al. 1999). Abundant fruit bodies formed when the transformants were selfed, and many showed some degree of fertility (1–10 % of wild-type ascospore production). In the reverse experiment, a *mat*-deleted *C. luttrellii* strain carrying *C. heterostrophus MAT1-1-1* can mate with a *mat*-deleted *C. luttrellii* strain carrying *C. heterostrophus MAT1-2-1* (Lu et al. 2011). The fertility of the cross is similar to that of the selfed wild-type *C. luttrellii*, except the asci often contain fewer than the eight expected ascospores. **These experiments show that a heterothallic species can become homothallic and vice versa via exchange of *MAT* loci. However, these exchanges result in quantitative and qualitative defects in the progeny. Therefore, mating types must undergo fine-tuned adaptation to perform optimally when placed in a new context.**

The deletion of one mating type was performed in the homothallic *Gibberella zeae*, which carries closely linked *MAT1-1* and *MAT1-2* in a single nucleus (Lee et al. 2003). Targeted gene replacement was used to differentially delete *MAT1-1* or *MAT1-2* from a wild-type haploid *MAT1-1,MAT1-2* strain. The resulting *MAT1-1,ΔMAT1-2* and *ΔMAT1-1,MAT1-2* strains were found to be completely self-sterile yet able to cross with each other. Fruit body formation was 10–20 % relative to wild-type selfers, and the fertility of the fruit bodies (in terms of ascospore production) was about 10% that of the wild type. As above, this experiment indicates fine-tuned adaptation of the *MAT* genes to optimise the sexual cycle in a homothallic species. **Crosses between *MAT1-1,ΔMAT1-2* and *ΔMAT1-1,MAT1-2* strains with a wild-type self-fertile strain have a striking feature. They yield only biparental progeny, and no asci resulting from selfing of wild-type nuclei are observed in perithecia.** This observation indicated a preference for karyogamy between nuclei from the *mat*-deleted parent and the wild type, rather than between like nuclei from the homothallic wild-type parent that is able to self. The mechanism for the preference among nuclei is currently unknown but may be related to the *MAT* gene-mediated nucleus recognition proposed in heterothallic species (Debuchy and Turgeon 2006). Similar observations were obtained for the homothallic *A. nidulans*, where deletion of either the resident *MAT1* (α1 domain) or *MAT2* (MATA_HMG-box) gene resulted in defects in sexual development (Paoletti et al. 2007). In both *ΔMAT1, MAT2* and *MAT1,ΔMAT2* strains, it was found that fruit bodies (cleistothecia) were still produced under conditions inducing the sexual cycle. However, these were lower in number and, in the case of *ΔMAT1,MAT2*, were smaller than those of control strains. Critically, cleistothecia were sterile, being devoid of ascospores. However, it was then possible to cross the resultant *MAT1,ΔMAT2* and *ΔMAT1,MAT2* strains

to produce cleistothecia containing ascospores, i.e. there was a switch from a homothallic to a heterothallic lifestyle as a result of manipulation of *MAT* genes. Remarkably, Czaja et al. (2014) have also recently reported that the human SRY protein (part of the HMG-box family) is able to functionally replace the *A. nidulans* MAT2 (synonym matA) protein to initiate early stages in the sexual cycle (Czaja et al. 2014). Ascospore production is defective, but a hybrid MATA protein containing the SRY HMG-box is fully capable of driving both early and late stages of sexual development.

There have been several attempts to convert heterothallic *P. anserina* and *C. heterostrophus* isolates to homothallism by introducing the *MAT1-1* idiomorph in a *MAT1-2* strain and vice versa, resulting in transgenic strains carrying resident and ectopic opposite idiomorphs (Coppin et al. 1993; Picard et al. 1991; Turgeon et al. 1993). Selfing of these transgenic strains results in a high number of fruit bodies but very low ascospore production. When the transgenic strains were outcrossed, normal fertility was obtained when resident idiomorphs of the transgenic strains were required to function. By contrast, fertility was low when ectopic idiomorphs were required to function. These ectopically integrated idiomorphs confer normal fertility to strains that have a deletion of the resident idiomorph, indicating that reduced fertility was not due to a deficiency in expression of the ectopic idiomorph, but instead that expression of the resident idiomorph is partially dominant (Coppin et al. 1993; Wirsel et al. 1996). **This dominance of the resident idiomorph with respect to the ectopic one is termed interference (Wirsel et al. 1996)**. To eliminate this dominant effect, the *MAT1-2* idiomorph was integrated immediately downstream of the resident *MAT1-1* idiomorph in *P. anserina* (Xie et al. 2015, submitted). Ironically, this strain displayed low fertility in outcrosses with *MAT1-1* and *MAT1-2* tester strains as well as in selfing strains (1% of a wild-type cross), suggesting that this structural organisation of mating-type genes affects the expression of both *MAT1-1* and *MAT1-2*. Crossing between the *MAT1-1,MAT1-2* strain and heterothallic tester strains results mainly in selfing of the *MAT1-1,MAT1-2* nuclei (Xie et al. 2015, submitted), a behaviour that contrasts with the preference for karyogamy between nuclei from the *mat*-deleted parent and wild type observed in the homothallic *G. zeae* (Lee et al. 2003). In conclusion, all attempts to obtain a fertile homothallic strain by manipulation of the mating-type genes of a heterothallic species have so far resulted in wild-type or higher than wild-type fertilisation ability (in terms of numbers of fruit bodies formed), but have failed to restore the normal post-fertilisation developmental processes that lead to ascospore formation.

Finally, the effects of overexpression of *MAT* genes in a homothallic species have been investigated in *A. nidulans*. **It was found that simultaneous overexpression of both *MAT1* and *MAT2* resulted in suppression of vegetative growth and the induction of sexual development under conditions not normally favourable for sex (Paoletti et al. 2007)**. The same study found that sexual reproduction was correlated with significantly increased expression of the *MAT* genes and key genes of a pheromone response MAP-kinase signalling pathway, indicating that selfing in *A. nidulans* involves activation of the same mating pathways characteristic of sex in heterothallic species. However, unlike heterothallic species, aspects of pheromone signalling appeared to be independent of *MAT* control because no clear differences were apparent in pheromone precursor and receptor gene expression despite deletion or overexpression of the resident *MAT* loci. This again suggests possible adaptation and fine-tuning of the role of *MAT* genes to optimise the sexual cycle in a homothallic species. It was speculated that *MAT* gene expression may therefore be primarily required for later stages of sexual development, rather than mating, as a possible adaptation for homothallism (Paoletti et al. 2007).

Taken together, these experiments clearly indicate that the switch from heterothallism to homothallism is associated with adaptations that involve the two idiomorphs and the *MAT* target genes. It is likely that there are as many accompanying adaptation mechanisms as there are switching events. One of the most interesting hypothetical mechanisms assumed that genetically self-compatible *MAT1-1,MAT1-*

2 nuclei become functionally self-incompatible (Metzenberg and Glass 1990). This could be achieved by an epigenetic process that inactivates the *MAT1-1* or the *MAT1-2* functions, as proposed by C. Scazzocchio for *A. nidulans* (Scazzocchio 2006). It seems unlikely that this mechanism is ubiquitous in homothallic species or operates in species that harbour fused *MAT1-1* and *MAT1-2* mating-type genes. However, this epigenetic mechanism may explain the preference for karyogamy between nuclei from the *mat*-deleted parent and the wild type, as observed in *G. zeae* (Lee et al. 2003). The *MAT1-1,MAT1-2* nuclei may undergo random epigenetic inactivation of *MAT1-1* or *MAT1-2*, and *MAT1-1,ΔMAT1-2* nuclei may recognise their compatible *MAT1-2* partner (or *MAT1-1* partner for *ΔMAT1-1,MAT1-2* nuclei) and form ascogenous hyphae and biparental progeny. Epigenetic silencing of *MAT* genes may not exist in heterothallic species, explaining the preference for selfing of *MAT1-1,MAT1-2* nuclei in a similar experiment in *P. anserina* (Xie et al. 2015, submitted).

3. Switching Lifestyle

a) Bidirectional Switching in Budding and Fission Yeasts

Similar to other traits, mating type is passed on from mother to daughter cells during vegetative growth and is transmitted to progeny according to Mendel's rules during sexual reproduction. But in some species, mating type can be altered during vegetative growth or sexual reproduction, in a process termed 'mating-type switching'. Mating-type switching has been thoroughly investigated in the budding yeast *S. cerevisiae* and the fission yeast *Schizosaccharomyces pombe* (Herskowitz 1988; Klar 2007). These two species contain one expressed mating-type locus and two silenced loci that contain opposite *MAT* sequences. A homologous recombination event replaces the expressed *MAT* allele with the opposite *MAT* allele from one of the silenced loci. **Mating-type switching in the budding and fission yeasts always occurs during mitosis and is bidirectional, which means that a cell lineage may display multiple and successive mating-type changes.** After mating, these yeasts display conventional, bipolar inheritance of their mating-type alleles.

b) Unidirectional Switching of Homothallism to Heterothallism

Mating-type switching in the Pezizomycotina differs from yeasts and displays three specific features: (i) switching occurs always during sexual reproduction; (ii) switching occurs from one lifestyle to another, i.e. from homothallism to heterothallism or vice versa; and (iii) switching is unidirectional and cannot be reversed. Examples include *Chromocrea spinulosa*, various *Ceratocystis* species, *Fusarium subglutinans* (Sordariomycetes) and *Sclerotinia trifoliorum* (Leotiomycetes), which display mating-type switching from homothallism to heterothallism during sexual reproduction (Perkins 1987; Tolmsoff 1983). Among the best-studied examples of mating-type switching in filamentous ascomycetes is *Ceratocystis spinulosa*, where upon selfing, four of the eight ascospores in each ascus are homothallic and the other four ascospores are heterothallic (Mathieson 1952). Heterothallic isolates cannot mate with one another, but heterothallic isolates can mate with homothallic isolates, where the same ratio of homothallic to heterothallic ascospores in each ascus is repeated (Fig. 14.4). The organisation of the mating-type locus in *C. spinulosa* has been determined (Turgeon and Debuchy 2007). Homothallic isolates have a *MAT1-2-1* gene that is adjacent to a complete set of *MAT1-1* genes including *MAT1-1-1*, *MAT1-1-2* and *MAT1-1-3*, whereas heterothallic isolates have all three *MAT1-1* genes but lack the *MAT1-2-1* gene. Direct repeats are present on either side of *MAT1-2-1* in homothallic strains (Turgeon and Debuchy 2007). Recombination between the two repeats would result in the elimination of *MAT1-2-1* and could account for the missing *MAT1-2-1* in heterothallic isolates.

Ceratocystis fimbriata is similar to *C. spinulosa* in that the progeny of homothallic strains includes both homothallic and heterothallic strains (Mathieson 1952; Olson 1949). Heterothallic strains can only mate with homothallic strains, again resulting in both homothallic and heterothallic progeny (Turgeon and Debuchy

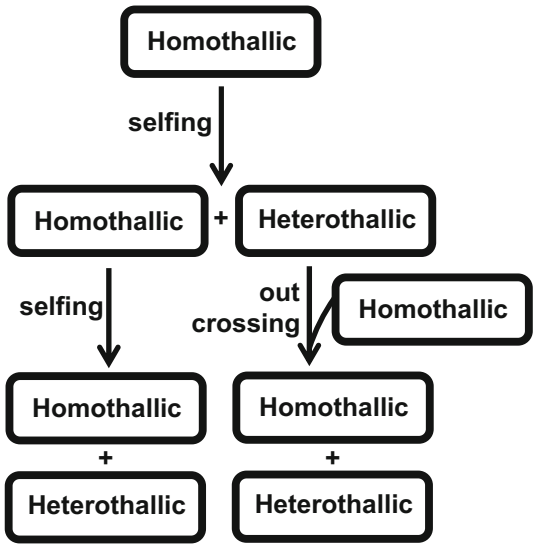


Fig. 14.4 Mating-type switching in *Ceratocystis* species, *C. spinulosa* and *Sclerotinia trifoliorum*. Progeny from selfing homothallics are either homothallic or heterothallic. Heterothallics can only mate with homothallics, again resulting in homothallic and heterothallic progeny. There is a 1:1 ratio of homothallics to heterothallics in tetrads of *C. spinulosa* and *S. trifoliorum* and in perithecia of *C. coerulescens*. The ratio varies from 1:1 to 1:9 between perithecia of *C. fimbriata*. Homothallic and heterothallic progeny differ in morphology, which is indicative of the pleiotropic effects of *MAT*. Mating-type switching appears to be due to deletion of a *MAT* gene in homothallics, which is caused by a recombination event between direct repeats flanking the deleted gene

2007; Webster 1967) (Fig. 14.4). As in *C. spinulosa*, *C. fimbriata* heterothallics are lacking the *MAT1-2-1* gene (Witthuhn et al. 2000), which in homothallic isolates is located between two direct repeats that are separated by 3.6 kb. The repeats measure 260 bp, and recombination between the two repeats would result in the excision of the intervening DNA, including *MAT1-2-1* (Wilken et al. 2014). But unlike *C. spinulosa*, the ratio of homothallic to heterothallic strains in *C. fimbriata* is not constant but depends on the perithecia and ranges from 1:1 to 1:9, with 3:2 being the most common (Webster and Butler 1967). Tetrad analyses are not possible in *Ceratocystis*, since the asci disintegrate before ascospores fully mature. Other species of *Ceratocystis*, including *Ceratocystis coerulescens*, *Ceratocystis pinicola* and *Ceratocystis virescens* also practise mating-type switching (Harrington and McNew 1997; Witthuhn et al. 2000), which results in the formation of heterothallic strains that may lack *MAT1-2-1*, as also seen with *C. fimbriata*.

Little is known about *MAT* architecture in *S. trifoliorum*, but mating compatibilities and the ratio of homothallic to heterothallic progeny (Uhm and Fujii 1983) are as observed in *C. spinulosa* (Fig. 14.4), suggesting a similar mechanism. Mating-type switching has also been reported in *F. subglutinans* (Leslie et al. 1986), but very few details have been described.

In *C. fimbriata*, *C. coerulescens*, *C. spinulosa* and *S. trifoliorum*, mating type correlates with phenotype, which suggests a pleiotropic effect of *MAT*. In *C. fimbriata* and in *C. coerulescens*, homothallic strains grow faster than heterothallic strains (Harrington and McNew 1997; Webster and Butler 1967), and in *C. fimbriata*, colony morphology differs between homothallic and heterothallic strains (Webster and Butler 1967). In *C. spinulosa* and *S. trifoliorum*, homothallic ascospores are larger than heterothallic ascospores (Uhm and Fujii 1983). Pleiotropic effects of *MAT* are known from the homothallic *Coniochaeta tetraspora*, where only half of the ascospores in each ascus are viable and all viable ascospores are homothallic (Raju and Perkins 2000). The pleiotropic effects of *MAT* may be mediated by altered *MAT* gene expression between different *MAT* region arrangements as described for *Sclerotinia sclerotiorum* below.

c) Structural Mating-Type Rearrangement Without Lifestyle Switching

***S. sclerotiorum* is a filamentous ascomycete that does not undergo mating-type switching, but the *S. sclerotiorum MAT* region undergoes regular rearrangements that correlate with *MAT* gene expression patterns (Chitrampalam et al. 2013), which could account for mating-type switching in other species.** *S. sclerotiorum* is homothallic and contains fused *MAT1-1* and *MAT1-2* idiomorphs. A 3.6 kb region, the inversion region, is flanked by 250 bp inverted repeats and spans across *MAT1-2-1*, *MAT1-2-4* and part of *MAT1-1-1*. Upon selfing, the inversion region is inverted in half of the progeny in each ascus and among random ascospore progeny. The 1:1

segregation pattern of forward to inverted inversion region is repeated when the progeny self and is independent of the orientation of the inversion region in the parent. Expression of *MAT1-1-1* and *MAT1-2-1* differs depending on the orientation of the inversion region. For instance in isolates with the inversion, the MAT1-1-1 α1 domain is shortened to only 15 residues. Thus, isolates with the inversion could be expected to be functionally *MAT1-2-1* heterothallic, but they remain homothallic. No phenotypic differences are known between strains that differ in the orientation of the inversion region (Chitrampalam et al. 2013).

d) Switching of Heterothallism to Unisexual Mating

In some heterothallic Ascomycetes, individuals derived from a homokaryotic ascospore are able to engage in sexual reproduction by mating with themselves or with a partner of the same mating type. This sexual lifestyle was investigated thoroughly in the heterothallic *B. cinerea*. Seven out of 105 asci from ten different crosses contained progeny that mated with both *MAT1-1* and *MAT1-2* strains (Faretra and Pollastro 1996). Two of these dual mater strains were crossed to a *MAT1-2* reference strain, which resulted in a 1:1 segregation for *MAT1-1* and *MAT1-2*, as well as the appearance of six dual mater progeny among the 128 ascospores examined between the two crosses (Faretra and Pollastro 1996). Four dual mater strains were investigated by sequencing of the *MAT* regions and were found to have standard heterothallic *MAT* arrangements, containing either *MAT1-1* or *MAT1-2* idiomorphs (Amselem et al. 2011). Thus, the mechanistic basis of this functional switching, from heterothallism to homothallism, does not involve bringing together complementary *MAT* genes and therefore is different from homothallism. In the heterothallic *Sordaria brevicollis*, uncrossed perithecia of one mating type are able to form a few ascospores (Robertson et al. 1998). Meiosis occurs, but the frequency of recombination is lower than in heterothallic crosses. *Cryphonectria parasitica* also appears to lack a regular mechanism for mating-type switching. Ascospores in general are heterothallic, and only very few are capable of self-mating. Mating is either due to heterokaryosis established during ascospore formation or results from an unknown mechanism in ascospores that have only one mating type (McGuire et al. 2004).

The ability of same-sex mating was termed unisexual mating (Wang and Lin 2011). This denomination should be restricted to species which have a heterothallic structural organisation of mating-type genes, to avoid confusion with homothallism. According to this restriction, *Neurospora* spp., which contain only *mat A* (e.g. *N. africana*) (Wik et al. 2008), should be considered homothallic species, as initially described (Glass et al. 1990b). To date, no structural rearrangement can explain the switch from heterothallism to unisexual mating in some individuals. Unlike mating-type switching from homothallism to heterothallism, the switch from heterothallism to unisexual mating is reversible. It is possible that unisexual mating results from an epigenetic mechanism, which converts a functional heterothallic individual to a homothallic one.

e) The Timing and Mechanism of Mating-Type Switching

All evidence points to the association of mating-type switching with the sexual state. In *C. fimbriata*, *C. coerulescens* and *C. spinulosa*, homothallics do not mutate into heterothallics during vegetative growth (Harrington and McNew 1997; Mathieson 1952; Webster 1967), and the *S. sclerotiorum MAT* inversion region is stable during vegetative growth (Chitrampalam et al. 2013). Harrington and McNew proposed that in *C. coerulescens*, switching occurs prior to dikaryon formation (Harrington and McNew 1997). This is an attractive possibility, since the switched, heterothallic nuclei could then pair up with homothallic nuclei during the dikaryotic stage (Shiu and Glass 2000). This would ensure that inside each ascus, one homothallic nucleus and one heterothallic nucleus entered into karyogamy. A constant 1:1 segregation ratio of homothallic to heterothallic progeny would ensue, as is observed for asci of *C. spinulosa* and *S. trifoliorum* (Mathieson 1952; Uhm and Fujii 1983) and possibly for *C. coer-*

ulescens perithecia (Harrington and McNew 1997). Association between homothallic and heterothallic nuclei may be less stringent in *C. fimbriata*, since the ratio of homothallic to heterothallic ascospores differs between perithecia (Webster and Butler 1967).

Various mechanisms have been proposed to explain mating-type switching in filamentous Ascomycetes (Perkins 1987; Tolmsoff 1983). As compared to budding and fission yeasts, few details are known, but evidence suggests the involvement of recombination between repeats at *MAT*. Analyses of the stability of repeated sequences during the sexual cycle in the non-mating-type switchers *P. anserina* (Bouhouche et al. 2004; Coppin-Raynal et al. 1989; Picard et al. 1987) and *N. crassa* (Selker 1990) provided numerous examples for premeiotic DNA deletions, which were most likely mediated by intra-chromosomal recombination between direct repeats. **We thus propose that mating-type switching as described for *C. spinulosa* (Mathieson 1952) and *C. fimbriata* (Webster 1967) relies on a premeiotic repeat-mediated, intra-chromosomal recombination mechanism similar to that evidenced in *N. crassa* and *P. anserina*.** In *C. spinulosa* and *C. fimbriata*, mating-type switching is unidirectional, because the repeats involved are direct repeats and the intervening DNA is deleted upon recombination. Inverted repeats have not been associated with mating-type switching but could result in reversible mating-type switching. This is because recombination between inverted repeats causes the DNA between repeats to invert, a process that is reversible as described for *S. sclerotiorum MAT* (Chitrampalam et al. 2013).

In *B. cinerea* and *C. parasitica*, mating-type switching does not alter the structure of *MAT*, which suggests that an epigenetic mechanism may be involved. Epigenetic modification of DNA associated with the sexual state was observed in *Ascobolus immersus*, where de novo premeiotic methylation (MIP) results in silencing of repeated genes (Rossignol and Faugeron 1995). **We thus propose that MIP might trigger the reversible mating-type switching observed in *B. cinerea* and potentially in *C. parasitica*.**

4. Asexual Lifestyle and Cryptic Sexuality

a) Definitions and Use of *MAT* Genes to Investigate Asexuality

It has been estimated that a surprisingly high proportion, as many as 20%, of all fungal species exhibit a purely asexual lifestyle with no sexual state having been described (Hawksworth et al. 1995). Phylogenetic analysis has shown that almost all species that were once classified in the subdivision Deuteromycotina (the so-called 'Fungi Imperfecti') are actually placed within the sexual Pezizomycotina grouping (Schoch et al. 2009). Indeed, taxonomic changes under the 'one fungus, one name' proposal might lead to supposedly asexual species being given genus epithets once reserved only for species with described sexual states (Hawksworth et al. 2011). **This lack of sexual reproduction in so many Pezizomycotina is very surprising given the many supposed benefits of sex in fungi, even if only intermittent and involving self-fertilisation (homothallism) [reviewed by Billiard et al. (2012), Dyer and Paoletti (2005) and Lee et al. (2010)].** Asexuality has also been viewed as an evolutionary dead end due to limited opportunities to generate genetic diversity and the problems associated with accumulation of deleterious mutations (the Muller's and Kondrashov's ratchet effects) (Lobuglio and Taylor 2002; Normark et al. 2003). **However, there has been accumulating evidence over recent years that many Pezizomycotina thought to have a purely asexual lifestyle might in fact have the ability to undergo sexual reproduction. Thus, they are described as exhibiting 'cryptic sexuality' (Dyer and O'Gorman 2012; Ene and Bennett 2014; Kück and Pöggeler 2009), here defined as meaning that they have genetic, physiological and/or morphological characteristics associated specifically with sexual species, without a sexual state yet having been described.** Therefore, these species can be said to show signs of sexuality, indicating that there might be possible occurrence in nature of an as yet unobserved and undescribed sexual state that would contribute to the generation of genetic diversity and evolution of the species in question. **Various criteria (sex tests) have**

been used to provide evidence of cryptic sexuality in supposed asexual species (Dyer and O'Gorman 2011; Varga et al. 2014). These include the detection of recombination from population genetic analyses, observation of the initial morphological stages of sexual development, identification of genes required for sexual reproduction in the genome and demonstration of functional expression of such sex-related genes. **Of relevance to the current chapter is that the analysis of the presence and distribution of mating-type genes has become the most commonly used method to gain insights into cryptic sexuality**, with a number of discoveries made about asexual lifestyles in the Pezizomycotina based on *MAT* gene studies as follows.

b) Presence of Impaired Mating-Type Genes

It is generally accepted that asexual Pezizomycotina is derived from earlier sexual ancestor lineages (Geiser et al. 1996; Taylor et al. 1999). **One possible explanation for the evolution of asexuality is that *MAT* genes regulating key sexual developmental processes might have become mutated and therefore impaired in function, thus leading to an asexual lifestyle.** There is evidence of such mutation of mating-type genes in the asexual yeast *Candida parapsilosis* (Logue et al. 2005). However, in all studies to date where *MAT* regions have been sequenced from asexual Pezizomycotina, there has been no such evidence for such mutations, with all sequenced genes appearing to code for intact, functional MAT proteins (see below). The only possible exception was with the phytopathogen *Phoma clematidina* in which the majority of isolates sampled failed to amplify a *MAT* gene product, and it was speculated that corruption of *MAT* genes might be the cause of asexuality (Woudenberg et al. 2011). However, the *MAT* regions were not sequenced to test this hypothesis.

c) Presence of Only One Mating-Type Gene in Populations

An alternative explanation for asexuality in certain species is that they have evolved from a heterothallic ancestor(s) of a single mating type, and therefore extant populations will be composed of isolates harbouring only one type of *MAT* gene. Consequently, the species would be obligately asexual even if the genome contained all genes, except that of the compatible mating type, for sexual development. There is evidence for such an explanation for an asexual lifestyle in certain of the Pezizomycotina based on studies of the presence and distribution of *MAT* genes. King et al. found that all sampled isolates of the phytopathogens *Rhynchosporium orthosporum* and *Rhynchosporium lolii* from a diverse range of hosts and geographical locations throughout Europe were exclusively of the *MAT1-1* genotype (King et al. 2014). This finding was confirmed by BLAST analyses of draft genome sequences of these species, which failed to detect the presence of any *MAT1-2-1* gene homologue. Furthermore, it was not possible to detect expression of the *MAT1-1-1* gene under laboratory conditions. Similarly, Christiansen et al. found that surveyed isolates of the oat pathogen *Cochliobolus victoriae* were entirely of the *MAT1-2* genotype and suggested that this species had arisen by horizontal gene transfer of DNA conferring pathogenicity on oats into a single female sterile *MAT1-2* ancestral strain of *Cochliobolus carbonum* (Christiansen et al. 1998). Groenewald et al. also reported that all worldwide isolate samples of *Cercospora apicola* were of the *MAT1-2* genotype, while all German isolates of *Cercospora apii* were of the *MAT1-1* genotype, both of these asexual species being pathogenic on celery (Groenewald et al. 2006). Most recently, Brännström et al. found that a sample of 218 individuals of the lichen-forming fungus *Thamnolia vermicularis* covering the whole of the northern, and parts of the southern, hemisphere were entirely of the *MAT1-2* genotype (Brännström et al. 2015). Further examples of the possible presence of only one mating type in populations leading to asexuality include various *Fusarium* and *Penicillium* species (Covert et al. 2007; Kerenyi et al. 2004; Lopez-Villavicencio et al. 2010), the antibiotic producer *Acremonium chrysogenum* (Pöggeler et al. 2008) and the ray blight fungus *Stagonosporopsis tanaceti* (Chilvers et al. 2014), but in these cases, sampling only covered a limited number of isolates or a limited geographic area

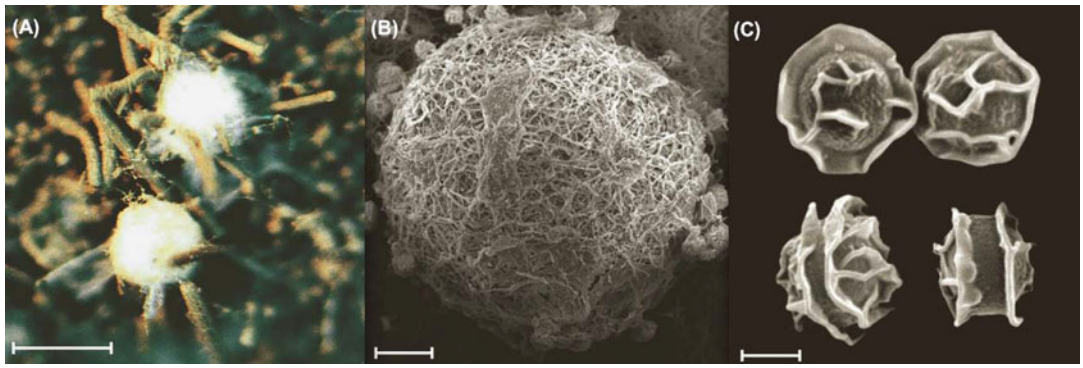


Fig. 14.5 Newly discovered sexual state of *Aspergillus fumigatus* (*Neosartorya fumigata*). (**a**) Two cleistothecia visible as white to cream hyphal aggregations against *darker green* conidia in the background; scale bar indicates 400 μm. (**b**) Scanning electron micrograph of a cleistothecium, showing the outer peridium wall of interwoven hyphae; scale bar indicates 100 μm. (**c**) Scanning electron micrograph of four ascospores showing spore ornamentation; scale bar indicates 2 μm. Adapted from O'Gorman et al. (2009)

so the results are less conclusive. Intriguingly, some yeasts exhibit unisexual reproduction between partners of the same mating type as a solution to same-sex incompatibility (Ene and Bennett 2014; Heitman et al. 2014), but such a phenomenon has not yet been described in filamentous ascomycetes other than with mating-type switching (see Sect. 3).

d) Presence of Complementary Mating-Type Genes Leads to Discovery of Sex

Arguably, the greatest insights into cryptic sexuality have arisen from studies where detection of both *MAT1-1* and *MAT1-2* isolates within global populations has ultimately led to the discovery of a previously unknown sexual stage. An understanding of *MAT* gene presence has been critical in such studies because a knowledge of the mating-type identity of strains has allowed directed crosses to be set up between known *MAT1-1* and *MAT1-2* isolates with potential sexual compatibility, whereas prior to this, many wasteful attempted crosses might have been tried with isolates that were of the same mating type, i.e. were sexually incompatible (Houbraken and Dyer 2015). A sexual revolution based on *MAT* gene analysis has been seen particularly regarding supposed asexual *Aspergillus* and *Penicillium* species (Dyer and O'Gorman 2011). For example, the opportunistic human pathogen *Aspergillus fumigatus* was long considered a purely asexual organism. However, genome analysis combined with experimental work demonstrated the presence of both *MAT1-1* and *MAT1-2* isolates within global populations in an approximately 1:1 ratio together with evidence for expression of *MAT1-1* and *MAT1-2* genes in vitro, consistent with sexual reproduction (Galagan et al. 2005; Paoletti et al. 2005). O'Gorman et al. later identified a recombinant population in Ireland and set up crosses between *MAT1-1* and *MAT1-2* Irish isolates under a variety of conditions (O'Gorman et al. 2009). A major finding was then made, being that prolonged incubation on a particular oatmeal substrate led to induction for the first observed time of sexual reproduction in the species with the newly identified sexual state being named *Neosartorya fumigata* (Fig. 14.5). Using a similar approach, Ramirez-Prado et al. were first able to demonstrate that the asexual species *Aspergillus flavus* and *Aspergillus parasiticus* both contained an idiomorphic *MAT* locus arrangement characteristic of heterothallic sexual species and that *MAT1-1* and *MAT1-2* genes were expressed in the species under laboratory conditions (Ramirez-Prado et al. 2008). In subsequent studies crossing known *MAT1-1* and *MAT1-2* isolates, Horn et al. were then able to induce sexual reproduction for the first observed time in these species, involving the production of a *Petromyces* sexual state (Horn et al. 2009a, b). Böhm et al. used similar methodology to induce sexual reproduction in the

antibiotic producer *P. chrysogenum* for the first time (Böhm et al. 2013). A *MAT* locus had previously been identified in *P. chrysogenum* (Henk et al. 2011; Hoff et al. 2008), and crossing of certain *MAT1-1* and *MAT1-2* isolates was found to lead to production of cleistothecia containing recombinant progeny. This finding was of industrial importance because the sexual cycle now offers a novel means of strain improvement. Further examples where a knowledge of *MAT* gene presence (from PCR amplification and sequencing work) has recently allowed sexual states to be identified for the first time include the aflatoxin producer *Aspergillus nomius* (Horn et al. 2011), the opportunistic pathogens *Aspergillus lentulus* and *Aspergillus terreus* (Arabatzis and Velegraki 2013; Swilaiman et al. 2013), two members of the black aspergilli *Aspergillus tubingensis* and *Aspergillus sclerotiicarbonarius* (Darbyshir et al. 2013; Horn et al. 2013), the emerging pathogens *Aspergillus felis* and *Fusarium keratoplasticum* (Barrs et al. 2013; Short et al. 2013) and *Aspergillus wyomingensis* (Nováková et al. 2014). All of these examples illustrate that many fungal species currently considered to be asexual are instead likely to exhibit cryptic sexuality. There is therefore the possibility of identifying a sexual stage if conditions to trigger sex can be identified, with the analysis of *MAT* gene presence and their use in identifying compatible mating partners being a key first step in such investigations (Houbraken and Dyer 2015).

e) Presence of Both Mating Types but No Sexual State Yet Identified

Although it has been possible to induce a sexual cycle in some previously considered asexual species, **the majority of studies to date involving the analysis of *MAT* organisation and the presence in the asexual Pezizomycotina have so far only been able to provide evidence of cryptic sexuality**. Numerous studies have shown that taxonomically diverse asexual species contain apparently functional *MAT* genes (based on sequence analysis) which are located in *MAT* idiomorphic regions whose structure resembles those of known sexual heterothallic species. There have also been a series of reports that *MAT* genes are expressed at the RNA level, and some studies have even shown evidence of functionality based on gene manipulation work. Furthermore, studies of field distributions of the complementary mating types have often shown a near 1:1 ratio of *MAT1-1*:*MAT1-2* isolates, consistent with sexual reproduction and data for known sexual species (Dyer et al. 2001; Waalwijk et al. 2002).

Debuchy and Turgeon previously described how either *MAT* genes alone or idiomorph regions have been sequenced from a range of asexual loculoascomycete, sordariomycete, leotiomycete and eurotiomycete species (Debuchy and Turgeon 2006). Where complete idiomorphs were sequenced, asexual species such as *Fusarium oxysporum*, for example, were found to contain *MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3* and *MAT1-2-1* genes that were structurally indistinguishable from those of related *Gibberella* sexual species (Yun et al. 2000). Furthermore, Debuchy and Turgeon described how for most asexual species where gene expression was assayed, such as *Bipolaris sacchari* and *Alternaria alternata* (Arie et al. 2000), it was possible to detect transcription of *MAT* genes, importantly suggesting that the asexual lifestyle does not arise from a transcriptional deficiency of these genes. Since then, various other studies have yielded similar data. Complementary *MAT1-1* and *MAT1-2* genes have been identified from asexual *Cercospora*, *Penicillium* and *Fusarium* species (Groenewald et al. 2006; Henk and Fisher 2011; Hughes et al. 2014; Lopez-Villavicencio et al. 2010), and diagnostic tests have shown a near 1:1 ratio of both mating types in field populations in most examples. Similarly, complete *MAT1-1* and *MAT1-2* genes have also been identified from asexual *Aspergillus*, *Rhynchosporium* and *Ulocladium* species, and in these instances, evidence of transcription of the *MAT* genes was also provided (Geng et al. 2014; King et al. 2014; Wada et al. 2012). **Houbraken and Dyer suggested that the term 'proto-heterothallic' be used to describe such asexual species where genetic evidence, such as the presence of complementary *MAT* loci, indicates the presence of a sexual cycle to distinguish these species from heterothallic**

species with an already described sexual state (Houbraken and Dyer 2015).

Interestingly, it is noted that investigations of *MAT* gene occurrence have so far failed to reveal any asexual species with both *MAT1-1* and *MAT1-2* family genes in the same genome, i.e. a homothallic arrangement of *MAT* genes. Given that homothallism seems to be a derived state from heterothallism as a result of evolutionary selection to facilitate sexual spore production (see Sect. II.B.2) (Billiard et al. 2012; Murtagh et al. 2000), it is conceivable that the same selective pressures would act to maintain sexual reproduction in homothallic species and therefore asexual species are unlikely to be derived from homothallic ones. But if such a homothallic mating-type organisation of *MAT* genes was found in a species lacking a sexual state, then the term 'proto-homothallic' could be applied (Houbraken and Dyer 2015).

f) Functionality of *MAT* Genes from Asexual Species

In a limited number of studies, evidence of the functionality of *MAT* genes from asexual species has also been provided. Arie et al. demonstrated that *MAT* genes from *A. alternata* were able to regulate sexual development by heterologous expression in a close sexual relative *C. heterostrophus*, where both *MAT1-1* and *MAT1-2* genes restored sexual fertility to a *MAT* null strain (Arie et al. 2000). Hornok's lab compared gene expression of a wild-type *MAT1-2* isolate of *F. verticillioides* versus a Δ*MAT1-2* gene deletant strain and found differential expression of 216 genes, providing evidence that the *MAT1-2* gene was acting as a transcription factor (Keszthelyi et al. 2007; Waalwijk et al. 2006). Pöggeler et al. showed that *A. chrysogenum* contains transcribed *MAT1-1* family genes and that when the *MAT1-1-1* gene was heterologously expressed in a *MAT* deletant strain of *P. anserina*, it was able to restore sexual fertility (Pöggeler et al. 2008). Most recently, Wada et al. demonstrated the presence of both *MAT1-1* and *MAT1-2* idiomorphs in *Aspergillus oryzae*, that both *MAT1-1* and *MAT1-2* genotypes were present in a large sampling of industrial strains of the fungus and that the *MAT* genes were expressed under in vitro conditions (Wada et al. 2012). They then created *MAT1-1* and *MAT1-2* isogenic strains and used DNA microarray experiments to show 33 genes were differentially regulated over tenfold between the different mating types. These included a pheromone precursor gene, indicating that the *MAT* genes were indeed functionally regulating cellular processes related to sexual development.

g) Future Prospects

Taken as a whole, studies using *MAT* genes to investigate the asexual lifestyle have in most cases revealed evidence of cryptic sexuality in the species involved and in a growing number of examples have led to induction of a complete sexual cycle. This indicates that perhaps the majority of the 'asexual' Pezizomycotina has the potential for sexual reproduction. However, it is cautioned that some species may prove to be genuinely asexual, especially given that there are some long-term evolutionary benefits to asexuality (Normark et al. 2003). Extensive attempts to induce sexual reproduction between *MAT1-1* and *MAT1-2* isolates of a series of asexual *Penicillium*, *Fusarium* and *Aspergillus* species have failed despite using environmental conditions and crossing protocols that have triggered sex in closely related species (P. S. Dyer and K. Kitamoto, unpublished data) (Hughes et al. 2014; Lopez-Villavicencio et al. 2010). Meanwhile, genetic manipulation of *MAT* genes has so far failed to induce sexual reproduction in asexual species. For example, expression of the *C. heterostrophus MAT1-1* and *MAT1-2* genes failed to induce a sexual cycle in the related asexual *B. sacchari* (Sharon et al. 1996). Thus, asexuality might be due to mutation in genes other than the *MAT* genes required for sexual reproduction. Although this review focuses on the role of *MAT* genes, it is noted that over 70 other genes have so far been characterised which are also required for normal sexual development (Dyer and O'Gorman 2012). Therefore, it is important to consider *MAT* genes as just one part, albeit a very significant one, of the overall sexual developmental machinery. It is also conceivable that *MAT* genes might have been subverted to control other cellular processes in asexual fungi,

although there is no evidence to date of this occurrence in filamentous fungi.

III. Mating-Type Circuits (Functions and Regulation) and Evolution

A. Regulation of Mating-Type Genes

1. Budding Yeast

The *S. cerevisiae* system comprises haploid ***a*** and ***α*** cells that mate spontaneously even on rich medium, which is generally considered as repressing sexual reproduction. This observation suggests that mating-type genes are constitutively transcribed. No significant transcription activation is observed for mating-type genes in medium deprived of nitrogen (Adomas et al. 2010; Gasch et al. 2000), a condition that typically induces sexual reproduction. There is also no induction of mating-type gene transcription in ***a*** and α cells after exposure to pheromones (Roberts et al. 2000). **Taken together, these observations confirm that mating-type genes are constitutively expressed in *S. cerevisiae*, although this expression can be modulated to some extent.** For example, GAT3 and RTG3 were found to regulate the expression of *MAT**a**1* (Lee et al. 2002), and *MAT*α1 is cell cycle regulated (Spellman et al. 1998). It is not clear how these modulations are related to mating. In diploid ***a***/α cells, the transcription of the *MAT*α*1* activator gene is repressed by the **a**1/α2 heterodimer, which has a binding site in the *MAT*α*1* promoter (Galgoczy et al. 2004).

2. Fission Yeast

The mating types of *S. pombe* are termed plus (P) and minus (M), with sexual identity determined by genes present at a single *MAT* locus. Either *P* or *M* sequences are present, each encoding two proteins designated by P or M, and a suffix (c or m), in the respective mating types (Kelly et al. 1988). Pc and Mc are needed for conjugation and meiosis, and Pm and Mm are specifically involved in meiosis. Unlike *S. cerevisiae*, *S. pombe* does not mate spontaneously; the expression of *Pc* and *Mc* is regulated by the transcription factor SpSte11 (Sugimoto et al. 1991). Transcription of *SpSte11* and phosphorylation of its product are regulated by numerous signalling pathways, such as the nitrogen starvation and mating pheromone response pathways (Otsubo and Yamamoto 2013). The two mating-type genes specifically involved in meiosis are not directly controlled by SpSte11, but their expression requires a pheromone signal, which in turn is dependent on SpSte11, Mc and Pc (Willer et al. 1995). Pm and Mm are not sufficient for meiosis; SpSte11 controls the expression of *mei2*, an RNA-binding protein, and the transcription of its RNA cofactor, meiRNA (Mata and Bahler 2006), both of which promote meiosis (Watanabe and Yamamoto 1994). **SpSte11, therefore, appears to be the master regulator of conjugation and meiosis, acting upstream of mating-type genes**. This idea is supported by the ectopic expression of SpSte11, which leads to sexual differentiation irrespective of nutritional conditions (Sugimoto et al. 1991). SpSte11 should not be confused with its *S. cerevisiae* homonym, the STE11 mitogen-activated MAPKKK (Merlini et al. 2013); SpSte11 is an HMG-box transcription factor that belongs to the MATA_HMG-box subfamily and recognises a ten-base DNA motif, the TR-box (5′-TTCTTTGTTY-3′) (Sugimoto et al. 1991).

3. Pezizomycotina

A functional analysis of HMG-box genes in *P. anserina* indicated that an SpSte11 orthologue, PaSte11 (Pa_1_13940), controls the transcription of the mating-type genes that are essential for fertilisation (Ait Benkhali et al. 2013). It binds to a target DNA sequence (5′-TTTCTTTGTT-3′) that is very similar to the TR-box of SpSte11 (see above). **PaSte11 is at the hub of a network of several HMG-box factors involved in the control of the sexual cycle.** Although PaSte11 appears to be a pivotal regulator of the sexual cycle, similar to its orthologue in *S. pombe*, the overexpression of PaSte11 does not trigger sexual differentiation unlike SpSte11 where overexpression in *S. pombe* has this effect (Sugimoto et al. 1991). However, deregulation of

SpSte11 results in heterogeneous aberrant growth phenotypes, a feature also observed in *P. anserina* strains overexpressing PaSte11. It is likely that the control of sexual differentiation requires finely tuned Ste11 expression. **An exhaustive analysis of HGM-boxes in *P. anserina* revealed the critical role of this superfamily in sexual development: 11 of 12 HMG-box genes are involved in sexual reproduction in this fungus (Ait Benkhali et al. 2013), with similar results recently obtained with the homothallic *A. nidulans* (A. Ashour, N. Salih and P.S. Dyer, unpublished results).**

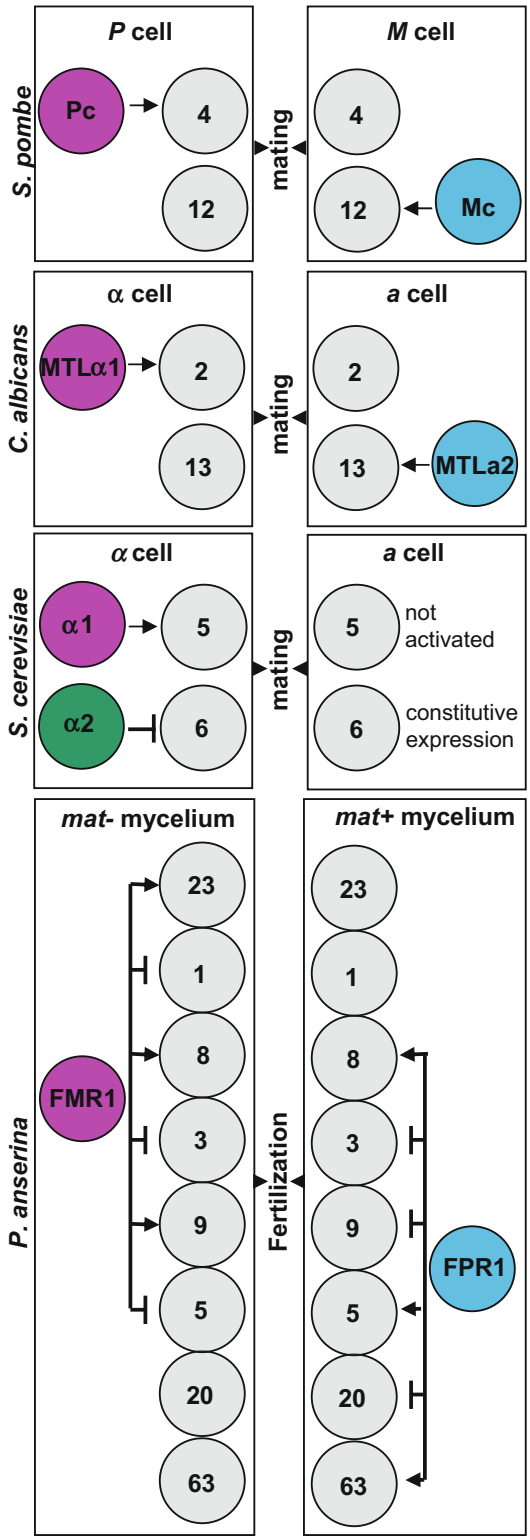
4. Evolution of Regulatory Circuits in Opisthokonta

***P. anserina* and *S. pombe* diverged 550 million years ago (Berbee and Taylor 2010). The functional conservation of PaSte11 and SpSte11 over such a prolonged time frame is a striking exception to the general observation that developmental pathways involved in sex are highly variable, even among closely related lineages.** A critical question is whether Ste11 regulates mating-type expression in other ascomycete species. FUNGIpath (Grossetete et al. 2010) failed to find an SpSte11 orthologue in *S. cerevisiae* or *Candida albicans* but identified one (YALI0D22660g) in *Yarrowia lipolytica*, which is a basal taxa within the Saccharomycotina lineage (Fitzpatrick et al. 2006; James et al. 2006). No functional analysis has determined whether this orthologue of SpSte11 regulates mating-type genes in *Y. lipolytica*. Although orthologues of PaSte11 are ubiquitous in the Pezizomycotina, evidence supporting their role in mating-type control is scarce. Functional analysis of transcription factors in the homothallic species *Fusarium graminearum* hints at such a role for Ste11 (*FGSG_01366*). In *F. graminearum*, the deletion of *FGSG_01366* recapitulates the phenotype of the deletion of mating-type genes (Son et al. 2011). This observation strongly suggests that FGSG_01366 also controls the expression of mating-type genes. *N. crassa* has an orthologue of PaSte11 (NCU09387) and one paralogue (NCU02326) (Ait Benkhali et al. 2013; Iyer et al. 2009). *NCU09387* mutations result in strains that can mate, but fruit body development is blocked before ascospore formation (Johnson 1979). The function of *NCU02326* has not yet been investigated. The transcription of these two genes during sexual development was investigated (Wang et al. 2014). *NCU02326* expression decreased after crossing and was maintained at a low level during perithecial development, while *NCU09387* expression was upregulated after crossing. These expression patterns suggest that NCU02326 and NCU09387 regulate the expression of mating-type genes during early and mid-early sexual development, respectively.

HMG-box genes also play a critical role in vertebrate sex determination. *Sry*, the mammalian Y-chromosomal testis-determining HMG-box gene, is an activator of *Sox9* (Koopman 2010). *Sox9* is also conserved among non-mammalian vertebrate species and has an ancestral and pivotal role in sex determination (Koopman 2010). The *P. anserina* and *S. pombe* mating-type genes and their upstream regulatory factor form a module of HMG-box genes that is similar to the *Sry*/*Sox9* module. SRY/SOX9 and the fungal mating-type proteins belong to different HMG-box subfamilies (Soullier et al. 1999) and are therefore not orthologues, suggesting that sex regulation in fungi and animals is analogous. **Nevertheless, an ancestral origin of sex regulation in Opisthokonta was proposed based on functional analyses (Czaja et al. 2014).** A hybrid MAT1-1-1 protein carrying the SRY HMG-box is able to drive a complete sexual cycle in *A. nidulans*. Although functional conservation is not a conclusive argument for common ancestry, a phylogenetic analysis supports the possible ancestral origin of sex regulation in Opisthokonta: the mating-type protein of an early diverged fungus is classified within the SRY/SOX9 subfamily (Ait Benkhali et al. 2013; Martin et al. 2010). The discovery of a mating-type regulator that belongs to the SRY/SOX9 subfamily in this fungus would provide conclusive evidence for an evolutionary link between the fungal *MAT* locus and animal sex regulation.

B. Regulation and Diversity of Mating-Type Target Genes

Given that mating-type genes are defined by their role in determining sexual identity, it



might be envisaged that the main target genes regulated by *MAT* genes would be those involved directly in mating processes, for example, genes involved with pheromone signalling and cell fusion. **However, there is evidence from recent studies from the Pezizomycotina indicating that a remarkably high number of genes can be regulated by *MAT* genes and that relatively complex regulatory circuits are present compared to the relatively few genes known to be regulated from yeast mating systems, as described below**. Indeed, as mating-type genes are also implicated in later stages of sexual development, *MAT* genes will undoubtedly regulate a wider diversity of target genes. However, we limit our review to the genes involved in mating and fertilisation because experimental studies have not yet been conducted to identify *MAT*-regulated target genes required for post-fertilisation functions in the Pezizomycotina.

The discovery of a potential sexual cycle in *C. albicans* (Hull et al. 2000; Magee and Magee 2000) prompted a dissection of the cell type-specification circuit in this yeast. The functions of the mating-type transcription factors are relatively straightforward. MATα1 and MAT**a**2 activate α− and **a**-specific genes, respectively (Fig. 14.6) (Scannell and Wolfe 2004). There are 15 target cell-specific genes that encode pheromones and their cell surface receptors as well as the factors required for processing and exporting pheromone (Tsong et al. 2003). The *C. albicans* mating-type circuit is considered to represent the ancestral paradigm. By contrast, the *S. cerevisiae* lineage has undergone further evolution involving the loss of the *MATa2* gene

Fig. 14.6 Cell-type regulation and target gene numbers in *Schizosaccharomyces pombe*, *Candida albicans*, *Saccharomyces cerevisiae* and *Podospora anserina*. Mating-type transcription factors are enclosed in *coloured circles*: *magenta*, α1 domain; *cyan*, MATA_HMG-box; and *green*, homeodomain. *Grey circles* indicate different types of target genes, and enclosed figures indicate their sample size. Mating-type transcription factors are specific to each cell type, whereas mating-type target genes are present in both cell types. *Arrows* with *heads* and *blunt ends* indicate activation and repression, respectively. Adapted from Bidard et al. (2011)

and consequent specific rewiring of the regulatory circuits. This rewiring of the *MAT* locus has resulted in the constitutive expression of **a**-specific genes in **a**-cells, while in α-cells, MATα2 represses the **a**-specific genes (Fig. 14.6) (Scannell and Wolfe 2004). As in *C. albicans*, the MATα1 transcription factor activates α-specific genes. **Among the 11 mating-type target genes, ten are well known for their role in mating, while the other has no obvious effect in this process.**

In *S. pombe*, the mating-type regulators Mc and Pc appear to be activators of M- and P-specific genes, respectively (Fig. 14.6). The induction of 12 M-specific genes and 4 P-specific genes by SpSte11 overexpression was cell type dependent (Mata and Bahler 2006). Note this strategy precludes the identification of genes that are repressed by Mc and Pc. **Among these 16 cell type-specific genes, three proved to be dispensable for mating and two were essential for viability.**

The first large-scale analysis of mating-type target genes in a filamentous fungus was reported by Hornok's lab in *Fusarium verticillioides* (Keszthelyi et al. 2007; Waalwijk et al. 2006). They identified a total of 216 target genes of MAT1-2-1 by screening 7680 ESTs on a macroarray. Among the 216 target genes, 120 were activated by MAT1-2-1 and 96 were repressed. **As *F. verticillioides* was supposedly asexual, the authors assumed that the mating-type genes were conserved in this fungus owing to their involvement in general metabolism, as evidenced by the large number of targets not directly involved in the mating process.** A later study using microarray analysis in the asexual *A. oryzae* also revealed that 1,155 genes were differentially regulated by *MAT1-1* and *MAT1-2*, with 33 genes showing a greater than tenfold difference in expression according to whether these were in a *MAT1-1* or *MAT1-2* genetic background (Wada et al. 2012). The target genes included a pheromone precursor gene, but most genes were of unknown function. **Surprisingly, further identification of mating-type target genes in sexual fungi such as *S. macrospora* (Klix et al. 2010; Pöggeler et al. 2006), *P. anserina* (Bidard et al. 2011) and *N. crassa* (Wang et al. 2012) revealed that these transcription factors control a very large number of genes not directly involved in the mating process.** In *P. anserina*, 157 genes are differentially transcribed in opposite mating types. Only a few of these genes are directly involved in mating. The transcriptome profiling of mating-type mutants indicated that MAT1-1-1 (also called FMR1) and MAT1-2-1 (also called FPR1) have activating and repressing functions, and all combinations of these two effects were observed on the target genes (Bidard et al. 2011) (Fig. 14.6). An additional layer of complexity was revealed by an interspecific comparison of mating-type-regulated genes (Bidard et al. 2011). The search for common target genes revealed a significant number of orthologous pairs in the comparisons between *P. anserina* and *F. verticillioides* or *S. macrospora*. Orthologous pairs of genes that are essential for mating typically have similar expression patterns across species. For instance, the transcription of genes encoding lipophilic pheromones is activated by FPR1 in *P. anserina* and MAT1-2-1 (also called mat a-1) in *N. crassa*. Similarly, the genes encoding hydrophilic pheromones are activated by FMR1 in *P. anserina* and MAT1-1-1 (also called mat A-1) in *N. crassa*. **Most of the orthologous pairs obtained from interspecific comparisons did not display conserved transcriptional profiles.** For example, a gene from one pair is activated in a given species and repressed in another one. This surprising observation indicates that the binding site is conserved, but some yet unknown factor determines the transcriptional pattern of the gene. The most recent studies in this area have concerned studies to identify genes regulated by the *MAT* genes of *P. chrysogenum*. It was shown that the *MAT1-2-1* gene was required for sexual development but in addition regulated light-dependent asexual sporulation, agglutination of conidiospores and aspects of secondary metabolism with relevance to biotechnology and influenced pellet morphology in submerged culture (Böhm et al. 2013, 2015). In a pioneering study, ChIP-seq was then used to identify target genes of the α-domain *MAT1-1-1* gene. A total of 254 genes were identified, with the vast majority of these genes found to have functions beyond sexual reproduction, such as an involvement with asexual sporulation and amino acid, iron

and secondary metabolism. Most significantly, further bioinformatic and experimental analysis led to the identification of a MAT1-1-1 binding motif (CTATTGAG) which showed a high degree of conservation within the Pezizomycotina, suggesting that MAT1-1-1 acts as master switch for development and metabolism in this fungal group (Becker et al. 2015).

Evidence to date has therefore shown that most mating-type target genes have putative functions that are not suggestive of a direct role in the mating process. Nevertheless, a possible involvement of *MAT* genes in regulating aspects of sexual reproduction was tested by deletion analysis in *A. nidulans* and *P. anserina*. **Orthologues of the mating-type target genes identified in *A. oryzae* (Wada et al. 2012) were deleted in *A. nidulans* (Salih and Dyer 2015; Salih et al. 2015). A range of effects was observed, from no obvious impact, to moderate loss or gain of fertility, to complete loss of sexuality.** By contrast, the deletion of 29 mating-type target genes in *P. anserina* did not affect mating and fertility (Bidard et al. 2011). However, the experiments performed in *A. nidulans* indicate that detection of loss and gain of fertility requires careful examination and slight, but significant, changes in levels of fertility may have been overlooked in *P. anserina*. To explain the numerous and diverse genes controlled by the mating-type transcription factors, we suggest that some of these might originate from the 'spurious' transcription factor binding sites that have been reported to occur throughout the genomes of various species (Spivakov 2014). The binding of transcription factors to these spurious binding sites typically occurs without transcription. In fungi with short intergenic sequences, e.g. ~ 2.8 kb in *P. anserina* (Silar et al. 2003), spurious binding could not occur outside gene regulatory regions and, thus, may affect the expression of various genes. Some of these genes may be unrelated to fertility, while the expression of others may affect the sexual process. Selection then may finely tune the expression of this latter category to optimise the sexual process according to the lifestyle and genomic context of the species.

In addition to acting alone to regulate developmental processes, one final issue is to what degree the MAT1-1 and MAT1-2 proteins might physically interact with other sex-related proteins to mediate gene transcription. It is known from the *S. cerevisiae* yeast model, for example, that MAT1-1 alpha domain protein forms a complex with the MCM1 and STE12 proteins to activate α1-domain-specific genes (Debuchy et al. 2010). Similar physical interactions between an MCM1 homologue and MAT1-1-1 and an STE12 homologue of *S. macrospora* have also been described (Nolting and Pöggeler 2006a, 2006b), suggesting a similar interaction throughout the Pezizomycotina. In addition, an interaction between the SMTA-1 and SMTa-1 proteins of *S. macrospora* has been reported (Jacobsen et al. 2002). In *G. zeae*, a direct interaction between MAT1-1-1 and MAT1-2-1 was not observed, but MAT1-1-2 interacts with all the other MAT transcription factors, which may bring MAT1-1-1 and MAT1-2-1 together (Zheng et al. 2013). Intriguingly, this suggests that MAT proteins of homothallic species might form such heterodimers in the dikaryotic ascogenous hyphae to control gene expression linked to further sexual development. This would be analogous to the formation of the MATα2-MAT**a**1 heterodimer of *S. cerevisiae*, which indirectly activates meiosis by repressing haploid-specific genes. However, no interaction between the FPR1 and FMR1 proteins of *P. anserina* could be detected via yeast two-hybrid studies, although an interaction between the FMR1 and SMR2 proteins was observed (Coppin et al. 1997). The MAT A-1 and MAT a-1 proteins of *N. crassa* have been found to interact in the yeast two-hybrid assay (Badgett and Staben 1999). However, mutations that interfere with the interaction eliminate vegetative incompatibility but not mating, suggesting that this interaction is not essential for the sexual cycle.

IV. Conclusion

Significant progress has been made since the previous edition of *The Mycota* published in 2006. The most conspicuous advances have concerned firstly our understanding of the evolution of mating-type regions in the fungal kingdom and secondly the identification of the

spectrum of target genes regulated by *MAT* genes.

The evolutionary trajectory of mating types can now be drawn as a continuous line of HMG-box gene history spanning from the early diverged fungi to the Pezizomycotina. By contrast, the relationship of the HMG-box mating-type genes with sex determination in vertebrates, and with mating types in the Basidiomycota, remains to be elucidated. Some commonalities point to a relationship between the sex determination mechanism in vertebrates, but the evidence for an ancestral origin of sex in the Opisthokonta is not conclusive. The acquisition of mating types in the Basidiomycota is not consistent with the continuous linear evolution of HMG-box genes. Understanding why and how HMG-box mating types in the Basidiomycota have been evicted by homeobox genes is critical to complete the history of mating-type evolution and may provide a clue to the yet unexplained presence of homeobox genes in yeast (Saccharomycotina and Taphrinomycotina) mating-type loci.

In the previous edition of *The Mycota*, the conclusion called for an effort to characterise the target genes of mating-type transcription factors. The use of microarray analyses, next-generation genome sequencing, RNA-seq and ChIP-seq technologies has now provided insights into this area and is continuing to do so. Transcriptome analyses have revealed a surprisingly high number and diversity of mating-type target genes and a complex nature of regulation. It is therefore perhaps ironic that mating-type genes are so named, given the emerging observation that probably the majority of genes regulated by *MAT* genes in the Pezizomycotina appear not to be directly related to sex and mating. Indeed, the fact that *MAT* genes appear to regulate processes as diverse as asexual sporulation and aspects of primary and secondary metabolism (Böhm et al. 2013, 2015; Becker et al. 2015) might explain the paradox why functional *MAT* genes are conserved and expressed in supposedly asexual species. Thus, in the future mating-type genes are likely to attract the attention of researchers with interests beyond those of the traditional areas of mating and sexual development.

Finally, concerning the specific topic of sexual development, we wish to highlight that analyses of *MAT* gene function have so far been limited to initial mating and fertilisation stages. Filamentous ascomycetes display novel developmental steps after fertilisation, including mitotic divisions of parental nuclei in a syncytium and formation of dikaryotic ascogenous hyphae. These specialised hyphae contain one nucleus from each parent. The mechanism for selection of the two parental nuclei from the syncytium is not yet known. Several models based on (i) nucleus identity determined by *MAT* genes (Arnaise et al. 1997; Papazian 1956), (ii) the formation of heterodimers between MAT proteins (Metzenberg and Glass 1990) and (iii) random distribution of nuclei to form biparental ascogenous hyphae and abortion of those hyphae that are not formed from one *MAT1-1* and one *MAT1-2* nucleus (Kronstad and Staben 1997) have been proposed, but genetic, transcriptomic and functional approaches are still needed to resolve these questions. The association between mating-type genes and processes that take place during syncytium and ascogenous hypha formation (e.g. repeat-induced point mutation and excision of repeated sequences) also needs investigation. The functional analysis of mating-type genes during fruit body development is certainly a field that would bring many exciting surprises. Investigations into the evolution of the developmental functions of mating-type genes from early diverged fungi to the Pezizomycotina and during the switch from heterothallism to homothallism are also likely to result in significant discoveries, as has already been illustrated in yeast species (Scannell and Wolfe 2004; Tsong et al. 2003, 2006, 2007; Tuch et al. 2008).

References

Adomas AB, Lopez-Giraldez F, Clark TA, Wang Z, Townsend JP (2010) Multi-targeted priming for genome-wide gene expression assays. BMC Genomics 11:477

Ait Benkhali J, Coppin E, Brun S, Peraza-Reyes L, Martin T, Dixelius C, Lazar N, van Tilbeurgh H, Debuchy R (2013) A network of HMG-box transcription factors regulates sexual cycle in the fungus *Podospora anserina*. PLoS Genet 9, e1003642

Amselem J, Cuomo CA, van Kan JAL, Viaud M, Benito EP, Couloux A, Coutinho PM, de Vries RP, Dyer PS, Fillinger S, Fournier E, Gout L, Hahn M, Kohn L, Lapalu N, Plummer KM, Pradier J-M, Quévillon E, Sharon A, Simon A, ten Have A, Tudzynski B, Tudzynski P, Wincker P, Andrew M, Vr A, Beever RE, Beffa R, Benoit I, Bouzid O, Brault B, Chen Z, Choquer M, Jr C, Cotton P, Danchin EG, Da Silva C, Al G, Giraud C, Giraud T, Gonzalez C, Grossetete S, Güldener U, Henrissat B, Howlett BJ, Kodira C, Kretschmer M, Lappartient A, Leroch M, Levis C, Mauceli E, Cc N, Oeser B, Pearson M, Poulain J, Poussereau N, Quesneville H, Rascle C, Schumacher J, Ba S, Sexton A, Silva E, Sirven C, Soanes DM, Talbot NJ, Templeton M, Yandava C, Yarden O, Zeng Q, Rollins JA, Lebrun M-H, Dickman M (2011) Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genet 7, e1002230

Arabatzis M, Velegraki A (2013) Sexual reproduction in the opportunistic human pathogen *Aspergillus terreus*. Mycologia 105:71–79

Arie T, Kaneko I, Yoshida T, Noguchi M, Nomura Y, Yamaguchi I (2000) Mating-type genes from asexual phytopathogenic ascomycetes *Fusarium oxysporum* and *Alternaria alternata*. Mol Plant Microbe Interact 13:1330–1339

Arnaise S, Debuchy R, Picard M (1997) What is a *bona fide* mating-type gene? Internuclear complementation of *mat* mutants in *Podospora anserina*. Mol Gen Genet 256:169–178

Arnaise S, Zickler D, Le Bilcot S, Poisier C, Debuchy R (2001) Mutations in mating-type genes of the heterothallic fungus *Podospora anserina* lead to self-fertility. Genetics 159:545–556

Badgett TC, Staben C (1999) Interaction between and transactivation by mating type polypeptides of *Neurospora crassa*. Fungal Genet Newslett. Suppl 42: Abstract #127

Barrs VR, van Doorn TM, Houbraken J, Kidd SE, Martin P, Pinheiro MD, Richardson M, Varga J, Samson RA (2013) *Aspergillus felis* sp. nov., an emerging agent of invasive aspergillosis in humans, cats, and dogs. PLoS One 8, e64871

Becker K, Beer C, Freitag M, Kuck U (2015) Genome-wide identification of target genes of a mating-type α-domain transcription factor reveals functions beyond sexual development. Mol Microbiol 96:1002–1022

Berbee ML, Taylor JW (2010) Dating the molecular clock in fungi—how close are we? Fungal Biol Rev 24:1–16

Bergero R, Charlesworth D (2009) The evolution of restricted recombination in sex chromosomes. Trends Ecol Evol 24:94–102

Bidard F, Ait Benkhali J, Coppin E, Imbeaud S, Grognet P, Delacroix H, Debuchy R (2011) Genome-wide gene expression profiling of fertilization competent mycelium in opposite mating types in the heterothallic fungus *Podospora anserina*. PLoS One 6, e21476

Bihon W, Wingfield MJ, Slippers B, Duong TA, Wingfield BD (2014) *MAT* gene idiomorphs suggest a heterothallic sexual cycle in a predominantly asexual and important pine pathogen. Fungal Genet Biol 62:55–61

Billiard S, Lopez-Villavicencio M, Hood ME, Giraud T (2012) Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J Evol Biol 25:1020–1038

Böhm J, Hoff B, O'Gorman CM, Wolfers S, Klix V, Binger D, Zadra I, Kurnsteiner H, Poggeler S, Dyer PS, Kück U (2013) Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus *Penicillium chrysogenum*. Proc Natl Acad Sci USA 110:1476–1481

Böhm J, Dahlmann TA, Gümüşer KU (2015) A *MAT1-2* wild-type strain from *Penicillium chrysogenum*: functional mating-type locus characterization, genome sequencing and mating with an industrial penicillin-producing strain. Mol Microbiol 95:859–874

Bouhouche K, Zickler D, Debuchy R, Arnaise S (2004) Altering a gene involved in nuclear distribution increases the repeat-induced point mutation process in the fungus *Podospora anserina*. Genetics 167:151–159

Brännström IO, Ament SL, Spribille T, Scofield DG, Johannesson H (2015) Constraints on sex by a single mating-type: a case study from lichenized fungi. Fungal Genet Reports 60(Suppl): Abstract p40

Brefort T, Muller P, Kahmann R (2005) The high-mobility-group domain transcription factor Rop1 is a direct regulator of *prf1* in *Ustilago maydis*. Eukaryot Cell 4:379–391

Butler G (2010) Fungal sex and pathogenesis. Clin Microbiol Rev 23:140–159

Charlesworth D, Charlesworth B, Marais G (2005) Steps in the evolution of heteromorphic sex chromosomes. Heredity (Edinb) 95:118–128

Chilvers MI, Jones S, Meleca J, Peever TL, Pethybridge SJ, Hay FS (2014) Characterization of mating type genes supports the hypothesis that *Stagonosporopsis chrysanthemi* is homothallic and provides evidence that *Stagonosporopsis tanaceti* is heterothallic. Curr Genet 60:295–302

Chitrampalam P, Inderbitzin P, Maruthachalam K, Wu BM, Subbarao KV (2013) The *Sclerotinia sclerotiorum* mating type locus (*MAT*) contains a 3.6-kb region that is inverted in every meiotic generation. PLoS One 8, e56895

Christiansen SK, Wirsel S, Yun SH, Yoder OC, Turgeon BG (1998) The two *Cochliobolus* mating type genes are conserved among species but one is missing in *C. victoriae*. Mycol Res 102:919–929

Contamine V, Zickler D, Picard M (2004) The *Podospora rmp1* gene implicated in nucleus-mitochondria cross-talk encodes an essential pro-

tein whose subcellular location is developmentally regulated. Genetics 166:135–150

Coppin E, Arnaise S, Contamine V, Picard M (1993) Deletion of the mating-type sequences in *Podospora anserina* abolishes mating without affecting vegetative functions and sexual differentiation. Mol Gen Genet 241:409–414

Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61:411–428

Coppin-Raynal E, Picard M, Arnaise S (1989) Transformation by integration in *Podospora anserina*. III. Replacement of a chromosome segment by a two-step process. Mol Gen Genet 219:270–276

Covert SF, Aoki T, O'Donnell K, Starkey D, Holliday A, Geiser DM, Cheung F, Town C, Strom A, Juba J, Scandiani M, Yang XB (2007) Sexual reproduction in the soybean sudden death syndrome pathogen *Fusarium tucumaniae*. Fungal Genet Biol 44:799–807

Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14:1188–1190

Czaja W, Miller KY, Skinner MK, Miller BL (2014) Structural and functional conservation of fungal MatA and human SRY sex-determining proteins. Nat Commun 5:5434

Darbyshir HL, van den Vondervoort PJI, Dyer PS (2013) Discovery of a sexual reproduction in the black aspergilli. Fungal Genet Reports 60(Suppl): Abstract #687

Debuchy R, Turgeon BG (2006) Mating-type structure, evolution and function in Euascomycetes. In: Kües U, Fischer R (eds) The Mycota I. Growth, differentiation and sexuality, 2nd edn. Springer, Berlin, Heidelberg, pp 293–323

Debuchy R, Berteaux-Lecellier V, Silar P (2010) Mating systems and sexual morphogenesis in Ascomycetes. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Washington, DC, pp 501–535

Dodge BO (1942) Heterokaryotic vigor in *Neurospora*. BUll Torrey Bot Club 69:75–91

Dyer PS (2008) Evolutionary biology: genomic clues to original sex in fungi. Curr Biol 18:R207–R209

Dyer PS, O'Gorman CM (2011) A fungal sexual revolution: *Aspergillus* and *Penicillium* show the way. Curr Opin Microbiol 14:649–654

Dyer PS, O'Gorman CM (2012) Sexual development and cryptic sexuality in fungi: insights from *Aspergillus* species. FEMS Microbiol Rev 36:165–192

Dyer PS, Paoletti M (2005) Reproduction in *Aspergillus fumigatus*: sexuality in a supposedly asexual species? Med Mycol 43(Suppl 1):S7–S14

Dyer PS, Furneaux PA, Douhan G, Murray TD (2001) A multiplex PCR test for determination of mating type applied to the plant pathogens *Tapesia yallundae* and *Tapesia acuformis*. Fungal Genet Biol 33:173–180

Dyer PS, Paoletti M, Archer DB (2003) Genomics reveals sexual secrets of *Aspergillus*. Microbiology 149:2301–2303

Ellison CE, Stajich JE, Jacobson DJ, Natvig DO, Lapidus A, Foster B, Aerts A, Riley R, Lindquist EA, Grigoriev IV, Taylor JW (2011) Massive changes in genome architecture accompany the transition to self-fertility in the filamentous fungus *Neurospora tetrasperma*. Genetics 189:55–69

Ene IV, Bennett RJ (2014) The cryptic sexual strategies of human fungal pathogens. Nat Rev Microbiol 12:239–251

Faretra F, Pollastro S (1996) Genetic studies of the phytopathogenic fungus *Botryotinia fuckeliana* (*Botrytis cinerea*) by analysis of ordered tetrads. Mycol Res 100:620–624

Fedorova ND, Khaldi N, Joardar VS, Maiti R, Amedeo P, Anderson MJ, Crabtree J, Silva JC, Badger JH, Albarraq A, Angiuoli S, Bussey H, Bowyer P, Cotty PJ, Dyer PS, Egan A, Galens K, Fraser-Liggett CM, Haas BJ, Inman JM, Kent R, Lemieux S, Malavazi I, Orvis J, Roemer T, Ronning CM, Sundaram JP, Sutton G, Turner G, Venter JC, White OR, Whitty BR, Youngman P, Wolfe KH, Goldman GH, Wortman JR, Jiang B, Denning DW, Nierman WC (2008) Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*. PLoS Genet 4, e1000046

Fitzpatrick DA, Logue ME, Stajich JE, Butler G (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol Biol 6:99

Fraser JA, Stajich JE, Tarcha EJ, Cole GT, Inglis DO, Sil A, Heitman J (2007) Evolution of the mating type locus: insights gained from the dimorphic primary fungal pathogens *Histoplasma capsulatum*, *Coccidioides immitis*, and *Coccidioides posadasii*. Eukaryot Cell 6:622–629

Freihorst D, Fowler TJ, Bartholomew K, Marjatta R, Stephen Horton J, Kothe E (2016) The mating type genes of the basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 329–349

Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, Batzoglou S, Lee SI, Basturkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Scazzocchio C, Farman M, Butler J, Purcell S, Harris S, Braus GH, Draht O, Busch S, D'Enfert C, Bouchier C, Goldman GH, Bell-Pedersen D, Griffiths-Jones S, Doonan JH, Yu J, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Penalva MA, Oakley BR, Momany M, Tanaka T, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes M, Paoletti M, Fischer R, Miller B, Dyer P, Sachs MS, Osmani SA, Birren BW (2005) Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438:1105–1115

Galgoczy DJ, Cassidy-Stone A, Llinas M, O'Rourke SM, Herskowitz I, DeRisi JL, Johnson AD (2004) Genomic dissection of the cell-type-specification circuit in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 101:18069–18074

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11:4241–4257

Geiser DM, Timberlake WE, Arnold ML (1996) Loss of meiosis in *Aspergillus*. Mol Biol Evol 13:809–817

Geng Y, Li Z, Xia LY, Wang Q, Hu XM, Zhang XG (2014) Characterization and phylogenetic analysis of the mating-type loci in the asexual ascomycete genus *Ulocladium*. Mycologia 106:649–665

Glass NL, Grotelueschen J, Metzenberg RL (1990a) *Neurospora crassa A* mating-type region. Proc Natl Acad Sci USA 87:4912–4916

Glass NL, Metzenberg RL, Raju NB (1990b) Homothallic Sordariaceae from nature- the absence of strains containing only the a mating type sequence. Exp Mycol 14:274–289

Groenewald M, Groenewald JZ, Harrington TC, Abeln EC, Crous PW (2006) Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. Fungal Genet Biol 43:813–825

Grognet P, Bidard F, Kuchly C, Tong LC, Coppin E, Benkhali JA, Couloux A, Wincker P, Debuchy R, Silar P (2014) Maintaining two mating types: structure of the mating type locus and its role in heterokaryosis in *Podospora anserina*. Genetics 197:421–432

Grossetete S, Labedan B, Lespinet O (2010) FUNGIpath: a tool to assess fungal metabolic pathways predicted by orthology. BMC Genomics 11:81

Hagen DC, Bruhn L, Westby CA, Sprague GF Jr (1993) Transcription of alpha-specific genes in *Saccharomyces cerevisiae*: DNA sequence requirements for activity of the coregulator alpha 1. Mol Cell Biol 13:6866–6875

Hallatschek O, Nelson DR (2008) Gene surfing in expanding populations. Theor Popul Biol 73:158–170

Harrington TC, McNew DL (1997) Self-fertility and unidirectional mating-type switching in *Ceratocystis coerulescens*, a filamentous ascomycete. Curr Genet 32:52–59

Hartmann HA, Kahmann R, Bolker M (1996) The pheromone response factor coordinates filamentous growth and pathogenicity in *Ustilago maydis*. EMBO J 15:1632–1641

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) Ainsworth and Bisby's dictionary of the fungi, 8th edn. CABI Publishing, Wallingford, UK

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, Abaci O, Aime C, Asan A, Bai FY, de Beer ZW, Begerow D, Berikten D, Boekhout T, Buchanan PK, Burgess T, Buzina W, Cai L, Cannon PF, Crane JL, Damm U, Daniel HM, van Diepeningen AD, Druzhinina I, Dyer PS, Eberhardt U, Fell JW, Frisvad JC, Geiser DM, Geml J, Glienke C, Grafenhan T, Groenewald JZ, Groenewald M, de Gruyter J, Gueho-Kellermann E, Guo LD, Hibbett DS, Hong SB, de Hoog GS, Houbraken J, Huhndorf SM, Hyde KD, Ismail A, Johnston PR, Kadaifciler DG, Kirk PM, Koljalg U, Kurtzman CP, Lagneau PE, Levesque CA, Liu X, Lombard L, Meyer W, Miller A, Minter DW, Najafzadeh MJ, Norvell L, Ozerskaya SM, Ozic R, Pennycook SR, Peterson SW, Pettersson OV, Quaedvlieg W, Robert VA, Ruibal C, Schnurer J, Schroers HJ, Shivas R, Slippers B, Spierenburg H, Takashima M, Taskin E, Thines M, Thrane U, Uztan AH, van Raak M, Varga J, Vasco A, Verkley G, Videira SI, de Vries RP, Weir BS, Yilmaz N, Yurkov A, Zhang N (2011) The amsterdam declaration on fungal nomenclature. IMA Fungus 2:105–112

Heitman J (2010) Evolution of eukaryotic microbial pathogens via covert sexual reproduction. Cell Host Microbe 8:86–99

Heitman J, Kronstad JW, Taylor JW, Casselton LA (2007) Sex in fungi, molecular determination and evolutionary implication. ASM Press, Washington, DC

Heitman J, Carter DA, Dyer PS, Soll DR (2014) Sexual reproduction of human fungal pathogens. In: Casadevall A, Mitchell AP, Berman J, Kwon-Chung KJ, Perfect JR, Heitman J (eds) Fungal pathogens. Cold Spring Harbor Laboratory Press, New York, pp 41–59

Henk DA, Fisher MC (2011) Genetic diversity, recombination, and divergence in animal associated *Penicillium dipodomyis*. PLoS One 6, e22883

Henk DA, Eagle CE, Brown K, Van Den Berg MA, Dyer PS, Peterson SW, Fisher MC (2011) Speciation despite globally overlapping distributions in *Penicillium chrysogenum*: the population genetics of Alexander Fleming's lucky fungus. Mol Ecol 20:4288–4301

Herskowitz I (1988) Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol Rev 52:536–553

Hoff B, Poggeler S, Kuck U (2008) Eighty years after its discovery, Fleming's *Penicillium* strain discloses the secret of its sex. Eukaryot Cell 7:465–470

Hoffmann B, Eckert SE, Krappmann S, Braus GH (2001) Sexual diploids of *Aspergillus nidulans* do not form by random fusion of nuclei in the heterokaryon. Genetics 157:141–147

Horn BW, Moore GG, Carbone I (2009a) Sexual reproduction in *Aspergillus flavus*. Mycologia 101:423–429

Horn BW, Ramirez-Prado JH, Carbone I (2009b) Sexual reproduction and recombination in the aflatoxin-

producing fungus *Aspergillus parasiticus*. Fungal Genet Biol 46:169–175

Horn BW, Moore GG, Carbone I (2011) Sexual reproduction in aflatoxin-producing *Aspergillus nomius*. Mycologia 103:174–183

Horn BW, Olarte RA, Peterson SW, Carbone I (2013) Sexual reproduction in *Aspergillus tubingensis* from section *Nigri*. Mycologia 105:1153–1163

Houbraken J, Dyer PS (2015) Induction of the sexual cycle in filamentous ascomycetes. In: van den Berg MA, Maruthachalam K (eds) Genetic transformation systems in fungi, vol 2. Fungal biology. Springer International Publishing, Switzerland, pp 23–46

Howe H (1964) Vegetative traits associated with mating type in *Neurospora tetrasperma*. Mycologia 56:519–525

Hughes TJ, O'Donnell K, Sink S, Rooney AP, Scandiani MM, Luque A, Bhattacharyya MK, Huang X (2014) Genetic architecture and evolution of the mating type locus in fusaria that cause soybean sudden death syndrome and bean root rot. Mycologia 106:686–697

Hull CM, Raisner RM, Johnson AD (2000) Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. Science 289:307–310

Idnurm A, Walton FJ, Floyd A, Heitman J (2008) Identification of the sex genes in an early diverged fungus. Nature 451:193–196

Inderbitzin P, Harkness J, Turgeon BG, Berbee ML (2005) Lateral transfer of mating system in *Stemphylium*. Proc Natl Acad Sci USA 102:11390–11395

Ironside JE (2010) No amicable divorce? Challenging the notion that sexual antagonism drives sex chromosome evolution. Bioessays 32:718–726

Iyer SV, Ramakrishnan M, Kasbekar DP (2009) *Neurospora crassa fmf-1* encodes the homologue of the *Schizosaccharomyces pombe* Ste11p regulator of sexual development. J Genet 88:33–39

Jackson D, Lawson T, Villafane R, Gary L (2013) Modeling the structure of yeast MATα1: an HMG-box motif with a C-terminal helical extension. Open J Biophys 3:1–12

Jacobsen S, Wittig M, Pöggeler S (2002) Interaction between mating-type proteins from the homothallic fungus *Sordaria macrospora*. Curr Genet 41:150–158

Jacobson DJ (2005) Blocked recombination along the mating-type chromosomes of *Neurospora tetrasperma* involves both structural heterozygosity and autosomal genes. Genetics 171:839–843

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443:818–822

Johnson TE (1979) A *Neurospora* mutation that arrests perithecial development as either male or female parent. Genetics 92:1107–1120

Kanematsu S, Adachi Y, Ito T (2007) Mating-type loci of heterothallic Diaporthe spp.: homologous genes are present in opposite mating-types. Curr Genet 52:11–22

Kasuga T, Townsend JP, Tian C, Gilbert LB, Mannhaupt G, Taylor JW, Glass NL (2005) Long-oligomer microarray profiling in *Neurospora crassa* reveals the transcriptional program underlying biochemical and physiological events of conidial germination. Nucleic Acids Res 33:6469–6485

Kelley LA, Sternberg MJ (2009) Protein structure prediction on the Web: a case study using the Phyre server. Nat Protoc 4:363–371

Kelly M, Burke J, Smith M, Klar A, Beach D (1988) Four mating-type genes control sexual differentiation in the fission yeast. EMBO J 7:1537–1547

Kerenyi Z, Moretti A, Waalwijk C, Olah B, Hornok L (2004) Mating type sequences in asexually reproducing *Fusarium* species. Appl Environ Microbiol 70:4419–4423

Keszthelyi A, Jeney A, Kerenyi Z, Mendes O, Waalwijk C, Hornok L (2007) Tagging target genes of the MAT1-2-1 transcription factor in *Fusarium verticillioides* (*Gibberella fujikuroi* MP-A). Antonie van Leeuwenhoek 91:373–391

King KM, West JS, Fitt BDL, Dyer PS (2014) Differences in *MAT* gene distribution and expression between *Rhynchosporium* on grasses. Plant Pathol 64 (2):344–354

Klar AJ (2007) Lessons learned from studies of fission yeast mating-type switching and silencing. Annu Rev Genet 41:213–236

Klix V, Nowrousian M, Ringelberg C, Loros JJ, Dunlap JC, Poggeler S (2010) Functional characterization of *MAT1-1*-specific mating-type genes in the homothallic ascomycete *Sordaria macrospora* provides new insights into essential and nonessential sexual regulators. Eukaryot Cell 9:894–905

Koopman P (2010) HMG domain superfamily of DNA-bending proteins: HMG, UBF, TCF, LEF, SOX, SRY and related proteins. Encyclopedia of life sciences. Wiley, Chichester

Kronstad JW, Staben C (1997) Mating type in filamentous fungi. Annu Rev Genet 31:245–276

Kruzel EK, Giles SS, Hull CM (2012) Analysis of *Cryptococcus neoformans* sexual development reveals rewiring of the pheromone-response network by a change in transcription factor identity. Genetics 191:435–449

Kück U, Pöggeler S (2009) Cryptic sex in fungi. Fungal Biol Rev 23:86–90

Lahn BT, Page DC (1999) Four evolutionary strata on the human X chromosome. Science 286:964–967

Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, Gerber GK, Hannett NM, Harbison CT, Thompson CM, Simon I, Zeitlinger J, Jennings EG, Murray HL, Gordon DB, Ren B, Wyrick JJ, Tagne JB, Volkert TL, Fraenkel E, Gifford DK, Young RA (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science 298:799–804

Lee J, Lee T, Lee YW, Yun SH, Turgeon BG (2003) Shifting fungal reproductive mode by manipulation of mating type genes: obligatory heterothallism of *Gibberella zeae*. Mol Microbiol 50:145–152

Lee SC, Ni M, Li W, Shertz C, Heitman J (2010) The evolution of sex: a perspective from the fungal kingdom. Microbiol Mol Biol Rev 74:298–340

Leslie JF, Hwang F, Doe FJ (1986) Homothallic and heterothallic isolates of *Fusarium subglutinans*. Phytopathology 76:1142

Li W, Metin B, White TC, Heitman J (2010) Organization and evolutionary trajectory of the mating type (MAT) locus in dermatophyte and dimorphic fungal pathogens. Eukaryot Cell 9:46–58

Liberti D, Rollins JA, Harmon PF (2012) Evidence for morphological, vegetative, genetic, and mating-type diversity in *Sclerotinia homoeocarpa*. Phytopathology 102:506–518

Lobuglio KF, Taylor JW (2002) Recombination and genetic differentiation in the mycorrhizal fungus *Cenococcum geophilum* Fr. Mycologia 94:772–780

Logue ME, Wong S, Wolfe KH, Butler G (2005) A genome sequence survey shows that the pathogenic yeast *Candida parapsilosis* has a defective MTLa1 allele at its mating type locus. Eukaryot Cell 4:1009–1017

Lopez-Villavicencio M, Aguileta G, Giraud T, de Vienne DM, Lacoste S, Couloux A, Dupont J (2010) Sex in *Penicillium*: combined phylogenetic and experimental approaches. Fungal Genet Biol 47:693–706

Lu SW, Yun SH, Lee T, Turgeon BG (2011) Altering sexual reproductive mode by interspecific exchange of *MAT* loci. Fungal Genet Biol 48:714–724

Magee BB, Magee PT (2000) Induction of mating in *Candida albicans* by construction of MTLa and MTLα strains. Science 289:310–313

Maheshwari R (1999) Microconidia of *Neurospora crassa*. Fungal Genet Biol 26:1–18

Mandel MA, Barker BM, Kroken S, Rounsley SD, Orbach MJ (2007) Genomic and population analyses of the mating type loci in *Coccidioides species* reveal evidence for sexual reproduction and gene acquisition. Eukaryot Cell 6:1189–1199

Marais G, Galtier N (2003) Sex chromosomes: how X-Y recombination stops. Curr Biol 13:R641–643

Marcou D, Masson A, Simonet JM, Piquepaille G (1979) Evidence for non-random spatial distribution of meiotic exchanges in *Podospora anserina*: comparison between linkage groups 1 and 6. Mol Gen Genet 176:67–79

Martin T, Lu SW, van Tilbeurgh H, Ripoll DR, Dixelius C, Turgeon BG, Debuchy R (2010) Tracing the origin of the fungal α1 domain places its ancestor in the HMG-box superfamily: implication for fungal mating-type evolution. PLoS One 5, e15199

Martin SH, Wingfield BD, Wingfield MJ, Steenkamp ET (2011) Structure and evolution of the *Fusarium* mating type locus: new insights from the *Gibberella fujikuroi* complex. Fungal Genet Biol 48:731–740

Mata J, Bahler J (2006) Global roles of Ste11p, cell type, and pheromone in the control of gene expression during early sexual differentiation in fission yeast. Proc Natl Acad Sci USA 103:15517–15522

Mathieson MJ (1952) Ascospore dimorphism and mating type in *Chromocrea spinulosa* (Fuckel) Petch n. comb. Ann Bot 16:449–466

McGuire IC, Marra RE, Milgroom MG (2004) Mating-type heterokaryosis and selfing in *Cryphonectria parasitica*. Fungal Genet Biol 41:521–533

Menkis A, Jacobson DJ, Gustafsson T, Johannesson H (2008) The mating-type chromosome in the filamentous ascomycete *Neurospora tetrasperma* represents a model for early evolution of sex chromosomes. PLoS Genet 4, e1000030

Merlini L, Dudin O, Martin SG (2013) Mate and fuse: how yeast cells do it. Open Biol 3:130008

Metzenberg RL, Glass NL (1990) Mating type and mating strategies in *Neurospora*. Bioessays 12:53–59

Murata Y, Fujii M, Zolan ME, Kamada T (1998) Molecular analysis of *pcc1*, a gene that leads to A-regulated sexual morphogenesis in *Coprinus cinereus*. Genetics 149:1753–1761

Murtagh GJ, Dyer PS, Crittenden PD (2000) Sex and the single lichen. Nature 404:564

Newmeyer D, Taylor CW (1967) A pericentric inversion in Neurospora, with unstable duplication progeny. Genetics 56:771–791

Ni M, Feretzaki M, Sun S, Wang X, Heitman J (2011) Sex in fungi. Annu Rev Genet 45:405–430

Nolting N, Pöggeler S (2006a) A MADS box protein interacts with a mating-type protein and is required for fruiting body development in the homothallic ascomycete *Sordaria macrospora*. Eukaryot Cell 5:1043–1056

Nolting N, Pöggeler S (2006b) A STE12 homologue of the homothallic ascomycete *Sordaria macrospora* interacts with the MADS box protein MCM1 and is required for ascosporogenesis. Mol Microbiol 62:853–868

Normark BB, Judson OP, Moran NA (2003) Genomic signatures of ancient asexual lineages. Biol J Linn Soc 79:69–84

Nováková A, Hubka V, Dudová Z, Matsuzava T, Kubátová A, Yaguchi T, Kolařík M (2014) New species in *Aspergillus* section *Fumigati* from reclamation sites in Wyoming (U.S.A.) and revision of *A. viridinutans* complex. Fungal Divers 64:253–274

Nygren K, Strandberg R, Wallberg A, Nabholz B, Gustafsson T, Garcia D, Cano J, Guarro J, Johannesson H (2011) A comprehensive phylogeny of *Neurospora* reveals a link between reproductive mode and molecular evolution in fungi. Mol Phylogenet Evol 59:649–663

O'Gorman CM, Fuller HT, Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 457:471–474

Olson E (1949) Genetics of *Ceratostomella*. I. Strains in *Ceratostomella fimbriata* (Ell. & Hals) Elliott from sweet potatoes. Phytopathology 39:548–561

Otsubo Y, Yamamoto M (2013) Signaling pathways for fission yeast sexual differentiation at a glance. J Cell Sci 125:2789–2793

Palmer JM, Kubatova A, Novakova A, Minnis AM, Kolarik M, Lindner DL (2014) Molecular characterization of a heterothallic mating system in *Pseudogymnoascus destructans*, the fungus causing white-nose syndrome of bats. G3 (Bethesda) 4:1755–1763

Paoletti M, Rydholm C, Schwier EU, Anderson MJ, Szakacs G, Lutzoni F, Debeaupuis JP, Latge JP, Denning DW, Dyer PS (2005) Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr Biol 15:1242–1248

Paoletti M, Seymour FA, Alcocer MJ, Kaur N, Calvo AM, Archer DB, Dyer PS (2007) Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. Curr Biol 17:1384–1389

Papazian HP (1956) *DIVISION OF MYCOLOGY*: Sex and cytoplasm in the fungi. Trans N Y Acad Sci 18:388–397

Pattemore JA, Hane JK, Williams AH, Wilson BA, Stodart BJ, Ash GJ (2014) The genome sequence of the biocontrol fungus *Metarhizium anisopliae* and comparative genomics of *Metarhizium* species. BMC Genomics 15:660

Peraza-Reyes L, Malagnac F (2016) Sexual development in fungi. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 407–455

Perkins DD (1987) Mating-type switching in filamentous ascomycetes. Genetics 115:215–216

Picard M, Debuchy R, Julien J, Brygoo Y (1987) Transformation by integration in *Podospora anserina*. II. Targeting to the resident locus with cosmids and instability of the transformants. Mol Gen Genet 210:129–134

Picard M, Debuchy R, Coppin E (1991) Cloning the mating types of the heterothallic fungus *Podospora anserina*: developmental features of haploid transformants carrying both mating types. Genetics 128:539–547

Pittenger TH, Atwood KC (1956) Stability of nuclear proportions during growth of *Neurospora* heterokaryons. Genetics 41:227–241

Pöggeler S, Kück U (2000) Comparative analysis of the mating-type loci from *Neurospora crassa* and *Sordaria macrospora*: identification of novel transcribed ORFs. Mol Gen Genet 263:292–301

Pöggeler S, Nowrousian M, Ringelberg C, Loros JJ, Dunlap JC, Kück U (2006) Microarray and real-time PCR analyses reveal mating type-dependent gene expression in a homothallic fungus. Mol Genet Genomics 275:492–503

Pöggeler S, Hoff B, Kuck U (2008) Asexual cephalosporin C producer *Acremonium chrysogenum* carries a functional mating type locus. Appl Environ Microbiol 74:6006–6016

Pontecorvo G (1946) Genetic systems based upon heterokaryosis. Cold Spring Harb Symp Quant Biol 11:193–201

Pontecorvo G, Roper JA, Hemmons LM, Macdonald KD, Bufton AW (1953) The genetics of *Aspergillus nidulans*. Adv Genet 5:141–238

Raju NB (1992) Functional heterothallism resulting from homokaryotic conidia and ascospores in *Neurospora tetrasperma*. Mycol Res 96:103–116

Raju NB, Perkins DD (1994) Diverse programs of ascus development in pseudohomothallic species of *Neurospora*, *Gelasinospora*, and *Podospora*. Dev Genet 15:104–118

Raju NB, Perkins DD (2000) Programmed ascospore death in the homothallic ascomycete *Coniochaeta tetraspora*. Fungal Genet Biol 30:213–221

Ramirez-Prado JH, Moore GG, Horn BW, Carbone I (2008) Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet Biol 45:1292–1299

Remenyi A, Lins K, Nissen LJ, Reinbold R, Scholer HR, Wilmanns M (2003) Crystal structure of a POU/HMG/DNA ternary complex suggests differential assembly of Oct4 and Sox2 on two enhancers. Genes Dev 17:2048–2059

Roberts CJ, Nelson B, Marton MJ, Stoughton R, Meyer MR, Bennett HA, He YD, Dai H, Walker WL, Hughes TR, Tyers M, Boone C, Friend SH (2000) Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. Science 287:873–880

Robertson SJ, Bond DJ, Read ND (1998) Homothallism and heterothallism in *Sordaria brevicollis*. Mycol Res 102:1215–1223

Roper M, Ellison C, Taylor JW, Glass NL (2011) Nuclear and genome dynamics in multinucleate ascomycete fungi. Curr Biol 21:R786–793

Roper M, Simonin A, Hickey PC, Leeder A, Glass NL (2013) Nuclear dynamics in a fungal chimera. Proc Natl Acad Sci USA 110:12875–12880

Rossignol JL, Faugeron G (1995) MIP: an epigenetic gene silencing process in *Ascobolus immersus*. Curr Top Microbiol Immunol 197:179–191

Rydholm C, Dyer PS, Lutzoni F (2007) DNA sequence characterization and molecular evolution of *MAT1* and *MAT2* mating-type loci of the self-compatible ascomycete mold *Neosartorya fischeri*. Eukaryot Cell 6:868–874

Salih N, Dyer P (2015) Identification of novel genes regulating sexual reproduction in *Aspergillus* species. Fungal Genet Reports 60 (Suppl): Abstract #575

Salih N, Ashour A, Wada R, Maruyama J, Kitamoto K, Dyer P (2015) Identification of novel genes regulating sexual development in *Aspergillus* species by functional analysis of transcripts differentially regulated by mating-type loci. Fungal Genet Reports 60 (Suppl): Abstract #170

Samils N, Gioti A, Karlsson M, Sun Y, Kasuga T, Bastiaans E, Wang Z, Li N, Townsend JP, Johannesson H (2013) Sex-linked transcriptional divergence in the hermaphrodite fungus *Neurospora tetrasperma*. Proc Biol Sci 280:20130862

Samils N, Oliva J, Johannesson H (2014) Nuclear interactions in a heterokaryon: insight from the model *Neurospora tetrasperma*. Proc Biol Sci 281

Scannell DR, Wolfe K (2004) Rewiring the transcriptional regulatory circuits of cells. Genome Biol 5:206

Scazzocchio C (2006) *Aspergillus* genomes: secret sex and the secrets of sex. Trends Genet 22:521–525

Schneider TD, Stephens RM (1990) Sequence logos: a new way to display consensus sequences. Nucleic Acids Res 18:6097–6100

Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lucking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239

Selker EU (1990) Premeiotic instability of repeated sequences in *Neurospora crassa*. Annu Rev Genet 24:579–613

Sharon A, Yamaguchi K, Christiansen S, Horwitz BA, Yoder OC, Turgeon BG (1996) An asexual fungus has the potential for sexual development. Mol Gen Genet 251:60–68

Shiu PK, Glass NL (2000) Cell and nuclear recognition mechanisms mediated by mating type in filamentous ascomycetes. Curr Opin Microbiol 3:183–188

Short DP, O'Donnell K, Thrane U, Nielsen KF, Zhang N, Juba JH, Geiser DM (2013) Phylogenetic relationships among members of the *Fusarium solani* species complex in human infections and the descriptions of *F. keratoplasticum* sp. nov. and *F. petroliphilum* stat. nov. Fungal Genet Biol 53:59–70

Silar P, Barreau C, Debuchy R, Kicka S, Turcq B, Sainsard-Chanet A, Sellem CH, Billault A, Cattolico L, Duprat S, Weissenbach J (2003) Characterization of the genomic organization of the region bordering the centromere of chromosome V of *Podospora anserina* by direct sequencing. Fungal Genet Biol 39:250–263

Singh G, Dyer PS, Ashby AM (1999) Intra-specific and inter-specific conservation of mating-type genes from the discomycete plant-pathogenic fungi *Pyrenopeziza brassicae* and *Tapesia yallundae*. Curr Genet 36:290–300

Son H, Seo YS, Min K, Park AR, Lee J, Jin JM, Lin Y, Cao P, Hong SY, Kim EK, Lee SH, Cho A, Lee S, Kim MG, Kim Y, Kim JE, Kim JC, Choi GJ, Yun SH, Lim JY, Kim M, Lee YH, Choi YD, Lee YW (2011) A phenome-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. PLoS Pathog 7, e1002310

Soullier S, Jay P, Poulat F, Vanacker JM, Berta P, Laudet V (1999) Diversification pattern of the HMG and SOX family members during evolution. J Mol Evol 48:517–527

Souza CA, Silva CC, Ferreira AV (2003) Sex in fungi: lessons of gene regulation. Genet Mol Res 2:136–147

Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol Biol Cell 9:3273–3297

Spivakov M (2014) Spurious transcription factor binding: non-functional or genetically redundant? Bioessays 36:798–806

Staben C, Yanofsky C (1990) *Neurospora crassa a* mating-type region. Proc Natl Acad Sci USA 87:4917–4921

Sugimoto A, Iino Y, Maeda T, Watanabe Y, Yamamoto M (1991) *Schizosaccharomyces pombe ste11*$^{+}$ encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. Genes Dev 5:1990–1999

Sun S, Hsueh YP, Heitman J (2012) Gene conversion occurs within the mating-type locus of *Cryptococcus neoformans* during sexual reproduction. PLoS Genet 8, e1002810

Swilaiman SS, O'Gorman CM, Balajee SA, Dyer PS (2013) Discovery of a sexual cycle in *Aspergillus lentulus*, a close relative of *A. fumigatus*. Eukaryot Cell 12:962–969

Talbert PB, Henikoff S (2010) Centromeres convert but don't cross. PLoS Biol 8, e1000326

Taylor J, Jacobson D, Fisher M (1999) The evolution of asexual fungi: Reproduction, Speciation and Classification. Annu Rev Phytopathol 37:197–246

Terhem RB, Stassen J, Van Kan JA (2013) Functional analysis of genes in the mating-type locus of *Botrytis cinerea*. Fungal Genet Reports 60S:147

Tolmsoff WJ (1983) Heteroploidy as a mechanism of variability among fungi. Annu Rev Phytopathol 21:317–340

Tsong AE, Miller MG, Raisner RM, Johnson AD (2003) Evolution of a combinatorial transcriptional circuit: a case study in yeasts. Cell 115:389–399

Tsong AE, Tuch BB, Li H, Johnson AD (2006) Evolution of alternative transcriptional circuits with identical logic. Nature 443:415–420

Tsong AE, Brian BT, Johnson AD (2007) Rewiring transcriptional circuitry: mating-type regulation in *Saccharomyces cerevisiae* and *Candida albicans* as a model for evolution. In: Heitman J, Kronstad JW, Taylor JW, Casselton LA (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 75–89

Tuch BB, Galgoczy DJ, Hernday AD, Li H, Johnson AD (2008) The evolution of combinatorial gene regulation in fungi. PLoS Biol 6, e38

Turgeon BG, Debuchy R (2007) *Cochliobolus* and *Podospora*: mechanism of sex determination and the evolution of reproductive lifestyle. In: Heitman J, Kronstad JW, Taylor JW, Casselton LA (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 93–121

Turgeon BG, Yoder OC (2000) Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet Biol 31:1–5

Turgeon BG, Bohlmann H, Ciuffetti LM, Christiansen SK, Yang G, Schafer W, Yoder OC (1993) Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. Mol Gen Genet 238:270–284

Uhm JY, Fujii H (1983) Heterothallism and mating type mutation in *Sclerotinia trifoliorum*. Phytopathology 73:569–572

Urban M, Kahmann R, Bolker M (1996) Identification of the pheromone response element in *Ustilago maydis*. Mol Gen Genet 251:31–37

van Houte LP, Chuprina VP, van der Wetering M, Boelens R, Kaptein R, Clevers H (1995) Solution structure of the sequence-specific HMG box of the lymphocyte transcriptional activator Sox-4. J Biol Chem 270:30516–30524

Varga J, Szigeti G, Baranyi N, Kocsube S, O'Gorman CM, Dyer PS (2014) Aspergillus: sex and recombination. Mycopathologia 178:349–362

Waalwijk C, Mendes O, Verstappen EC, de Waard MA, Kema GH (2002) Isolation and characterization of the mating-type idiomorphs from the wheat septoria leaf blotch fungus *Mycosphaerella graminicola*. Fungal Genet Biol 35:277–286

Waalwijk C, Keszthelyi A, van der Lee T, Jeney A, de Vries I, Kerenyi Z, Mendes O, Hornok L (2006) Mating type loci in *Fusarium*: structure and function. Mycotoxin Res 22:54–60

Wada R, Maruyama J, Yamaguchi H, Yamamoto N, Wagu Y, Paoletti M, Archer DB, Dyer PS, Kitamoto K (2012) Presence and functionality of mating type genes in the supposedly asexual filamentous fungus *Aspergillus oryzae*. Appl Environ Microbiol 78:2819–2829

Wang L, Lin X (2011) Mechanism of unisexual mating in *Cryptococcus neoformans*. Fungal Genet Biol 48:651–660

Wang Z, Kin K, Lopez-Giraldez F, Johannesson H, Townsend JP (2012) Sex-specific gene expression during asexual development of *Neurospora crassa*. Fungal Genet Biol 49:533–543

Wang Z, Lopez-Giraldez F, Lehr N, Farre M, Common R, Trail F, Townsend JP (2014) Global gene expression and focused knockout analysis reveals genes associated with fungal fruiting body development in *Neurospora crassa*. Eukaryot Cell 13:154–169

Watanabe Y, Yamamoto M (1994) S. pombe *mei2*$^{+}$ encodes an RNA-binding protein essential for premeiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. Cell 78:487–498

Webster RK (1967) The inheritance of sexuality, color and colony type in *Ceratocystis fimbriata*. Mycologia 59:222–234

Webster RK, Butler EE (1967) The origin of self-fertile, cross-fertile strains in *Ceratocystis fimbriata*. Mycologia 59:212–221

Werner MH, Huth JR, Gronenborn AM, Clore GM (1995) Molecular basis of human 46X, Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. Cell 81:705–714

Whittle CA, Nygren K, Johannesson H (2011) Consequences of reproductive mode on genome evolution in fungi. Fungal Genet Biol 48:661–667

Wik L, Karlsson M, Johannesson H (2008) The evolutionary trajectory of the mating-type (*mat*) genes in *Neurospora* relates to reproductive behavior of taxa. BMC Evol Biol 8:109

Wilken PM, Steenkamp ET, Wingfield MJ, de Beer ZW, Wingfield BD (2014) DNA loss at the *Ceratocystis fimbriata* mating locus results in self-sterility. PLoS One 9, e92180

Willer M, Hoffmann L, Styrkarsdottir U, Egel R, Davey J, Nielsen O (1995) Two-step activation of meiosis by the *mat1* locus in *Schizosaccharomyces pombe*. Mol Cell Biol 15:4964–4970

Wilson AM, Godlonton T, van der Nest MA, Wilken PM, Wingfield MJ, Wingfield BD (2015) Unisexual reproduction in *Huntiella moniliformis*. Fungal Genet Biol 80:1–9

Wirsel S, Turgeon BG, Yoder OC (1996) Deletion of the *Cochliobolus heterostrophus* mating-type (*MAT*) locus promotes the function of *MAT* transgenes. Curr Genet 29:241–249

Witthuhn RC, Harrington TC, Wingfield BD, Steimel JP, Wingfield MJ (2000) Deletion of the *MAT-2* mating-type gene during uni-directional mating-type switching in *Ceratocystis*. Curr Genet 38:48–52

Woo PC, Chong KT, Tse H, Cai JJ, Lau CC, Zhou AC, Lau SK, Yuen KY (2006) Genomic and experimental evidence for a potential sexual cycle in the pathogenic thermal dimorphic fungus *Penicillium marneffei*. FEBS Lett 580:3409–3416

Woudenberg JH, De Gruyter J, Crous PW, Zwiers LH (2011) Analysis of the mating-type loci of co-occurring and phylogenetically related species of *Ascochyta* and *Phoma*. Mol Plant Pathol 13:350–362

Xie N, Ruprich-Robert G, Chapeland-Leclerc F, Coppin E, Debuchy R, Silar P (2015, Submitted) A genetic screen uncovers inositol-phosphate signaling as essential for sexual reproduction in *Podospora anserina*

Yokoyama E, Arakawa M, Yamagishi K, Hara A (2006) Phylogenetic and structural analyses of the mating-type loci in Clavicipitaceae. FEMS Microbiol Lett 264:182–191

Yu JJ, Sun WX, Yu M, Yin XL, Meng XK, Zhao J, Huang L, Huang L, Liu Y (2015) Characterization of mating-type loci in rice false smut fungus *Villosiclava virens*. FEMS Microbiol Lett 362. doi: 10.1093/femsle/fnv014

Yuan YO, Stroke IL, Fields S (1993) Coupling of cell identity to signal response in yeast: interaction between the alpha 1 and STE12 proteins. Genes Dev 7:1584–1597

Yun SH, Berbee ML, Yoder OC, Turgeon BG (1999) Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc Natl Acad Sci USA 96:5592–5597

Yun SH, Arie T, Kaneko I, Yoder OC, Turgeon BG (2000) Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. Fungal Genet Biol 31:7–20

Zakharov IA (2005) Intratetrad mating and its genetic and evolutionary consequences. Russ J Genet 41:402–411

Zheng Q, Hou R, Juanyu Z, Ma J, Wu Z, Wang G, Wang C, Xu JR (2013) The *MAT* locus genes play different roles in sexual reproduction and pathogenesis in *Fusarium graminearum*. PLoS One 8, e66980

Zickler D (2006) Meiosis in mycelial fungi. In: Kües U, Fischer R (eds) The Mycota I. Growth, differentiation and sexuality, 2nd edn. Springer, Berlin, Heidelberg, pp 415–438

15 Fruiting Body Formation in Basidiomycetes

Jordi F. Pelkmans[1], Luis G. Lugones[1], Han A.B. Wösten[1]

CONTENTS

I. Introduction

Fruiting bodies have evolved in the fungal kingdom to produce and disperse sexual spores. These reproductive structures of the Homobasidiomycota generally contain specialised cells, basidia, in which the genetically different parental haploid nuclei fuse. The resulting diploid cells immediately undergo meiosis to form haploid basidiospores. In some cases, diploid nuclei are already formed in the vegetative mycelium, as in *Armillaria* species (Ullrich and Anderson 1978; Grillo et al. 2000). After discharge, germination of the haploid basidiospores results in homokaryotic mycelia. These mycelia can fuse and, depending on an often complex system of mating-type genes (see Freihorst et al. 2016), produce a heterokaryotic mycelium that is fertile. Consequently, it forms fruiting bodies under appropriate environmental conditions. Basidiomycetes behaving according to this scheme are in the majority and are called heterothallic (i.e. self-incompatible). From a teleological point of view, this makes sense because it ensures that the diploid basidia produce recombinant meiotic progeny. A minority of basidiomycetes (about 10 %; Whitehouse 1949) are homothallic (i.e. self-compatible). Recently, it was shown that unisexual reproduction also creates diversity (Ni et al. 2013), which would explain the existence of homothallic species from an evolutionary point of view.

This review discusses the regulation of fruiting body formation in homobasidiomycetes and the role structural proteins and enzymes play in this process. Related topics such as morphogenesis, cytology and mathematical modelling are discussed in Wells and Wells (1982), Moore et al. (1985), Wessels (1993), Chiu and Moore (1996), Moore (1998), Kües (2000), Meskauskas et al. (2004). Fruiting of commercially important species is reviewed in van Griensven (1988), Kües and Liu (2000), Kothe (2001) and Sánchez (2010).

II. Development of Emergent Structures

Formation of a Feeding Mycelium

Fruiting bodies develop from a vegetative mycelium. Formation of this mycelium starts with the germination of an asexual or sexual

[1]Microbiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands; e-mail: h.a.b.wosten@bio.uu.nl

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

spore. Hyphae growing out of these spores grow at their tips, while branching subapically (Wessels 1986, 1990). Hyphal fusion promotes the formation of an elaborate hyphal network (Chap. 6 in The Mycota, Volume VIII).

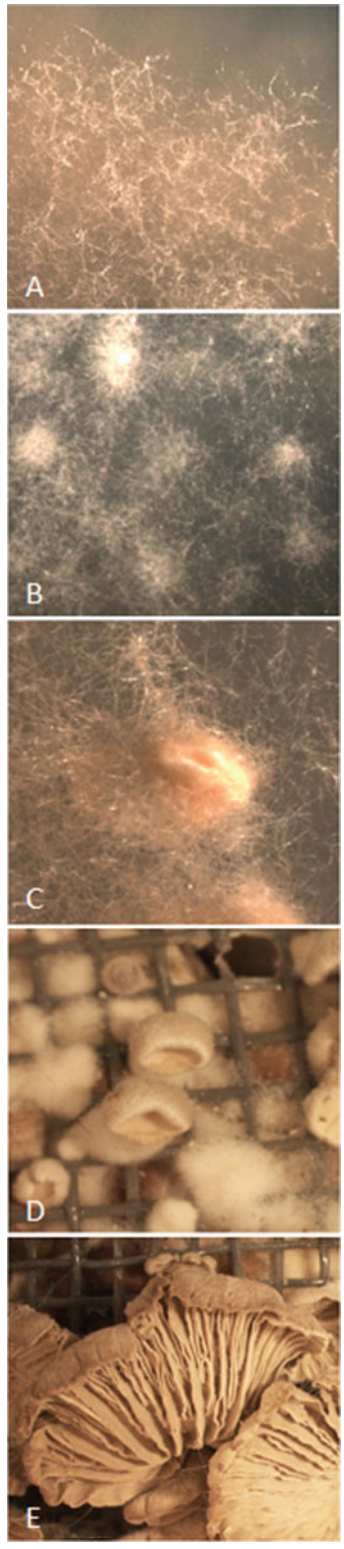
The vegetative mycelium degrades and colonises organic substrates by means of extracellular enzymes that are secreted at tips of growing hyphae (Wösten et al. 1991; Moukha et al. 1993). The degradation products are taken up and serve as nutrients. They can also be transported to other parts of the mycelium, which would explain why some fungi can grow for considerable distances over nonnutritive surfaces (Jennings 1984, see Chap. 9 in Volume I of The Mycota, 1994). Mass transport of water and nutrients through the mycelium implies that the cytoplasm is continuous within the mycelium. This would be mediated by the large pores (diameters up to 500 nm) within the septa that compartmentalise hyphae. These pores, however, are not always open, at least in *Schizophyllum commune* (van Peer et al. 2009). As many as 90 % of the subapical septa can be closed when *S. commune* is growing on glucose-containing medium. Septal closure is strongly reduced when *S. commune* is grown in the absence of this carbon source, while high temperature, hypertonic conditions or exposure to the antibiotic nourseothricin increase plugging incidence. So far, the plugging state of septa has not been assessed when *S. commune* (or other basidiomycetes) grows under natural conditions and how this affects long- and short-distance transport of nutrients. Closure of the septal pores is mediated by the septal pore cap (SPC) (van Driel et al. 2008; van Peer et al. 2010). Inactivation of *spc33* that encodes a structural SPC protein results in reduced vegetative growth and retarded fruiting body formation (van Peer et al. 2010). It was proposed that this is caused by decreased turgor pressure due to the inability to close the septal pores. Septal closure may also allow for differentiation of hyphae within the vegetative mycelium, as was shown in *Aspergillus niger* (Wösten et al. 2013).

Translocation of water and nutrients is essential for fruiting body development. Fruiting body primordia of *S. commune* (Wessels 1965) and *Coprinus cinereus* (*Coprinopsis cinerea*) (Moore 1998) can arise at the expense of polymeric constituents of the supporting mycelium, while expanding fruiting bodies grow at the expense of both supporting mycelium and abortive fruiting body primordia. At the moment, it is not clear how much of this is due to movement of cytoplasm or to degradation and resynthesis of cellular components. Woolston et al. (2011) showed in *A. bisporus* that the heterologous protein β-glucuronidase is transported from the vegetative mycelium into the fruiting body. No evidence was obtained for translocation of its mRNA.

Formation of Fruiting Bodies from the Vegetative Mycelium

Formation of fruiting bodies is a highly complex developmental process. A generalised scheme for formation of agaric fruiting bodies such as those of *C. cinereus* (Moore 1998; Kües 2000) is as follows: After a "critical mass" of vegetative mycelium has been formed, hyphae escape the substrate to grow into the air. These aerial hyphae form aggregates, which are called hyphal knots or nodules. Within the knots, hyphae aggregate forming a fruiting body initial. These initials are the first fruiting body-specific structures (Sánchez and Moore 1999; Sánchez et al. 2004; Kües et al. 2004). Initials can further develop into primordia. Primordia are characterised by the presence of all tissues that occur in a mature fruiting body. Only a fraction of the initials or primordia eventually will grow out into mature fruiting bodies. Possibly, stochastic processes and competition for translocated materials determine which initials further develop. Alternatively, and not mutually exclusive, developing fruiting bodies may repress outgrowth of neighbouring initials. Such a mechanism was proposed based on the observation that inactivation of *fst3* of *S. commune* resulted in a high number of fruiting bodies. These Δ*fst3* fruiting bodies were smaller than those of the wild type (Ohm et al. 2010).

The lower part of primordia will develop into the stipe of a mature fruiting body, while the cap will be formed from the upper part. Within the cap, different tissues develop, which are not formed from meristems, as in plants, but result from the interaction of



individual hyphae. The hymenium is one of the cap tissues, which consists of different cell types including the basidia. In the basidia, karyogamy and meiosis take place, ultimately resulting in basidiospores. The complexity of fruiting body development is also exemplified by the fact that formation of the different tissues overlaps in time. Moreover, cells in the developing mushroom differ in diameter, length, the number of septa, nuclei and vacuoles and molecular composition (e.g. the content of reserve carbohydrates; Moore 1998). The different cell types are the result of localised growth as well as apoptosis (Umar and van Griensven 1997; Wiemer et al. 2016).

Not all basidiomycetes follow the general morphogenetic pathway of fruiting body formation (Reijnders and Stafleu 1992; Watling 1996; Clémonçon 1997). For instance, the fruiting bodies of *S. commune* (Fig. 15.1) result from indeterminate growth of fruiting body primordia. Expansion of the cup-shaped primordia is not the result of intercalary growth but is due to continued apical growth and differentiation of hyphae in the primordium (Wessels 1993). Despite the variety in developmental programmes, their underlying regulation seems to be conserved, at least partly (see Sects. III.B and III.C).

III. Regulation of Fruiting Body Formation

A. Environmental Signals

Aerial growth is accompanied by drastic changes in exposure to oxygen, CO_2 and light. These environmental factors can exert a profound influence on fruiting body development. Moreover, temperature, humidity, volatiles, pH, salinity and availability of nutrients may play a decisive role (Madelin 1956; Manachère 1980; Kües and Liu 2000; Eastwood et al. 2013).

Fig. 15.1 Fruit body development of *S. commune*. Aerial hyphae (**a**) aggregate into stage I aggregates (**b**) from which stage II primordia develop (**c**). These primordia form *cup-shaped* structures (**d**) that further differentiate into mature fruiting bodies (**e**)

The development of fruiting bodies is often, if not always, the result of a combination of environmental conditions. For instance, fruiting in *Lentinula edodes* is induced by light, moisture and low temperature (Ishikawa 1967; Matsumoto and Kitamoto 1987; Nakazawa et al. 2008). In the case of *Agaricus bisporus*, it was proposed that the volatile 1-octen-3-ol controls the early differentiation of vegetative hyphae to multicellular knots. A drop in temperature would subsequently control the differentiation of primordia in this basidiomycete, while the carbon dioxide level would determine the number of fruiting bodies that develop (Eastwood et al. 2013). Molecular mechanisms underlying regulation of fruiting by CO_2 and light have been studied in some detail. This will be discussed in the following sections.

Carbon Dioxide as a Signal for Fruiting Body Formation

CO_2 represses fruiting body development in a variety of basidiomycetes including *A. bisporus* and *S. commune* (Niederpruem 1963; Raudaskoski and Viitanen 1982; van Griensven 1988; Eastwood et al. 2013). Carbonic anhydrase most likely functions in CO_2 sensing by converting this molecule in bicarbonate (HCO_3^-) (Bahn and Muhlschlegel 2006). This molecule stimulates adenylyl cyclase activity in *Candida albicans* and *Cryptococcus neoformans* (Klengel et al. 2005; Mogensen et al. 2006). Adenylyl cyclase may also be involved in CO_2 sensing in fruiting body formation (Eastwood et al. 2013). In agreement, high levels of intracellular cAMP, the product of adenylyl cyclase, resulting from expression of dominant active G protein α-subunits (SCGP-A and SCGP-C) reduce fruiting in *S. commune* (Yamagishi et al. 2002, 2004).

Light as a Signal for Fruiting Body Formation

Induction of primordia would be the only light-sensitive step in *S. commune* (Perkins 1969; Raudaskoski and Yli-Mattila 1985). Illumination for a few minutes was reported to be sufficient to induce primordia and, as a consequence, fruiting body development. However, recently, it was found that at least two developmental stages require light exposure at 2000 lux. Light is required for aggregate formation and for maturation of fruiting bodies (JF Pelkmans, unpublished data). Light is also required for several stages of fruiting body development in, for example, *Coprinus congregatus* (Manachère 1988) and *C. cinereus* (Tsusué 1969; Lu 1974; Kamada et al. 1978). Fruiting body formation in *C. cinereus* has at least five light-sensitive phases (Kües 2000; Lu 2000). Light is needed for the formation of initials, for maturation of primordia and for karyogamy. On the other hand, it negatively impacts hyphal knot formation and completion of meiosis. Thus, for fruiting bodies to develop, cycles of light and darkness are required. Light effects are local and are not spread systemically (Madelin 1956; Kertesz-Chaloupková et al. 1998).

Light is not required for the formation of mushrooms in *A. bisporus*. On the other hand, blue light initiates fruiting body formation in *C. cinerea* and *S. commune* (Perkins 1969; Durand 1985). The molecular mechanisms underlying blue light regulation in these basidiomycetes have been partly elucidated. Yet, blue light regulation is much better understood in *Aspergillus nidulans* and *Neurospora crassa* (Purschwitz et al. 2006; Bayram et al. 2010). We here briefly discuss what is known about light signalling in *A. nidulans* in relation to sexual development to illustrate how light signalling may operate in fruiting body formation in basidiomycetes. *A. nidulans* forms fruiting bodies preferentially in the dark. The phytochrome FphA is a red light receptor that represses sexual development (Blumenstein et al. 2005), while the blue light LreA/LreB receptor complex stimulates this process. The latter is concluded from inactivation of *lreA* or *lreB* (Purschwitz et al. 2008). Yet, light-regulated development is more complex as evidenced by the finding that fruiting body formation in a strain in which *lreA*, *lreB* and *fphA* are inactivated is less affected than in the Δ*lreA* and the Δ*lreB* strains. LreA, LreB and PhyA form a light-sensing complex that also includes an activator of sexual development called VeA (Kim et al. 2002). VeA is also part of a complex

with VelB, LaeA and VosA as core components (Bayram et al. 2010). Like the Δ*veA* strain, the Δ*velB* strain does not form fruiting bodies (Bayram et al. 2008b). LaeA is a negative regulator of sexual development (Sarikaya Bayram et al. 2010) as evidenced by the finding that fruiting body development in the Δ*laeA* strain is markedly increased in the light. Finally, *A. nidulans* has a second blue light sensor, CryA (Bayram et al. 2008a). CryA functions as a negative regulator of sexual development probably by integrating near-UV and blue light, resulting in repression of VeA-mediated initiation of sexual development.

C. cinerea has homologues for LreA, LreB, VeA, VelB, LaeA, a phytochrome and a cryptochrome-like photolyase. The role of these proteins is not yet known except for the LreA and LreB homologues, Dst1 and WC-2, respectively (Terashima et al. 2005; Nakazawa et al. 2011). Gene *dst2* has also been proposed to be a photomorphogenic protein based on its phenotype (Kuratani et al. 2010). Genes *dst1*, *wc-2* and *dst2* were identified in a homokaryotic fruiting strain (i.e. with constitutively active A and B mating-type pathways). This strain shows aberrant photomorphogenesis in that it forms dark stipes when grown continuously in the dark (Kamada et al. 2010). In other words, this strain does not need light for induction of initials but requires light for primordia maturation. Dark stipes in the wild type are obtained when cultures containing primordia are transferred to the dark. The pileus and stipe tissues at the upper part of the primordium remain rudimentary in the case of dark stipes, but the basal part of the primordium does elongate. Inactivation of *dst1*, *wc-2* or *dst2* in the homokaryotic fruiter impaired fruiting body development and resulted in the dark stipe phenotype when subjected to light.

The genome of *S. commune* contains orthologues of *lreA*, *lreB*, a putative cryptochrome, a phytochrome and genes encoding homologues of VeA, VelB and LaeA (Ohm et al. 2010). Moreover, *S. commune* contains a homologue of *dst2*. Genes *wc-1* and *wc-2* represent the *lreA* and *lreB* homologues of *S. commune*. Inactivation of these genes results in a blind phenotype. Dikaryons with both copies of *wc-1* or *wc-2* inactivated behave like monokaryons, unable to form mushrooms in white or blue light (Ohm et al. 2013; Fig. 15.2). The WC-1 and WC-2- proteins contain two and one protein-binding Per-Arnt-Sim (PAS) domain, respectively. One of the PAS domains in WC-1 is a modified version, called a light-oxygen-voltage (LOV) domain. This domain is expected to bind flavin adenine dinucleotide (FAD), which is a chromophore that detects blue light. The WC-2- protein has a predicted zinc finger domain of the GATA type. These data suggest that WC-1 is the light receptor, while WC-2 would have a role as a transcriptional regulator. Compared to the wild type, 183 and 244 genes are more than twofold up- and downregulated in the Δwc-2Δwc-2 dikaryon, respectively (Ohm et al. 2013). Hydrophobin genes are overrepresented in the downregulated genes (see Sect. IV. A). Moreover, expression of the transcription factor genes *c2h2* and *hom1*, known to be involved in fruiting body development (Ohm et al. 2011) (see Sect. III.C), is decreased in the Δ*wc-2*Δ*wc-2* dikaryon. Gene *cry1* is also downregulated in the Δ*wc-2*Δ*wc-2* dikaryon. This suggests that the encoding cryptochrome/photolyase plays a role in photobiology of *S. commune* (Ohm et al. 2013).

B. Mating-Type Genes as Master Regulators

Heterokaryons of heterothallic basidiomycetes are generally the life stage capable of fruiting. Fertile heterokaryons are the result of a mating between two homokaryons that have different mating-type genes (for further details, see Freihorst et al. 2016). Homokaryons of *S. commune* and *C. cinereus* contain one nucleus in each hyphal compartment and are therefore called monokaryons. On the other hand, the heterokaryons contain two nuclei (one of each mating partner) in each hyphal compartment and are, therefore, called dikaryons. The dikaryons of *S. commune* and *C. cinereus* are characterised by a clamp connection at each septum, which is formed during synchronous mitotic division of the two nuclei (Kües et al. 2016). Nuclear distribution and the presence of clamp connections are variable in heterobasidiomy-

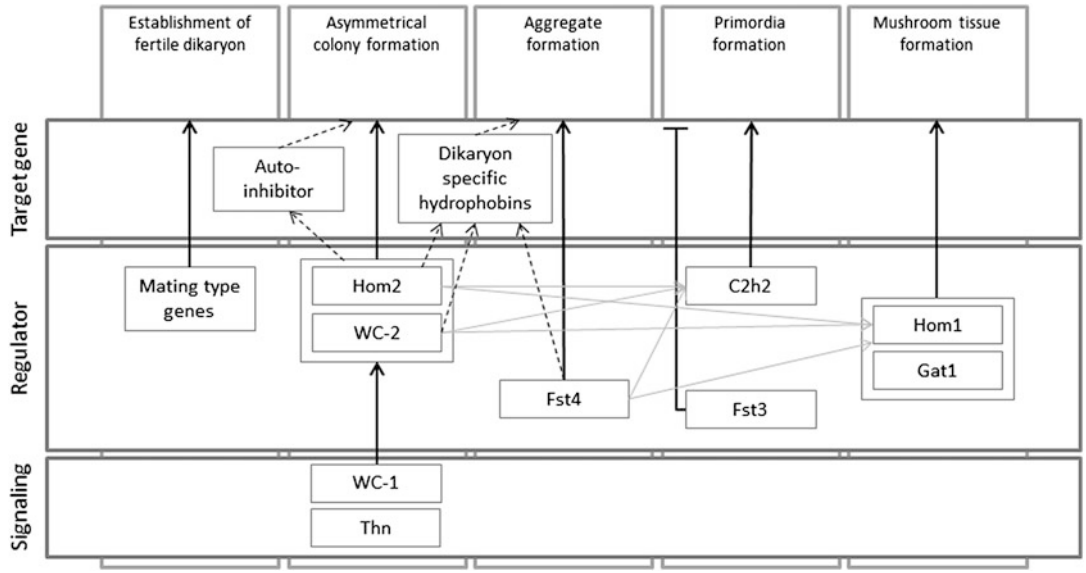


Fig. 15.2 Model of regulation of fruiting body formation in *S. commune*. Depicted are transcription factors and proteins involved in signalling as have been investigated in *S. commune*. *Thick lines* represent developmental stages in which these genes are involved. *Thin lines* represent interaction between transcription factors, as indicated by whole genome expression analysis (Ohm et al. 2011, 2013). *Dotted lines* represent regulation of target genes that are involved in specific stages of development

cetes. The homokaryon of *Agaricus bitorquis* is multikaryotic, while the fertile heterokaryon is dikaryotic but without clamp connections (Raper 1976). The fertile heterokaryon of *A. bisporus* grows directly from a basidiospore that contains two nuclei with different mating types. The heterokaryon is multikaryotic and, like *A. bitorquis*, has no clamp connections (Raper et al. 1972).

The mating-type genes are the master regulators of sexual development (see also Freihorst et al. 2016). When two homokaryons with different alleles for the *A* and *B* mating-type loci (in *S. commune* called *MATA* and *MATB*) fuse, a heterokaryon is formed with the potential to fruit. Nuclei that are exchanged migrate to the apical compartment of the recipient hypha, which is accompanied by septal dissolution. In the apical compartment, the donated and recipient nuclei pair and hyphal dissolution is switched off. In fact, new septa are formed that are more resistant to dissolution and which physically prevent nuclear migration (Wessels and Marchant 1974). The nuclei in a dikaryotic hyphal compartment divide synchronously. Nuclear division is accompanied by the formation of clamps. As a result, the apical and the subapical compartments contain nuclei of both mating types.

The molecular structure of the mating-type loci has been uncovered in, for instance, *S. commune* and *C. cinereus* (for a detailed overview and references, see Dyer et al. 2016). The *A* genes of these fungi encode homeodomain proteins of the HD1 and HD2 type. In heterokaryons with nuclei containing different *A* genes, these proteins form heterodimers that are active in clamp development and the initial pairing and migration of compatible nuclei. On the other hand, the *B* genes of *C. cinereus* and *S. commune* encode pheromones and G-coupled pheromone receptors. In heterokaryons with nuclei containing different *B* genes, pheromones from one nucleus interact with receptors encoded by the other nucleus and vice versa. These genes are involved in exchange and migration of the nuclei as well as fusion of the hook cell. Homokaryotic mutant strains have been isolated with a constitutive active *A* mating-type gene (referred to as $MATA^{con}$ in *S. commune*, *Amut* in *C. cinereus*) and/or *B* mating-type gene (referred to as $MATB^{con}$ in *S. commune*, *Bmut* in *C. cinereus*)

(Raper et al. 1965; Koltin 1970; Swamy et al. 1984). Activity of these loci is thus independent of a compatible allele donated by a sexual partner. The *S. commune* $MATA^{con}MATB^{con}$ dikaryotic homokaryon can form fruiting bodies. Likewise, *AmutBmut* homokaryons of *C. cinereus* form fruiting bodies like wild-type dikaryons (Swamy et al. 1984; Boulianne et al. 2000). Mutations in the *pcc1* gene of *C. cinereus* can also lead to fruiting in homokaryons (Uno and Ishikawa 1971; Muraguchi et al. 1999). The product of *pcc1* has been proposed to be a negative regulator of the *A* mating-type pathway (Kamada 2002; see below). The *frt1* gene of *S. commune* also initiates fruiting in a homokaryon. Certain homokaryons with introduced copies of this gene start to fruit independent of the mating-type loci (see next section).

S. commune and *C. cinereus* are examples of tetrapolar species that have two unlinked mating-type loci. Bipolar species exist within the agaricomycetes as well, where the *B* locus has lost its ability to distinguish self from nonself (James et al. 2006). This is in contrast to bipolar basidiomycetes that have linked all mating-type genes on a single locus (Bakkeren and Kronstad 1994; Lengerer et al. 2002).

C. Other Regulatory Genes

Regulatory Genes in Establishment of the Dikaryotic Mycelium

Establishment of the dikaryotic mycelium strongly impacts gene expression. For instance, 26 % of the genes of *S. commune* show a fold change of two or more upon mating (Erdmann et al. 2012). The *A* and *B* pathways regulate 27 % and 42 % of these genes, respectively, while the remaining 31 % are controlled by both mating-type loci. Among the *A*-activated genes is *clp3*, a homologue of *clp1* in *C. cinereus* (Inada et al. 2001). The latter gene was isolated by complementation of an *AmutBmut* mutant homokaryon that did not form clamps. Expression of *clp1* also depends on the *A* genes. Clamps are made independently from the homeodomain heterodimer when *clp1* is expressed from a constitutive promoter. The mode of action of Clp1 is not known, and it does not contain any known structural motifs. Gene *pcc1* (see previous section) is another gene that seems to be part of the *A*-regulated pathway (Murata et al. 1998). A homokaryotic strain with a mutated copy of this gene forms pseudoclamps, and it has the capacity to form fully differentiated fruiting bodies. These data and its expression in a wild-type homokaryon indicate that *pcc1* is a repressor of the fruiting pathway in the absence of a functional *A* complex (i.e. in the homokaryon). Gene *pcc1* likely encodes a transcription factor because of its HMG box motif and nuclear localisation signal. The presence of pheromone-responsive elements in the *pcc1* promoter suggests that it is a pheromone-responsive gene. Indeed, it is upregulated by a compatible *B*-mating interaction (Murata et al. 1998). The fact that *pcc1* is also upregulated by an activated *A* gene suggests that Pcc1 coordinates the activities of the *A* and *B* genes (Murata et al. 1998). This hypothesis is supported by the finding that the *pcc1* mutant homokaryon has the potential to form fruiting bodies. It has been suggested that the repressor activity of *pcc1* is released by a compatible *A* gene interaction via *clp1* (Kamada 2002).

FRT1 is a putative nucleotide-binding protein with a P-loop motif (Horton and Raper 1995). Its gene was identified by its fruiting phenotype when transformed into certain homokaryons of *S. commune* (Horton and Raper 1991). Experimental evidence indicates that homokaryotic strains that start to fruit have an endogenous *frt1* allele of a different kind (designated *frt1-2*; Horton et al. 1999). In contrast, strains possessing *frt1-1* do not fruit when transformed with this allele (Horton and Raper 1991). Homokaryons in which the *frt1* gene is disrupted are more fluffy compared to wild-type strains, and the Δ*frt1* aerial hyphae aggregate (Horton et al. 1999) as observed during the first stages of fruiting body development (Raudaskoski and Vauras 1982; van der Valk and Marchant 1978). This is accompanied by increased expression of the dikaryon-specific genes sc*1* and *sc4* (see Sect. IV). From these data, it was proposed that *FRT1* is part of a signal transduction pathway that represses expression of dikaryon-specific genes

in the monokaryon (Horton and Raper 1995). However, the absence of FRT1 is not sufficient to initiate full development of fruiting bodies. How can haploid fruiting be explained when *frt1-1* is transformed into a *frt1-2* strain? It has been proposed that these proteins dimerise and by this relieve repression of the dikaryon-specific genes. The heterodimer would also activate genes, resulting in the formation of mature fruiting bodies (Horton et al. 1999). Note that the absence of *frt1* did not affect fruiting in the dikaryon (Horton et al. 1999). This suggests that *frt1* is not a component of the pathway that leads to formation of fruiting bodies in the dikaryon.

Highly conserved small G proteins, called Ras, play an essential role in intracellular signalling (e.g. the MAPK, cAMP and Cdc42 signalling pathways). Two *ras* genes have been identified in nearly all fungi. The Ras protein exists in a GTP-bound active form and a GDP-bound inactive form. Guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) promote interconversion of the active and inactive forms of Ras, respectively. Deletion of *gap1* of *S. commune* resulted in hampered clamp, gill and spore formation (Schubert et al. 2006). Constitutively activated Ras1 resulted in dysfunctional dikaryotization, as nonself nuclei were not accepted. Furthermore, growth orientation, branching, fruiting body morphology and spore formation were affected (Knabe et al. 2013). Since deletion of *gap1* increases Ras1 activity, an overlap of phenotypical traits with the *ras1* overexpression strain is expected. Indeed, PKA levels were increased in both strains, linking these proteins to the cAMP signalling pathway. Expression analysis of above-mentioned transformant strains and a constitutively active Cdc42 mutant, involved in the MAPK pathway, revealed an overlap in target genes. This implies that Ras1 is also involved in MAPK signalling in *S. commune* (Weber et al. 2005).

Regulatory Genes Involved in Fruiting Body Formation in the Heterokaryon

The *thn1* gene of *S. commune* acts early in the developmental pathway of fruiting body formation. A mutation in *thn1* leads to pleiotropic effects including the absence of aerial growth in the homokaryon and, when homozygous, in the dikaryon (Raper and Miles 1958; Schwalb and Miles 1967; Wessels et al. 1991b). Thus, both aerial hyphae and fruiting body formation are affected. Thn1 is a putative Regulator of G-protein signaling protein that interacts with a Gα subunit of a heterotrimeric G protein. Such a protein converts an active GTP-bound Gα subunit into an inactive GDP-bound Gα. It was therefore proposed (Fowler and Mitton 2000) that *thn1* regulates a heterotrimeric G protein signalling pathway that functions in the decision of the vegetative mycelium to start the development of aerial hyphae and fruiting bodies. This function would be similar to that of FlbA in *A. nidulans* (Lee and Adams 1994). Transcriptome analysis of a *thn* mutant showed 114 genes, mostly involved in cellular responses that were affected by the mutation. This is in agreement with a role in G protein signalling (Erdmann et al. 2012). Most of the pleiotropic effects of the *thn* mutation are overcome by growing the mutant near a wild-type colony (Schuren 1999). A diffusible molecule smaller than 8 kDa would be responsible for this effect and may be part of the signalling cascade.

A set of transcription factor genes has been identified that is involved in different stages of fruiting body development in *S. commune* (Fig. 15.2) (Ohm et al. 2010, 2011, 2013). Inactivation of the homeodomain gene *hom2* results in symmetrical monokaryon-like colonies that form aerial hyphae but do not form aggregates, primordia and mature fruiting bodies. Fst4 is a zinc finger transcription factor positioned downstream of Hom2. Deletion of its encoding gene results in irregular dikaryon-like colonies that form aerial hyphae but do not form aggregates. Zinc finger protein C2h2 is involved in primordia formation. Inactivation of its encoding gene gives rise to irregular colonies that form aerial hyphae and aggregates. Deletion strains of *fst3*, *gat1* and *hom1* do form fruiting bodies. However, these fruiting bodies are smaller and are produced in a higher number. It was proposed that Fst3, a fungal-specific transcription factor, is a local repressor of primordia formation (Ohm et al. 2010). This would imply the existence of communication

between developing fruiting bodies. Such communication would ensure full outgrowth of mushrooms when resources are limited. Gat1, a GATA-type zinc finger protein, and Hom1, a homeodomain protein, might play a role in the expansion of the fruiting body. Expansion of fruiting bodies will go at the expense of outgrowth of neighbouring aggregates because of limited resources. Such a mechanism would not require communication between fruiting bodies. The *ich1* gene of *C. cinereus* also acts at later stages of fruiting body formation. Cap differentiation is blocked at an early stage of fruiting body differentiation in the *ich1* mutant (Muraguchi and Kamada 1998). In contrast to wild-type primordia, no rudimentary pileus is observed in the primordial shaft of the *ich1* mutant. The precise role of the gene is not yet known. It encodes a protein that contains a nuclear targeting signal and an S-adenosyl-L-methionine (SAM)-binding motif (Kües 2000), being characteristic for the enzyme family of methyltransferases (Faumann et al. 1999).

The model presented in Fig. 15.2 could also apply to other mushroom-forming fungi. The *hom2*, *hom1*, *fst3*, *fst4*, *c2h2* and *gat1* genes are basidiomycete-specific regulators (Todd et al. 2014). These and the other *S. commune* transcription factors that are involved in fruiting body development have homologues in *Laccaria bicolor* and in *A. bisporus* (Ohm et al. 2010; Morin et al. 2012). Similarly, homologues of *ich1* are present in *S. commune* and *A. bisporus*. Gene expression analysis of *A. bisporus* revealed 22 genes with homologues in *S. commune* that were significantly upregulated in mature fruiting bodies. This group includes *fst3*, *fst4*, *c2h2* and *hom1*. Similarly, homologues of *hom2*, *fst4*, *fst3*, *gat1* and *hom1* showed similar expression in *L. bicolor* (Morin et al. 2012), while homologues of *c2h2*, *fst3*, *hom1* and *gat1* showed similar expression in *C. cinereus* (Plaza et al. 2014).

Whole genome expression analysis revealed that 284 genes are differentially expressed in the monokaryotic stage of *S. commune* (Ohm et al. 2010), whereas 128 genes are differentially expressed during the aggregate stage. Genes involved in hydrophobins, protein and energy production are upregulated during aggregation, while genes involved in signal transduction, gene regulation, carbohydrate metabolism and cell wall biogenesis are downregulated. 467 genes were differentially expressed during primordia formation. Upregulated genes are involved in signal transduction, gene regulation, carbohydrate metabolism and cell wall biogenesis. Protein and energy production are downregulated. Of the 128 differentially expressed genes during fruiting body maturation, fatty acid metabolism is upregulated, while gene regulation and glucose, alcohol and amino acid metabolism are downregulated (Ohm et al. 2010). The high number of genes exclusively expressed during primordia development suggests this is a crucial developmental switch.

D. Nuclear Positioning

The *sc1*, *sc4* and *sc6* hydrophobin genes (see Sect. IV.A) as well as *sc7* and *sc14* are expressed in dikaryons (*MATA*-on *MATB*-on) of *S. commune* but not in monokaryons (*MATA*-off *MATB*-off) and *MATA*-on *MATB*-off or *MATA*-off *MATB*-on mycelia (Mulder and Wessels 1986; Wessels et al. 1995). In contrast, the *sc3* hydrophobin gene is active in the monokaryon and is downregulated in a *MATA*-off *MATB*-on mycelium (Ásgeirsdóttir et al. 1995). From this, it is expected that *sc3* would also be inactive in dikaryons (i.e. *MATA*-on *MATB*-on). Indeed, *sc3* mRNA levels are reduced in a fruiting dikaryon. However, under non-fruiting conditions (e.g. high CO_2 and darkness), high *sc3* expression is observed, while expression of *sc1*, *sc4*, *sc6*, *sc7* and *sc14* is relatively low (Wessels et al. 1987). Apparently, the *MATB* pathway, and possibly also the *MATA* pathway, is not active in at least part of the dikaryotic mycelium. This was explained by a regulatory mechanism that varies the distance between the nuclei in compartments of dikaryotic hyphae (Schuurs et al. 1998). The distance of nuclei in dikaryotic aerial hyphae is relatively large (>8 μm) and correlates with high *sc3* expression. In contrast, nuclear distance in fruiting bodies is small (<2 μm), correlating with low *sc3* and high *sc4* expression. Increased distance of the nuclei (>4 μm) would inactivate

the *MATB*-on pathway, and possibly also the *MATA*-on pathway, resulting in a monokaryon-like gene expression (Wessels et al. 1998).

IV. Structural Proteins and Enzymes Involved in Fruiting

A. Hydrophobins

Hydrophobins are secreted proteins that fulfil a wide spectrum of functions in fungal growth and development (Wessels 1997; Wösten and Wessels 1997; Wösten 2001, see Chap. 7 in Volume VIII, The Mycota). Class I and class II hydrophobins are distinguished based on hydropathy patterns and solubility characteristics (Wessels 1994). Basidiomycetes only have class I hydrophobins. These hydrophobins can affect hyphal wall composition in a soluble state (van Wetter et al. 2000b). However, most functions of these hydrophobins are based on their property to self-assemble into an amphipathic membrane at hydrophilic/hydrophobic interfaces (Wösten et al. 1993, 1994a, b, 1995, 1999). This ~10 nm thick membrane is highly insoluble and consists of a mosaic of amyloid-like fibrils called rodlets (Wösten et al. 1993; Wösten and de Vocht 2000; de Vocht et al. 2002; Butko et al. 2001; Scholtmeijer et al. 2009). Upon self-assembly at the interface between the hydrophilic cell wall and a hydrophobic environment (the air or the hydrophobic surface of a host), the hydrophilic side of the amphipathic membrane will face the cell wall, while the hydrophobic side becomes exposed. Aerial hyphae and spores thus become hydrophobic, while hyphae that grow over a hydrophobic substrate become attached.

The role of hydrophobins in mushroom-forming fungi has been best studied in *S. commune*. This fungus contains 13 hydrophobin genes (Ohm et al. 2010) including *sc1*, *sc3*, *sc4* and *sc6* (Mulder and Wessels 1986; Schuren and Wessels 1990; Wessels et al. 1995; de Vocht et al. 1998). The *sc1*, *sc4* and *sc6* hydrophobin genes are dikaryon-specific and are regulated by the mating-type genes (Ruiters et al. 1988), *fbf* (Springer and Wessels 1989), *thn* (Wessels et al. 1991b) and transcriptional regulators involved in pre-aggregate development *wc-2*, *hom2* and *fst4* (Ohm et al. 2011, 2013). On the other hand, *sc3* is expressed both in the monokaryon and the dikaryon (see Sect. III.D). It is regulated by *thn*, *wc-2*, *hom2* and *fst4* but not by *fbf*. Hydrophobins make up 6–8 % of protein synthesised by *S. commune* at the time of emergent growth (Wessels et al. 1991a, b). So far, the roles of SC3 and SC4 have been established and they will be discussed below.

Expression of *sc3* in monokaryons is induced after a feeding mycelium has been established (Mulder and Wessels 1986). SC3 secreted by submerged hyphae self-assembles at the medium-air interface, which is accompanied by a huge drop in water surface tension. This enables hyphae to breach the interface of the aqueous environment and the air to form aerial hyphae (Wösten et al. 1999). The water surface tension remains high in a Δ*sc3* strain and only few hyphae can escape the aqueous environment. In other words, in the absence of *sc3* expression, hyphae are forced to grow in the aqueous substrate. Expression of *sc3* is thus a main event in the onset of aerial growth. How the mycelium senses that the feeding mycelium is large enough to be able to support aerial growth by switching on SC3 production remains to be solved (Wösten and Willey 2000). SC3 secreted by aerial hyphae cannot diffuse into the medium but will be confronted with the cell wall-air interface. As a result, SC3 assembles at the surface of these hyphae making them hydrophobic (Wösten et al. 1994a). SC3 also lowers the water surface tension in the dikaryon allowing aerial hyphae to grow into the air (van Wetter et al. 2000a). The amount of SC4 in the medium is too low to complement for the absence of SC3 in a Δ*sc3*Δ*sc3* dikaryon. SC3 also coats aerial hyphae of the dikaryon and hyphae at the outer surface of fruiting bodies (Ásgeirsdóttir et al. 1995). SC4, but not SC3, is located in the fruiting body context, in which it lines air channels that traverse the plectenchyma (Lugones et al. 1999). The air channels of Δ*sc4*Δ*sc4* fruiting bodies readily fill with water in the absence of a hydrophobic coating (van Wetter et al. 2000a). SC4 thus ensures gas exchange in the fruiting body tissue under moist conditions.

The ABH1 hydrophobin of *A. bisporus* (Lugones et al. 1996; de Groot et al. 1996) is an orthologue of SC4 of *S. commune*. Expression of *abh1* starts prior to primordia formation and remains high during primordia and fruiting body formation (Eastwood et al. 2013). The protein not only lines air channels in the fruiting body tissue (Lugones et al. 1999) but also coats the outer surface of the mushroom (Lugones et al. 1996). The HypB hydrophobin of *A. bisporus* is located at the border of the cap and the stipe tissue and has been proposed to protect the mushroom against bacterial infection (de Groot et al. 1999).

The presence of multiple hydrophobin genes not only enables the fungus to produce hydrophobins at different stages of development (Kershaw et al. 1998) but also to form hydrophobins that are tailored to fulfil specific functions (van Wetter et al. 2000a). SC4 can substitute for SC3 in the formation of hydrophobic aerial hyphae, but hyphal attachment to hydrophobic surfaces is only partially restored. This is explained by a lower affinity of assembled SC4 for the cell wall of adhering hyphae when compared to SC3. Possibly, this is related to sugar-binding specificities of these hydrophobins (van Wetter et al. 2000a). The exposed carbohydrates of cell walls of aerial hyphae and hyphae in the fruiting body tissue may be different, requiring different lectin specificities to ensure strong binding to the cell wall. Evolution of hydrophobins to fulfil specific functions is also indicated by sequence analysis. SC3 of *S. commune* (de Vocht et al. 1998), ABH3 of *A. bisporus* (Lugones et al. 1998), COH1 of *C. cinereus* (Ásgeirsdóttir et al. 1997) and POH1 of *P. ostreatus* (Ásgeirsdóttir et al. 1998) that all function in the formation of aerial hyphae are more related to each other than SC3 and ABH3 to other hydrophobins of *S. commune* and *A. bisporus*, respectively. Similarly, the fruiting body-specific hydrophobins of *S. commune* cluster with *HypB* (de Groot et al. 1999), *ABH1* and *ABH2* (Lugones et al. 1996; de Groot et al. 1996) of *A. bisporus*.

With the established roles of SC3, SC4 and ABH1, we are only at the beginning of our understanding of the functions of hydrophobins in fruiting. Dikaryons express several hydrophobins at the same time that may have specific properties and/or are expressed at a particular place. For instance, it has been suggested that hydrophobins could be involved in aggregating aerial hyphae during fruiting body morphogenesis (see Chap. 21 in Volume I 1994). The situation is even more complex by the presence of proteins that can substitute for hydrophobins. For example, the SC15 protein can partly rescue the reduction of surface tension of the culture medium by SC3 (Lugones et al. 2004).

B. Lectins

Lectins are carbohydrate-binding proteins that play diverse roles in fungi (Singh et al. 2010). For instance, they function in parasitism against plants and insects, in predation of soil nematodes and in early stages of ectomycorrhizal symbiosis. Expression of galectin lectins is upregulated in multiple stages of fruiting in *C. cinereus* and *Agrocybe aegerita* (Boulianne et al. 2000; Luan et al. 2010). They may play a role in mushroom defence against predators and parasites but also seem to play a role in mushroom development. Addition of the galectin AAL of *A. aegerita* to an established mycelium induces aggregation and primordia formation both in *A. aegerita* and *Auricularia polytricha* (Sun et al. 2003; Luan et al. 2010). In contrast, the presence of the galectin at the moment of inoculation results in growth inhibition and repression of fruiting (Luan et al. 2010). These data show that temporal expression of galectins is important for their role in mushroom development.

C. Haemolysins

Haemolysins are pore-forming proteins that are known for their ability to lyse red blood cells. Aggregation of haemolysins in the plasma membrane results in ~4 nm wide pores that permeabilise the membrane (Nayak et al. 2013). Fungal homologues have been identified in the basidiomycetes *A. aegerita* (Fernandez Espinar and Labarère 1997; Berne et al. 2002),

P. ostreatus (Berne et al. 2002) and *S. commune* (Han et al. 2010). There is a strong evidence for the involvement of haemolysins in fruiting body formation. These proteins are specifically expressed in primordia and young fruiting bodies of *A. aegerita* and *P. ostreatus* (Vidic et al. 2005). In both species, the concentration of protein is highest at the edge of lamellae, in basidia and in developing basidiospores. Notably, external addition of the haemolysin ostreolysin of *P. ostreatus* to mycelium boosts fruiting initiation in this fungus and increases quantity and size of fruiting bodies (Berne et al. 2007).

D. Oxidative Enzymes

A role for laccases in oxidative cross-linking of hyphae in polypores, which become pigmented and woody by oxidation of phenolic compounds, was already proposed in 1967 (Bu'Lock 1967; Bu'Lock and Walker 1967). However, so far, proof of a role in mushroom development is lacking. Evidence for a role of cytochrome P450 enzymes in fruiting body formation is available. The *eln2-1* mutant of *C. cinereus* was isolated in a screen for developmental mutants (Muraguchi et al. 1999). This mutant is characterised by dumpy fruiting body primordia. Cell morphogenesis and tissue organisation are affected in the primordial shaft of the *eln2-1* mutant. As a result, the mature fruiting bodies have short stipes (Muraguchi and Kamada 2000). The *eln2* gene is constitutively expressed and encodes a novel type of cytochrome P450 enzyme. These enzymes are involved in the oxidative, peroxidative and reductive metabolism of numerous compounds. A deletion of 18 amino acids at the C-terminus is the cause of the mutant phenotype. Muraguchi and Kamada (2000) gave three explanations to explain the mutant phenotype. A changed catalytic activity may produce a toxic compound that affects development in the primordial shaft. Alternatively, activity of the truncated enzyme may not result in a product that is normally instrumental in development. Finally, the mutant enzyme may overproduce a normal metabolite or produce superoxide radicals.

E. Expansins

Expansins are nonenzymatic proteins that induce cell wall extension and cell wall modification (McQueen-Mason et al. 1992; Cosgrove 2005). These proteins are believed to act by disrupting non-covalent interactions between carbohydrate polymers (McQueen-Mason and Cosgrove 1995). Expansins were originally found in plants, but homologues have also been identified in fungi. Basidiomycetes do not appear to have strict homologues of expansins. Instead, they have expansin-like proteins named loosenins with an N-terminal domain similar to that of plant expansins. LOOS1 was first isolated from the basidiomycete *Bjerkandera adusta* (Quiroz-Castañeda et al. 2011), but they also occur in, for instance, *S. commune*, *A. bisporus*, *C. cinereus* and *L. bicolor* (Suzuki et al. 2014). The main function of expansin(-like) proteins in fungi is believed to facilitate degradation of plant-derived substrates. However, evidence accumulates that these proteins also play a role in morphogenesis and cell wall modification. Deletion of the expansin-like gene *eglD* in *A. nidulans* results in increased resistance against lysing enzymes. This observation combined with the exclusive presence of EglD in conidial cell walls suggests that this protein is involved in cell wall remodelling during germination (Bouzeralou et al. 2008). In basidiomycetes, repression of stipe wall extension by heat in *F. velutipes* and *C. cinereus* could be rescued by a snail expansin-like protein (Fang et al. 2014; Zhang et al. 2014). This suggests that expansin(-like) proteins are involved in the extension of fruiting bodies.

V. Conclusions

Establishment of the dikaryotic mycelium and formation of fruiting bodies are highly complex developmental programmes that are activated by a combination of environmental cues. A wide variety of proteins are expected to regulate and coordinate these programmes or to fulfil enzymatic conversions or structural roles. With the identification of the first genes involved in mushroom development, we are only at the beginning of understanding fruiting body for-

mation. The process of identification of genes will be accelerated by whole genome expression studies and increased availability of molecular tools to assign functions to genes.

Establishment of the dikaryon and emergence of fruiting bodies in basidiomycetes are regulated by the mating-type genes. These genes encode DNA-binding proteins and pheromones and their receptors. Regulation of fruiting by the mating-type genes is mediated by downstream transcription factors. Several genes encoding such regulatory proteins have now been identified. Regulatory circuits ultimately activate genes encoding structural proteins or enzymes that are involved in fruiting body formation. The role of hydrophobins is well established. They enable hyphae to escape the aqueous environment to allow fruiting body development. Moreover, they coat aerial structures and line air channels in mushrooms. The hydrophobic coating irreversibly directs growth of hyphae into the air, allows dispersal of spores and ensures gas exchange in fruiting bodies under humid conditions. Apart from hydrophobins, phenolics polymerised by the action of laccases may contribute to surface hydrophobicity of fruiting bodies. These enzymes have also been proposed to cross-link cell walls of hyphae in the fruiting bodies but this still has to be established. Experimental evidence indicates that cytochrome P450 enzymes, lectins, haemolysins and expansins also function in mushroom development. Lectins may be involved in aggregation of hyphae, haemolysins in signalling particularly to induce apoptosis of selected hyphae in the fruiting body, while expansins may be involved in cell wall modification and extension.

References

Ásgeirsdóttir SA, van Wetter MA, Wessels JGH (1995) Differential expression of genes under control of the mating-type genes in the secondary mycelium of *Schizophyllum commune*. Microbiology 141:1281–1288

Ásgeirsdóttir SA, Halsall JR, Casselton LA (1997) Expression of two closely linked hydrophobin genes of *Coprinus cinereus* is monokaryon-specific and down-regulated by the *oid-1* mutation. Fungal Genet Biol 22:54–63

Ásgeirsdóttir SA, de Vries OMH, Wessels JGH (1998) Identification of three differentially expressed hydrophobins in *Pleurotus ostreatus* (oyster mushroom). Microbiology 144:2961–2969

Bahn YS, Muhlschlegel FA (2006) CO_2 sensing in fungi and beyond. Curr Opin Microbiol 9:572–578

Bakkeren G, Kronstad JW (1994) Linkage of mating-type loci distinguishes bipolar from tetrapolar mating in basidiomycetous smut fungi. Proc Natl Acad Sci USA 91:7085–7089

Bayram Ö, Biesemann C, Krappmann S, Galland P, Braus GH (2008a) More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. Mol Biol Cell 19:3254–3262

Bayram O, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon NJ, Keller NP, Yu JH, Braus GH (2008b) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320:1504–1506

Bayram Ö, Braus GH, Fischer R, Rodriguez-Romero J (2010) Spotlight on *Aspergillus nidulans* photosensory systems. Fungal Genet Biol 47:900–908

Berne S, Križaj I, Pohleven F, Turk T, Maček P, Sepčić K (2002) *Pleurotus* and *Agrocybe* hemolysins, new proteins hypothetically involved in fungal fruiting. Biochem Biophys Acta 1570:153–159

Berne S, Pohleven J, Vidic I, Rebolj K, Pohleven F, Turk T, Maček P, Sonnenberg A, Sepčić K (2007) Ostreolysin enhances fruiting initiation in the oyster mushroom (*Pleurotus ostreatus*). Mycol Res 111(12):1431–1436

Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, Frankenberg-Dinkel N, Fischer R (2005) The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr Biol 15:1833–1838

Boulianne RP, Liu Y, Aebi M, Lu BC, Kües U (2000) Fruiting body development in *Coprinus cinereus*: regulated expression of two galectins secreted by a non classical pathway. Microbiology 146:1841–1853

Bouzeralou D, Billini M, Roumelioti K, Sophianopoulou V (2008) EglD, a putative endoglucanase, with an expansin like domain is localized in the conidial cell wall of *Aspergillus nidulans*. Fungal Genet Biol 45:839–850

Bu'Lock JD (1967) Essays in biosynthesis and microbial development. In: Squibb ER (ed) Essays in biosynthesis and microbial development, lectures on chemistry and microbial products. Wiley, New York, pp 1–18

Bu'Lock JD, Walker DC (1967) On chagi. J Chem Soc Sect C 5:336–338

Butko P, Buford JP, Goodwin JS, Stroud PA, McCormick CL, Cannon GC (2001) Spectroscopic evidence for amyloid-like interfacial self-assembly of hydrophobin Sc3. Biochem Biophys Res Commun 280:212–215

Chiu S-W, Moore D (eds) (1996) Patterns in fungal development. Cambridge University Press, Cambridge

Clémonçon H (1997) Anatomy of the hymenomycetes. An introduction to the cytology and plectology of crust fungi, bracket fungi, club fungi, Chantarelles, Agarics and Boletes. F. Flück-Wirth, Teufen, Switzerland

Cosgrove DJ (2005) Growth of the plant cell wall. Nat Rev Mol Cell Biol 6(11):850–861

de Groot PWJ, Schaap PJ, Sonnenberg ASM, Visser J, Van Griensven LJLD (1996) The *Agaricus bisporus hypA* gene encodes a hydrophobin and specifically accumulates in peel tissue of mushroom caps during fruit body development. J Mol Biol 257:1008–1018

de Groot PWJ, Roeven RT, Van Griensven LJ, Visser J, Schaap PJ (1999) Different temporal and spatial expression of two hydrophobin-encoding genes of the edible mushroom *Agaricus bisporus*. Microbiology 145:1105–1113

de Vocht ML, Scholtmeijer K, van der Vegte EW, de Vries OMH, Sonveaux N, Wösten HAB, Ruysschaert J-M, Hadziioannou G, Wessels JGH, Robillard GT (1998) Structural characterization of the hydrophobin SC3, as a monomer and after self-assembly at hydrophobic/hydrophilic interfaces. Biophys J 74:2059–2068

de Vocht ML, Reviakine I, Ulrich WP, Bergsma-Schutter W, Wösten HAB, Vogel H, Brisson A, Wessels JGH, Robillard GT (2002) Self-assembly of the hydrophobin SC3 proceeds via two structural intermediates. Protein Sci 11:1199–1205

Durand R (1985) Blue U.V.-light photoreception in fungi. Rev Physiol Végét 23:935–943

Dyer PS, Inderbitzin P, Debuchy R (2016) Mating-type structure, function, regulation and evolution in the pezizomycotina. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 351–385

Eastwood DC, Herman B, Noble R, Dobrovin-Pennington A, Sreenivasaprasad S, Burton KS (2013) Environmental regulation of reproductive phase change in *Agaricus bisporus* by 1-octen-3-ol, temperature and CO2. Fungal Genet Biol 55:54–66

Erdmann S, Freihorst D, Raudaskoski M, Schmidt-Heck W, Jung EM, Senftleben D, Kothe E (2012) Transcriptome and functional analysis of mating in the basidiomycete *Schizophyllum commune*. Eukaryot Cell 11:571–589

Fang H, Zhang W, Niu X, Liu Z, Lu C, Wei H, Yuan S (2014) Stipe wall extension of *Flammulina velutipes* could be induced by an expansin-like protein from *Helix aspersa*. Fungal Biol 118(1):1–11

Faumann EB, Blumenthal RM, Cheng XD (1999) Structure and evolution of AdoMet-dependent methyltransferases. In: Cheng XD, Blumenthal RM (eds) S-adenosylmethionine-dependent methyltransferases: structures and functions. World Scientific, Singapore, pp 1–38

Fernandez Espinar MT, Labarère J (1997) Cloning and sequencing of the *Aa-pri1* gene specifically expressed during fruiting initiation in the edible mushroom *Agrocybe aegerita*. Curr Genet 32:420–424

Fowler TJ, Mitton MF (2000) *Scooter*, a new active transposon in *Schizophyllum commune*, has disrupted two genes regulating signal transduction. Genetics 156:1585–1594

Freihorst D, Fowler TJ, Bartholomew K, Marjatta R, Stephen Horton J, Kothe E (2016) The mating type genes of the basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 329–349

Grillo R, Korhonen K, Hantula J, Hietala AM (2000) Genetic evidence of somatic haploidization in developing fruit bodies of *Armillaria tabescens*. Fungal Genet Biol 30:135–145

Han C-H, Zhang G-Q, Wang H-X, Ng TB (2010) Schizolysin, a hemolysin from the split gill mushroom *Schizophyllum commune*. FEMS Microbiol Lett 309(2):115–121

Horton JS, Raper CA (1991) A mushroom-inducing DNA sequence isolated from the basidiomycete *Schizophyllum commune*. Genetics 129:707–716

Horton JS, Raper CA (1995) The mushroom-inducing gene *FRT1* of *Schizophyllum commune* encodes a putative nucleotide binding protein. Mol Gen Genet 247:358–366

Horton JS, Palmer GE, Smith WJ (1999) Regulation of dikaryon-expressed genes by *FRT1* in the basidiomycete *Schizophyllum commune*. Fungal Genet Biol 26:33–47

Inada K, Morimoto Y, Arima T, Murata Y, Kamada T (2001) The *clp1* gene of the mushroom *Coprinus cinereus* is essential for *A*-regulated sexual development. Genetics 157:133–140

Ishikawa H (1967) Physiological and ecological studies on Lentinus edodes (Berk.) Sing. J Agric Lab 8:1–57

James TY, Srivilai P, Kües U, Vilgalys R (2006) Evolution of the bipolar mating system of the mushroom *Coprinellus disseminatus* from its tetrapolar ancestors involves loss of mating-type-specific pheromone receptor function. Genetics 172(3):1877–1891

Jennings DH (1984) Water flow through mycelia. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of fungal mycelia. Cambridge University Press, Cambridge, pp 143–164

Kamada T (2002) Molecular genetics of sexual development in the mushroom *Coprinus cinereus*. BioEssays 24:449–459

Kamada T, Kurita R, Takemaru T (1978) Effects of light on basidiocarp maturation in *Coprinus macrorhizus*. Plant Cell Physiol 19:263–275

Kamada T, Sano H, Nakazawa T, Nakahori K (2010) Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. Fungal Genet Biol 47:917–921

Kershaw MJ, Wakley G, Talbot NJ (1998) Complementation of the *mpg1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships between fungal hydrophobins. EMBO J 17:3838–3849

Kertesz-Chaloupková K, Walser PJ, Granado JD, Aebi M, Kües U (1998) Blue light overrides repression of a-sexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 23:95–109

Kim H, Han K, Kim K, Han D, Jahng K, Chae K (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80

Klengel T, Liang WJ, Chaloupka J, Ruoff C, Schröppel K, Naglik JR, Eckert SE, Mogensen EG, Haynes K, Tuite MF, Levin LR, Buck J, Mühlschlegel FA (2005) Fungal adenylyl cyclase integrates CO2 sensing with cAMP signaling and virulence. Curr Biol 15:2021–2026

Knabe N, Jung E-M, Freihorst D, Hennicke F, Horton JS, Kothe E (2013) A central role for Ras1 in morphogenesis of the basidiomycete *Schizophyllum commune*. Eukaryot Cell 12(6):941–952

Koltin Y (1970) Development of the *AmutBmut* strain of *Schizophyllum commune*. Arch Microbiol 74:123–128

Kothe E (2001) Mating-type genes for basidiomycete strain improvement in mushroom farming. Appl Microbiol Biotechnol 56:602–612

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U, Liu Y (2000) Fruiting body production in basidiomycetes. Appl Microbiol Biotechnol 54:141–152

Kües U, Künzler M, Bottoli APF, Walser PJ, Granado JD, Yi L, Bertossa RC, Ciardo D, Clergeot P-H, Loos S, Ruprich-Robert G, Aebi M (2004) Mushroom development in higher basidiomycetes; implications for human and animal health. In: Kushwaha RKS (ed) Fungi in human and animal health. Scientific, Jodhpur, India, pp 431–469

Kües U, Badalyan SM, Gießler A, Dörnte B (2016) Asexual sporulation in agaricomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 269–328

Kuratani M, Tanaka K, Terashima K, Muraguchi H, Nakazawa T, Nakahori K, Kamada T (2010) The *dst2* gene essential for photomorphogenesis of *Coprinopsis cinerea* encodes a protein with a putative FAD-binding-4 domain. Fungal Genet Biol 47:152–158

Lee BN, Adams TH (1994) Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation, leads to activation of *brlA* and premature initiation of development. Mol Microbiol 14:323–334

Lengerer KB, Fox DS, Fraser JA, Allen A, Forrester K, Dietrich FS, Heitman J (2002) Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. Eukaryot Cell 1(5):704–718

Lu BC (1974) Meiosis in Coprinus. Role of light on basidiocarp initiation, mitosis, and hymenium differentiation in *Coprinus lagopus*. Can J Bot 52:299–308

Lu BC (2000) The control of meiosis progression in the fungus *Coprinus cinereus* by light/dark cycles. Fungal Genet Biol 31:33–41

Luan R, Liang Y, Chen Y, Liu H, Jiang S, Che T, Wong B, Sun Hui H (2010) Opposing developmental functions of *Agrocybe aegerita* galectin (AAL) during mycelia differentiation. Fungal Biol 114(8):599–608

Lugones LG, Bosscher JS, Scholtmeijer K, de Vries OMH, Wessels JGH (1996) An abundant hydrophobin (ABH1) forms hydrophobic rodlet layers in *Agaricus bisporus* fruiting bodies. Microbiology 142:1321–1329

Lugones LG, Wösten HAB, Wessels JGH (1998) A hydrophobin (ABH3) secreted by the substrate mycelium of *Agaricus bisporus* (common white button mushroom). Microbiology 144:2345–2353

Lugones LG, Wösten HAB, Birkenkamp KU, Sjollema KA, Zagers J, Wessels JGH (1999) Hydrophobins line air channels in fruiting bodies of *Schizophyllum commune* and *Agaricus bisporus*. Mycol Res 103:635–640

Lugones LG, de Jong JF, de Vries OMH, Jalving R, Dijksterhuis J, Wösten HAB (2004) The SC15 protein of *Schizophyllum commune* mediates formation of aerial hyphae and attachment in the absence of the SC3 hydrophobin. Mol Microbiol 53(2):707–716

Madelin MF (1956) The influence of light and temperature on fruiting of *Coprinus lagopus* Fr. in pure culture. Ann Bot 20:467–480

Manachère G (1980) Conditions essential for controlled fruiting of macromycetes—A review. Trans Brit Mycol Soc 75:255–270

Manachère G (1988) Regulation of sporophore differentiation in some macromycetes, particularly in Coprini: An overview of some experimental studies from fruiting initiation to sporogenesis. Cryptogamie Mycol 9:291–323

Matsumoto T, Kitamoto Y (1987) Induction of fruitbody formation by water-flooding treatment in sawdust cultures of *Lentinus edodes*. Trans Mycol Soc Jpn 28:437–443

McQueen-Mason SJ, Cosgrove DJ (1995) Expansin mode of action on cell walls. Analysis of wall hydrolysis, stress relaxation, and binding. Plant Physiol 107(1):87–100

McQueen-Mason S, Durachko DM, Cosgrove DJ (1992) Two endogenous proteins that induce cell wall extension in plants. Plant Cell 4(11):1425–1433

Meskauskas A, McNulty LJ, Moore D (2004) Concerted regulation of all hyphal tips generates fungal fruit body structures: experiments with computer visualizations produced by a new mathematical model of hyphal growth. Mycol Res 108:341–353

Mogensen EG, Janbon G, Chaloupka J, Steegborn C, Fu MS, Moyrand F, Klengel T, Pearson DS, Geeves MA, Buck J, Levin LR, Mühlschlegel FA (2006) *Cryptococcus neoformans* senses CO_2 through the

carbonic anhydrase Can2 and the adenylyl cyclase Cac1. Eukaryot Cell 5:103–111

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge

Moore D, Casselton LA, Wood DA, Frankland JC (eds) (1985) Developmental biology of higher fungi. Cambridge University Press, Cambridge

Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL et al (2012) Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc Natl Acad Sci USA 109 (43):17501–17506

Moukha SM, Wösten HAB, Asther M, Wessels JGH (1993) In situ localization of lignin peroxidase excretion in colonies of *Phanerochaete chrysosporium* using sandwiched mode of culture. J Gen Microbiol 139:969–978

Mulder GH, Wessels JGH (1986) Molecular cloning of RNAs differentially expressed in monokaryons and dikaryons of *Schizophyllum commune*. Exp Mycol 10:214–227

Muraguchi H, Kamada T (1998) The *ich1* gene of the mushroom *Coprinus cinereus* is essential for pileus formation in fruiting. Development 125:3133–3141

Muraguchi H, Kamada T (2000) A mutation in the *eln2* gene encoding a cytochrome P450 of *Coprinus cinereus* affects mushroom morphogenesis. Fungal Genet Biol 29:49–59

Muraguchi H, Takemaru T, Kamada K (1999) Isolation and characterization of developmental variants in fruiting using a homokaryotic fruiting strain of Coprinus cinereus. Mycoscience 40:227–235

Murata Y, Fujii M, Zolan ME, Kamada T (1998) Molecular analysis of *pcc1*, a gene that leads to *A*-regulated sexual morphogenesis in *Coprinus cinereus*. Genetics 149:1753–1761

Nakazawa T, Miyazaki Y, Kaneko S, Shishido K (2008) Stimulative effects of light and a temperature downshift on transcriptional expressions of developmentally regulated genes in the initial stages of fruiting-body formation of the basidiomycetous mushroom *Lentinula edodes*. FEMS Microbiol Lett 289:67–71

Nakazawa T, Ando Y, Kitaaki K, Nakahori K, Kamada T (2011) Efficient gene targeting in Δ*Cc.ku70* or ΔCc. *lig4* mutants of the agaricomycete *Coprinopsis cinerea*. Fungal Genet Biol 48:939–946

Nayak AP, Green BJ, Beezhold DH (2013) Fungal hemolysins. Med Mycol 51:1–16

Ni M, Feretzaki M, Li W, Floyd-Averette A, Mieczkowski P, Dietrich FS, Heitman J (2013) Unisexual and heterosexual meiotic reproduction generate aneuploidy and phenotypic diversity de novo in the yeast *Cryptococcus neoformans*. PLoS Biol 11, e1001653

Niederpruem DJ (1963) Role of carbon dioxide in the control of fruiting of *Schizophyllum commune*. J Bacteriol 85:1300–1308

Ohm RA, de Jong JF, Lugones LG, Aerts A, Kothe E, Stajich JE, de Vries RP, Record E, Levasseur A, Baker SE, Bartholomew KA, Coutinho PM, Erdmann S, Fowler TJ, Gathman AC, Lombard V, Henrissat B, Knabe N, Kües U, Lilly WW, Lindquist E, Lucas S, Magnuson JK, Piumi F, Raudaskoski M, Salamov A, Schmutz J, Schwarze FW, vanKuyk PA, Horton JS, Grigoriev IV, Wösten HAB (2010) Genome sequence of the model mushroom Schizophyllum commune. Nat Biotechnol 28:957–963

Ohm RA, de Jong JF, de Bekker C, Wösten HAB, Lugones LG (2011) Transcription factor genes of *Schizophyllum commune* involved in regulation of mushroom formation. Mol Microbiol 81:1433–1445

Ohm RA, Aerts D, Wösten HAB, Lugones LG (2013) The blue light receptor complex WC-1/2 of *Schizophyllum commune* is involved in mushroom formation and protection against phototoxicity. Environ Microbiol 15:943–955

Perkins JH (1969) Morphogenesis in *Schizophyllum commune*. I. Effects of white light. Plant Physiol 44:1706–1711

Plaza DF, Lin CW, van der Velden NSJ, Aebi M, Künzler M (2014) Comparative transcriptomics of the model mushroom *Coprinopsis cinerea* reveals tissue-specific armories and a conserved circuitry for sexual development. BMC Genomics 15:492

Purschwitz J, Müller S, Kastner C, Fischer R (2006) Seeing the rainbow: light sensing in fungi. Curr Opin Microbiol 9:566–571

Purschwitz J, Müller S, Kastner C, Schöser M, Haas H, Espeso EA, Atoui A, Calvo AM, Fischer R (2008) Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. Curr Biol 18:255–259

Quiroz-Castañeda RE, Martínez-Anaya C, Cuervo-Soto LI, Segovia L, Folch-Mallol JL (2011) Loosenin, a novel protein with cellulose-disrupting activity from *Bjerkandera adusta*. Microb Cell Factories 10:8

Raper CA (1976) Sexuality and life cycle of the edible, wild *Agaricus bitorquis*. J Gen Microbiol 95:54–66

Raper JR, Miles PG (1958) The genetics of *Schizophyllum commune*. Genetics 43:530–546

Raper JR, Boyd DH, Raper CA (1965) Primary and secondary mutations at the incompatibility loci in *Schizophyllum*. Proc Natl Acad Sci USA 53:1324–1332

Raper CA, Raper JR, Miller RE (1972) Genetic analysis of the life cycle of *Agaricus bisporus*. Mycologia 64:1088–1117

Raudaskoski M, Vauras R (1982) Scanning electron microscope study of fruit body differentiation in *Schizophyllum commune*. Trans Br Mycol Soc 78:89–96

Raudaskoski M, Viitanen H (1982) Effects of aeration and light on fruit-body induction in *Schizophyllum commune*. Trans Br Mycol Soc 78:89–96

Raudaskoski M, Yli-Mattila T (1985) Capacity for photoinduced fruiting in the dikaryon of *Schizophyllum commune*. Trans Br Mycol Soc 85:145–151

Reijnders AFM, Stafleu JA (1992) The development of the hymenophoral trama in the Aphylophorales and Agaricales. Stud Mycol 34:1–109

Ruiters MHJ, Sietsma JH, Wessels JGH (1988) Expression of dikaryon-specific mRNAs of *Schizophyllum commune* in relation to incompatibility genes, light, and fruiting. Exp Mycol 12:60–69

Sánchez C (2010) Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl Microbiol Biotechnol 85(5):1321–1337

Sánchez C, Moore D (1999) Conventional histological stains selectively stain fruit body initials of basidiomycetes. Mycol Res 103:315–318

Sánchez C, Tellez-Tellez M, Diaz-Godinez G, Moore D (2004) Simple staining detects ultrastructural and biochemical differentiation of vegetative hyphae and fruit body initials in colonies of *Pleurotus pulmonarius*. Lett Appl Microbiol 38:483–487

Sarikaya Bayram O, Bayram O, Valerius O, Park HS, Irniger S, Gerke J, Ni M, Han KH, Yu JH, Braus GH (2010) LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. PLoS Genet 6, e1001226

Scholtmeijer K, de Vocht ML, Rink R, Robillard GT, Wösten HAB (2009) Assembly of the fungal SC3 hydrophobin into functional amyloid fibrils depends on its concentration and is promoted by cell wall polysaccharides. J Biol Chem 284:26309–26314

Schubert D, Raudaskoski M, Knabe N, Kothe E (2006) Ras GTPase-activating protein Gap1 of the homobasidiomycete *Schizophyllum commune* regulates hyphal growth orientation and sexual development. Eukaryot Cell 5(4):683–695

Schuren FHJ (1999) Atypical interactions between the *thn* and wild-type mycelia of *Schizophyllum commune*. Mycol Res 103:1540–1544

Schuren FHJ, Wessels JGH (1990) Two genes specifically expressed in fruiting dikaryons of *Schizophyllum commune*: homologies with a gene not regulated by mating-type genes. Gene 90:199–205

Schuurs TA, Dalstra HJP, Scheer JMJ, Wessels JGH (1998) Positioning of nuclei in secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. Fungal Genet Biol 23:150–161

Schwalb MN, Miles PG (1967) Morphogenesis of *Schizophyllum commune*. I. Morphological variation and mating behaviour of the *thin* mutation. Am J Bot 54:440–446

Singh RS, Bhari R, Kaur HP (2010) Mushroom lectins: current status and future perspectives. Crit Rev Biotechnol 30:99–126

Springer J, Wessels JGH (1989) A frequently occurring mutation that blocks the expression of fruiting genes in *Schizophyllum commune*. Mol Gen Genet 219:486–488

Sun H, Zhao CG, Tong X, Qi YP (2003) A lectin with mycelia differentiation and antiphytovirus activities from the edible mushroom *Agrocybe aegerita*. J Biochem Mol Biol 36:214–222

Suzuki H, Vuong TV, Gong Y, Chan K, Ho C-Y, Master ER, Kondo A (2014) Sequence diversity and gene expression analyses of expansin-related proteins in the white-rot basidiomycete, *Phanerochaete carnosa*. Fungal Genet Biol. doi:10.1016/j.fgb.2014.05.008

Swamy S, Uno I, Ishikawa T (1984) Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 130:3219–3224

Terashima K, Yuki K, Muraguchi H, Akiyama M, Kamada T (2005) The *dst1* gene involved in mushroom photomorphogenesis of *Coprinus cinereus* encodes a putative photoreceptor for blue light. Genetics 171:101–108

Todd RB, Zhou M, Ohm RA, Leeggangers HACF, Visser L, de Vries RP (2014) Prevalence of transcription factors in ascomycete and basidiomycete fungi. BMC Genomics 15:240

Tsusué YM (1969) Experimental control of fruit-body formation in *Coprinus macrorhizus*. Dev Growth Diff 11:164–178

Ullrich RC, Anderson JB (1978) Sex and diploidy in *Armillaria mellea*. Exp Mycol 2:119–129

Umar MH, van Griensven LJLD (1997) Morphogenetic cell death in developing primordia of *Agaricus bisporus*. Mycologia 89:274–277

Uno I, Ishikawa T (1971) Chemical and genetical control of induction of monokaryotic fruiting bodies in *Coprinus macrorhizus*. Mol Gen Genet 113:228–239

van der Valk P, Marchant R (1978) Hyphal ultrastructure in fruit body primordial of the basidiomycetes *Schizophyllum commune* and *Coprinus cinereus*. Protoplasma 95:57–72

van Driel KGA, Van Peer AF, Grijpstra J, Wösten HAB, Verkleij AJ, Müller WH, Boekhout T (2008) Septal pore cap protein SPC18, isolated from the basidiomycetous fungus *Rhizoctonia solani*, also resides in pore plugs. Eukaryot Cell 7(10):1865–1873

van Griensven LJLD (ed) (1988) The cultivation of mushrooms. Darlington Mushroom Laboratories, Rustington, Sussex

van Peer AF, Müller WH, Boekhout T, Lugones LG, Wösten HAB (2009) Cytoplasmic continuity revisited: closure of septa of the filamentous fungus *Schizophyllum commune* in response to environmental conditions. PLoS ONE 4(6)

van Peer AF, Wang F, van Driel KGA, de Jong JF, van Donselaar EG, Müller WH, Boekhout T, Lugones LG, Wösten HAB (2010) The septal pore cap is an organelle that functions in vegetative growth and mushroom formation of the wood-rot fungus *Schizophyllum commune*. Environ Microbiol 12 (4):833–844

van Wetter MA, Wösten HAB, Wessels JGH (2000a) SC3 and SC4 hydrophobins have distinct functions in formation of aerial structures in dikaryons of *Schizophyllum commune*. Mol Microbiol 36:201–210

van Wetter MA, Wösten HAB, Sietsma JH, Wessels JGH (2000b) Hydrophobin gene expression affects hyphal wall composition in *Schizophyllum commune*. Fungal Genet Biol 31:99–104

Vidic I, Berne S, Drobne D, Maček P, Frangež R, Turk T, Štrus J, Sepčić K (2005) Temporal and spatial expression of ostreolysin during development of the oyster mushroom (*Pleurotus ostreatus*). Mycol Res 109(3):377–382

Watling R (1996) Patterns in fungal development - fruiting patterns in nature. In: Chiu S-W, Moore D (eds) Patterns in fungal development. Cambridge University Press, Cambridge

Weber M, Salo V, Uuskallio M, Raudaskoski M (2005) Ectopic expression of a constitutively active Cdc42 small GTPase alters the morphology of haploid and dikaryotic hyphae in the filamentous homobasidiomycete *Schizophyllum commune*. Fungal Genet Biol 42(7):624–637

Wells K, Wells EK (eds) (1982) Basidium and basidiocarp. Springer, New York

Wessels JGH (1965) Morphological and biochemical processes in *Schizophyllum commune*. Wentia 13:1–113

Wessels JGH (1986) Cell wall synthesis in apical hyphal growth. Int Rev Cytol 104:37–79

Wessels JGH (1990) Role of cell wall architecture in fungal tip growth generation. In: Heath IB (ed) Tip growth of plant and fungal cells. Academic, San Diego, pp 1–29

Wessels JGH (1993) Fruiting in the higher fungi. Adv Microb Physiol 34:147–202

Wessels JGH (1994) Developmental regulation of fungal cell wall formation. Annu Rev Phytopathol 32:413–437

Wessels JGH (1997) Hydrophobins: proteins that change the nature of the fungal surface. Adv Microb Physiol 38:1–45

Wessels JGH, Marchant JR (1974) Enzymatic degradation in hyphal wall preparations from a monokaryon and a dikaryon of *Schizophyllum commune*. J Gen Microbiol 83:359–368

Wessels JGH, Mulder GH, Springer J (1987) Expression of dikaryon-specific and non-specific mRNAs of *Schizophyllum commune* in relation to environmental conditions and fruiting. J Gen Microbiol 133:2557–2561

Wessels JGH, de Vries OMH, Ásgeirsdóttir SA, Schuren FHJ (1991a) Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in *Schizophyllum*. Plant Cell 3:793–799

Wessels JGH, de Vries OMH, Ásgeirsdóttir SA, Springer J (1991b) The *thn* mutation of *Schizophyllum commune*, which suppresses formation of aerial hyphae, affects expression of the *Sc3* hydrophobin gene. J Gen Microbiol 137:2439–2445

Wessels JGH, Ásgeirsdóttir SA, Birkenkamp KU, de Vries OMH, Lugones LG, Scheer JMJ, Schuren FHJ, Schuurs TA, van Wetter M-A, Wösten HAB (1995) Genetic regulation of emergent growth in *Schizophyllum commune*. Can J Bot 73: S273–S281

Wessels JGH, Schuurs TA, Dalstra HJP, Scheer JMJ (1998) Nuclear distribution and gene expression in the secondary mycelium of *Schizophyllum commune*. In: Gow N, Robson G, Gadd G (eds) The fungal colony. Cambridge University Press, Cambridge, pp 302–325

Whitehouse HLK (1949) Multiple allelomorph heterothallism in the fungi. New Phytol 48:212–244

Wiemer M, Grimm C, Osiewacz HD (2016) Molecular control of fungal senescence and longevity. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 155–181

Woolston BM, Schlagnhaufer C, Wilkinson J, Larsen J, Shi Z, Mayer KM, Walters DS, Curtis WR, Romaine CP (2011) Long-distance translocation of protein during morphogenesis of the fruiting body in the filamentous fungus, *Agaricus bisporus*. PLoS ONE 6, e28412

Wösten HAB (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55:625–646

Wösten HAB, de Vocht ML (2000) Hydrophobins, the fungal coating unravelled. Biochim Biophys Acta Rev Biomembranes 1469:79–86

Wösten HAB, Wessels JGH (1997) Hydrophobins, from molecular structure to multiple functions in fungal development. Mycoscience 38:363–374

Wösten HAB, Willey JM (2000) Surface active proteins enable microbial aerial hyphae to grow into the air. Microbiology 146:767–773

Wösten HAB, Moukha SM, Sietsma JH, Wessels JGH (1991) Localization of growth and secretion of proteins in *Aspergillus niger*. J Gen Microbiol 137:2017–2024

Wösten HAB, de Vries OMH, Wessels JGH (1993) Interfacial self-assembly of a fungal hydrophobin into a hydrophobic rodlet layer. Plant Cell 5:1567–1574

Wösten HAB, Ásgeirsdóttir SA, Krook JH, Drenth JHH, Wessels JGH (1994a) The Sc3p hydrophobin self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. Eur J Cell Biol 63:122–129

Wösten HAB, Schuren FHJ, Wessels JGH (1994b) Interfacial self-assembly of a hydrophobin into an amphipathic membrane mediates fungal attachment to hydrophobic surfaces. EMBO J 13:5848–5854

Wösten HAB, Ruardy TG, van der Mei HC, Busscher HJ, Wessels JGH (1995) Interfacial self-assembly of a *Schizophyllum commune* hydrophobin into an insoluble amphipathic membrane depends on surface hydrophobicity. Coll Surf B Biointerface 5:189–195

Wösten HAB, van Wetter MA, Lugones LG, van der Mei HC, Busscher HJ, Wessels JGH (1999) How a fun-

gus escapes the water to grow into the air. Curr Biol 9:85–88

Wösten HAB, van Veluw GJ, de Bekker C, Krijgsheld P (2013) Heterogeneity in the mycelium: implications for the use of fungi as cell factories. Biotechnol Lett 35:1155–1164

Yamagishi K, Kimura T, Suzuki M, Shinmoto H (2002) Suppression of fruit-body formation by constitutively active G-protein alpha-subunits ScGP-A and ScGP-C in the homobasidiomycete *Schizophyllum commune*. Microbiology 148:2797–2809

Yamagishi K, Kimura T, Suzuki M, Shinmoto H, Yamaki KJ (2004) Elevation of intracellular cAMP levels by dominant active heterotrimeric G protein alpha Subunits ScGP-A and ScGP-C in homobasidiomycete, *Schizophyllum commune*. Biosci Biotechnol Biochem 68:1017–1026

Zhang W, Wu X, Zhou Y, Liu Z, Zhang W, Niu X, Zhao Y, Pei S, Zhao Y, Yuan S (2014) Characterization of stipe elongation of the mushroom *Coprinus cinereus*. Microbiology. doi:10.1099/mic.0.079418-0

16 Sexual Development in Fungi

Leonardo Peraza-Reyes[1], Fabienne Malagnac[2]

CONTENTS

[1]Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, 04510 México, D.F., Mexico; e-mail: lperaza@ifc.unam.mx
[2]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France; e-mail: fabienne.malagnac@u-psud.fr

I. Introduction

The kingdom Fungi is a vast and diverse group of eukaryotic organisms that exhibit a broad ecological distribution. Their metabolic and physiological resourcefulness enable them to colonize a wide variety of habitats and to establish intricate biological associations with most other organisms, which are crucial for ecosystem structure and functioning. Much of the success of fungi in nature is related to the large diversity of reproductive systems that has evolved throughout their evolution. Fungi are able to reproduce asexually by propagating vegetatively or by producing asexual propagules or spores,

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

which are frequently produced in large numbers and that allow extensive dissemination. In addition, most fungi are able to reproduce sexually. Sexual reproduction in fungi entails a large diversity of developmental strategies and mating systems, which correlates with their specific lifestyles. This includes inbreeding systems that permit a single individual to self-fertilize and to complete sexual reproduction on its own, notwithstanding outcrossing between genetically different individuals, as well as obligate outbreeding systems in which only genetically distinct compatible partners are able to mate (Ni et al. 2011; Billiard et al. 2012). On the end of this spectrum, elaborate multifactorial incompatibility systems have evolved in fungi, where a fungal species might posses over 20,000 different sexes (mating types), that enable to establish very high outbreeding rates (Kothe 1996). Moreover, breeding systems that allow self-sterility to be bypassed to different extent (pseudohomothallism, Billiard et al. 2012) or that generate ploidy changes and recombination without meiosis (i.e., parasexual reproduction, Sherwood and Bennett 2009) extend the diversity of reproductive systems evolved in fungi.

Sexual reproduction enables genetic recombination and the production of genetically diverse offspring, which has long been considered to facilitate adaptive evolution (Aanen and Hoekstra 2007). Besides, sexual reproduction can also reduce the accumulation of deleterious mutations (Bruggeman et al. 2003). In addition, sexual reproduction in fungi is associated to developmental roles such as the formation of survival/resistance structures (e.g., Doll et al. 2013; Coluccio et al. 2008; Aanen and Hoekstra 2007).

In fungi, sexual reproduction involves the differentiation of mating structures, the recognition of mating partners, and the fusion—in most fungi—of haploid cells (plasmogamy) and nuclei (karyogamy) to produce a diploid zygote, which subsequently undergoes meiosis. In most fungi, this process culminates with the formation of haploid sexual spores, which enclose the nuclear products of meiosis and permit the dissemination of the meiotic offspring (the sexual life cycles of two model ascomycete fungi are illustrated in Figs. 16.1 and 16.2). Sexual reproduction involves important changes in cellular functioning and architecture. This process requires precise coordination between the differentiation of multiple cell types and the progression of nuclei through karyogamy and meiosis and often must be orchestrated with the morphogenic process driving the formation of complex multicellular fruiting bodies, where sexual development takes place. The essence of sexual reproduction lays on meiosis, which constitutes the reductional division that allows, along with karyogamy, for the alternation of haploid and diploid phases and that provides the potential for genetic recombination. In this chapter, we describe the molecular and cellular bases of the developmental processes implicated in sexual reproduction in fungi, with an emphasis on the cell biology of the mating process and on the developmental events that connect this process to meiosis. Further detailed information on the genetic systems governing sexual identity and determination can be consulted elsewhere in this book.

II. Sexual Identity and Mate Recognition in Fungi

A. Sexual Compatibility in Fungi Is Defined by Mating-Type Loci

The cell identity implicated in sex determination in fungi is defined by a specialized genomic region known as the mating-type locus, which is designated by the MAT acronym. Two basic strategies defining mating behavior have evolved in fungi. Some fungi are able to self-mate and are referred to as homothallic, whereas other species are unable to mate between genetically identical cells and are, thus, self-sterile. Mating in these latter fungi, which are known as heterothallic, only takes place between compatible partners that differ at the mating-type locus (Figs. 16.1A and 16.2). In these fungi, the MAT locus of compatible strains encompasses alternative idiomorphic alleles, which are located at the same genomic position but that differ in sequence and information. So far, the mating system of all studied heterothallic ascomycetes and

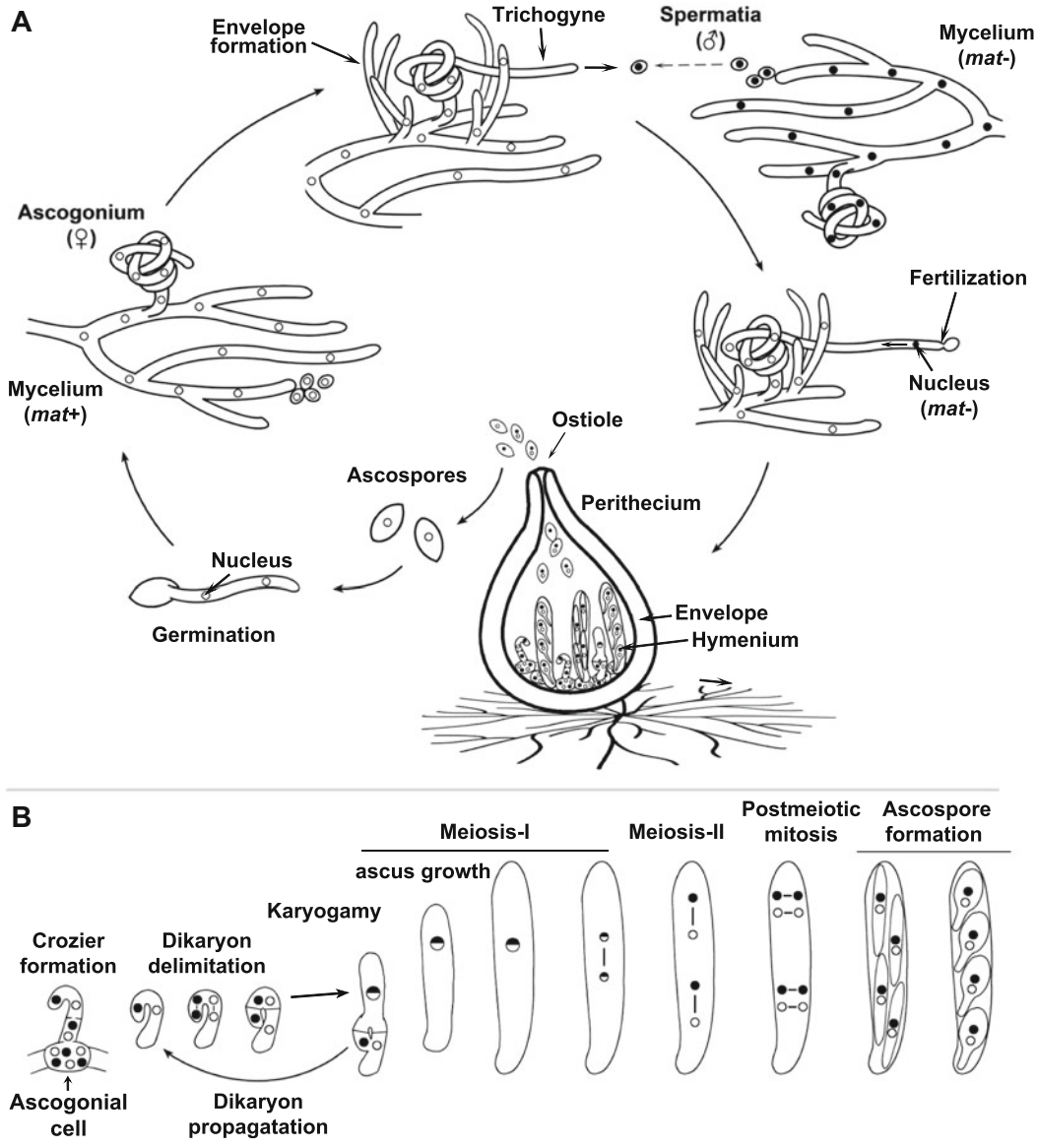


Fig. 16.1 Sexual development of a model ascomycete fungus. (**A**) The sexual life cycle of a mycelial heterothallic ascomycete. The somatic (vegetative) phase of most Pezizomycotina consists on haploid hyphal cells that grow by apical extension and producing ramifications to establish an intricate hyphal system known as the mycelium. In these fungi, sexual reproduction typically takes place inside of multicellular fruiting bodies emerging from the mycelium and known as ascomata (singular ascoma). These structures are formed by a sexual tissue (the hymenium) surrounded by a protective envelope of highly interwoven hyphae. In a number of Pezizomycotina, ascomata consist on piriform fructifications opening by an upper ostiole, which are known as perithecia (singular perithecium). Heterothallic ascomycetes possess two mating types, which are defined genetically (denoted in the figure as *MAT*+ and *MAT*– and depicted by different nuclear shading). Mycelia of either mating type differentiate both female gametangia (ascogonia) and male gametes, but mating only occurs between sexual structures of opposite mating type. The ascogonium emerges from vegetative hyphae as a single multinucleated hyphal branch that coils around itself. Ascogonia then recruit neighboring hyphae and become surrounded by a protective coat of aggregate hyphae, which after fertilization produces the perithecium enve-

zygomycetes is governed by a single MAT locus, which possesses two alternative versions and defines a bipolar mating system. In contrast, a multifactorial mating system composed of two MAT loci controls sexual compatibility in basidiomycete fungi. In these fungi, the two MAT loci might be located at distinct genomic positions and be, therefore, genetically unlinked. This arrangement results in a tetrapolar mating system, in which four different mating types are generated after meiosis. Nevertheless, the two MAT loci of some basidiomycetes have physically coalesced to constitute a single locus, which defines a bipolar system (Raudaskoski and Kothe 2010; Debuchy et al. 2010; Freihorst et al. 2016; Dyer et al. 2016, for a comprehensive analysis on mating-type structure, function, and evolution).

Self-fertility in fungi has evolved by different genetic and molecular mechanisms. The mating system of homothallic fungi is also controlled by the MAT locus. However, in these fungi, the MAT locus has adopted an arrangement that consents sexual reproduction to occur between genetically identical cells. For instance, the genetic mechanisms that allow for self-mating in ascomycete fungi include the presence of both mating-type idiomorphs in a single genome or the existence of a single MAT idiomorph that allows unisexual mating. Additionally, secondary homothallism has evolved in some fungal species by molecular mechanisms that allow heterothallism to be bypassed, such as mating-type switching or the co-segregation of compatible mating-type nuclei when sexual spores are made (Ni et al. 2011) (an example of the latter process is illustrated by the development of the pseudohomothallic ascomycete *Podospora anserina* in Fig. 16.1B).

B. Sexual Pheromones Manifest the Mating Identity of Fungal Cells

The mating-type loci genetically define the mating identity of individuals in fungi. However, this identity is expressed by means of distinctively produced sexual pheromones. In most fungi, sexual pheromones are produced in a mating-type-specific manner, and they constitute the main factor that allows fungi to detect mating partners and to establish mating compatibility. Nevertheless, among different evolutionary lineages, fungal pheromones exert their action at different levels of the sexual determination

Fig. 16.1 (continued) lope. Ascogonia also differentiate specialized hyphae—the trichogynes—that exhibit positive tropism toward opposite mating-type pheromone-producing cells. Trichogynes provide the female gametic cells, which grow toward and fuse with male fertilizing cells. Vegetative hyphal fragments or asexual spores can fertilize trichogynes, but some fungi differentiate specialized cells (antherida) or spores (spermatia) to fulfill this role. After fertilization, the male gametic nucleus is delivered through the trichogyne into the ascogonium. Male and female nuclei then divide within the coenocytic ascogonium before differentiating the sexual cell lineages that constitute the hymenium and where karyogamy, meiosis, and sexual spore (ascospore) formation takes place. Ultimately, the mature ascospores are forcibly expelled out of the perithecium. (**B**) Sexual development from the dikaryotic stage to ascospore differentiation. From *left* to *right*: Fertilized ascogonia present within ascomata contain male and female nuclei (of opposite mating type in heterothallic fungi), which migrate into ascogenous cells emerging from ascogonial cells. Ascogenous hyphae elongate and grow around producing hook-shaped cells called croziers. After coordinated mitoses (*lines* linking nuclei represent spindles) and septa formation, each crozier is separated into three cells: a penultimate (*upper*) binucleated cell flanked by to uninucleated cells. The two uninucleated cells fuse, and the terminal cell nucleus migrates into the basal cell, which regains the dikaryotic state and further produces new dikaryotic croziers. Concomitantly, the upper dikaryotic cell undergoes karyogamy and develops into an ascus, where meiosis takes place (the meiocyte). The meiotic-derived haploid nuclei are ultimately packaged into ascospores, but in many fungi, this process is preceded by additional mitoses. In the figure, which illustrates the development of *P. anserina*, a postmeiotic mitosis yields eight haploid nuclei, which are enclosed by pairs into ascospores. In this fungus, the high frequency of second meiotic division segregation of the *MAT* locus and the packaging of two non-sister nuclei per ascospore result in the formation of binucleated spores containing one nuclei of each mating type, which upon germination produce heterokaryotic self-fertile mycelium (pseudohomothallic inbreeding)

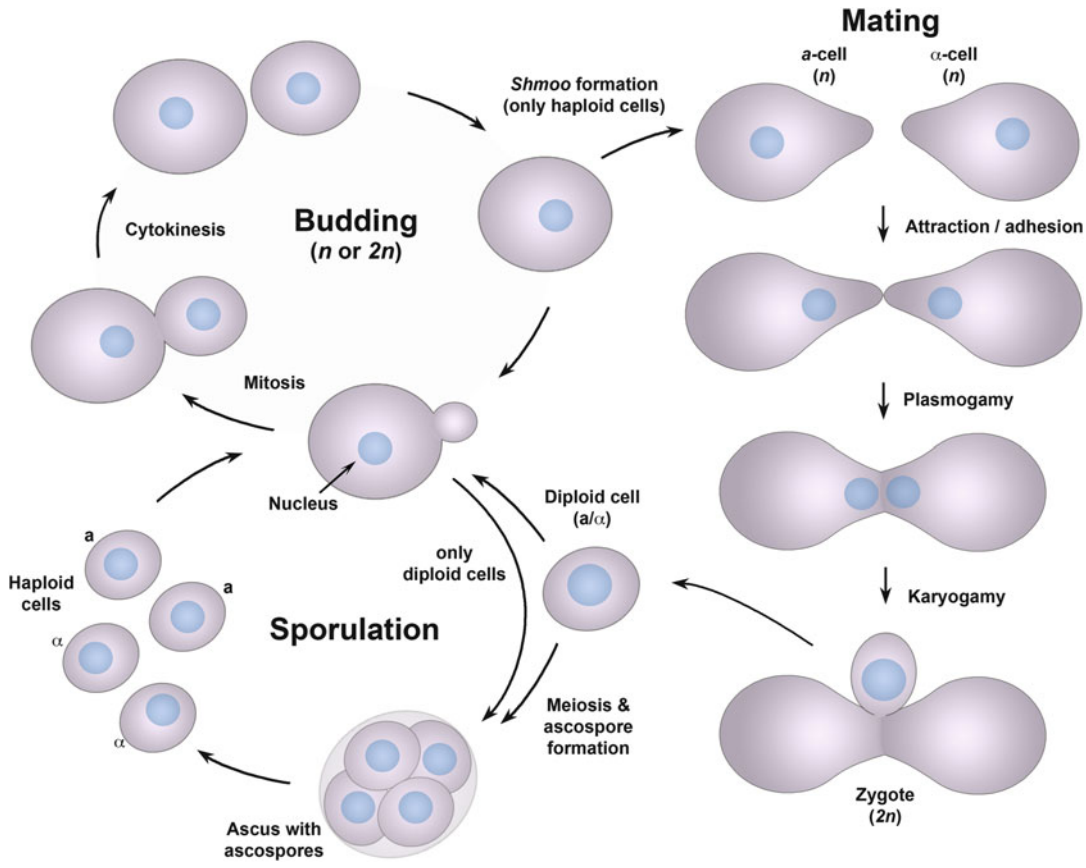


Fig. 16.2 The life cycle of *Saccharomyces cerevisiae*. The ascomycete yeast *S. cerevisiae* can proliferate mitotically by budding of either haploid or diploid cells. However, only haploid cells are able to reproduce sexually. Sexual reproduction is initiated when cells of opposite mating type (*S. cerevisiae* possesses two mating types, denoted as *a* and α) encounter each other, and the pheromones produced by one mating type induce the formation of *shmoos* in the opposite mating type. *Shmoos* are cells competent to mating that produce a mating projection toward an opposite mating-type pheromone source. Like this, the mating projections of opposite mating-type *shmoos* encounter each other and undergo plasmogamy, which is immediately followed by karyogamy. This results in the formation of a diploid zygote, which on favorable nutritional conditions propagates asexually by budding. When diploid cells are exposed to nutritional limitations, they undergo meiosis and pack their resulting nuclei into ascospores. The four resulting haploid ascospores are enclosed inside the original mother cell, the ascus. Finally, ascospores are released from asci and proliferate by budding

process. Furthermore, sexual pheromones consist on molecules of different chemical nature in different fungi. This indicates that **different pheromone-based communication systems have evolved in fungi**. The evolution of such systems has allowed consenting sexual reproduction among diverse fungi, which differ in evolutionary origin and lifestyle.

1. Sexual Pheromones in Chytridiomycetes and Zygomycetes

Fungal sexual pheromones were first identified and characterized in the Blastocladiomycota fungus *Allomyces macrogynus* (Machlis 1958; Machlis et al. 1966; Nutting et al. 1968). In this aquatic fungus, male and female gametes consist of motile uninucleated cells that bear a

single posterior flagellum. These gametes are produced in separate gametangia, which are superposed in a common hyphal branch (a terminal male gametangium laying on top of a subterminal female gametangium), and differ in size and subcellular architecture. **The female gametes of this fungus attract the smaller male gametes by producing a low molecular mass diffusible pheromone of sesquiterpene nature, which is known as sirenin** (Nutting et al. 1968; Machlis 1968). Sirenin secretion by female gametes begins before they are released from gametangia. This attracts male gametes to swarm around the developing female gametangia, ready to fertilize female gametes as soon as they emerge (Carlile 1996; Machlis 1958). Evidence also indicates that male cells also produce a female attractant, parisin (Pommerville and Olson 1987), but this pheromone has remained uncharacterized. Also uncharacterized has remained the role that these pheromones exert beyond gamete attraction, and whether these molecules participate in fertilization or postfertilization events is currently unknown.

Mating in zygomycete fungi has most intensely been studied in Mucoromycotina. In these fungi, mating typically takes place by conjugation between specialized sexually committed hyphae, which differentiate from the vegetative mycelium. These hyphae might consist on aerial hyphae, which are morphologically indistinguishable from the surrounding aerial mycelium, or on distinct specialized hyphal branches that emerge from vegetative hyphae. These specialized hyphae are known as zygophores, and they have the ability to grow toward each other to meet and engage in sexual reproduction. In heterothallic species, zygophores come from opposed parents, whereas in homothallic ones, they originate within a common mycelium. After their initial contact, zygophores differentiate into progametangia by swelling their apposed tips. Each progametangium then divides by forming a septum, resulting in the formation of two cells: the gametangium and its suspensor cell. Plasmogamy takes place at the site where the two gametangia make contact, and a zygospore further differentiates at this site. Zygospores are zygotic resting spores, which ultimately undergo karyogamy and meiosis (Wostemeyer and Schimek 2007; Idnurm et al. 2007).

The pheromones implicated in sexual determination have been identified in two zygomycete lineages—Mucormycotina and Mortierellomycotina—and in both these lineages, they consist on trisporic acid-based pheromones (Gooday and Carlile 1997; Schimek et al. 2003; Wostemeyer and Schimek 2007). These pheromones are capable of inducing zygophore formation, which is the earliest recognizable sexual differentiation event in these fungi, and directing the growth of the zygophores from opposed mating partner toward each other, a process that is known as zygotropism. Interestingly, externally added trisporic acid is able to induce the formation of zygophores irrespectively of the mating type of a strain (Van den Ende et al. 1970). Furthermore, trisporoid pheromones are also active in inducing zygophore formation of a mucoralean species even if produced in the mating reaction of a different one (Austin et al. 1969; Gooday 1968; Van Den Ende 1967). Trisporoid pheromones provide, however, a highly specific and stringent system for sexual communication, which allows induction of sexually committed hyphae only in the presence of a compatible partner and that permits to finely direct the mating cell growth, ensuring accurate zygotropism of the developing zygophores.

The specificity of the trisporoid pheromone system is provided by the regulation of the synthesis of trisporic acid (recently reviewed in Lee and Heitman 2014). This process involves the sequential action of specific enzymes, which are solely produced by a mating partner. In this system, a trisporoid precursor synthesized and released by a mating-type strain is taken up and modified by its compatible partner, which then produces a molecule that is active as a pheromone. This process results in the cooperative formation of a number of trisporic acid derivatives, which in turn stimulate the production of enzymes involved in trisporic acid synthesis in a mating-type-dependent manner (Sahadevan et al. 2013; Czempinski et al. 1996; Schimek et al. 2005; Wetzel et al. 2009). This results in a feedback trisporic acid pheromone production system, which ensures that no trisporic acid is

produced in unmated cultures. This system prevents sexual responses in single mating partner cultures and establishes a tight sexual communication that allows to accurately finding a compatible partner.

Currently, the processes underlying zygophore differentiation, as well as the mechanism by which trisporoid pheromones regulate zygotropism, is currently not known. Also currently unknown is whether trisporoid pheromones are directly involved in the mating process per se. Interestingly, in contrast to other fungi where the mating-type genes regulate pheromone production—like most Ascomycota fungi—in the zygomycete fungus *Mucor mucedo*, the mating-type genes are themselves regulated by trisporoid pheromones. This suggests a cooperative mate recognition system, in which pheromones could contribute to define sexual identity before the action of the mating-type genes. This could also indicate that, in addition to mate recognition, the mating-type genes of this fungus participate in later stages of sexual development (Wetzel et al. 2012).

2. Mating Pheromones in the Dikarya Fungi

The function of fungal sexual pheromones has been more thoroughly studied **in the Dikarya fungi, where pheromones controlling sexual compatibility are peptidic in nature**. These pheromones consist on short peptides, which are the processed products of pheromone precursor genes belonging to two dissimilar classes. The first class—known as *alpha*-class (after the *S. cerevisiae* paradigm; see below)—encodes for peptidic pheromones that are produced, processed, and secreted by the standard secretory pathway. In contrast, the second pheromone class (the *a*-class) consists on lipid-modified peptides, which are processed in the cytosol and are exported out of the cell through specific cell transporters. Whereas *alpha*-class pheromones are exclusively present in ascomycete fungi, the lipopeptidic *a*-class pheromones are present both in ascomycetes and basidiomycetes (Jones and Bennett 2011).

One gene of each pheromone class is present in a common genome of Ascomycota fungi (although paralogues may exist), irrespectively of its mating type. However, the expression of these genes in most fungi is mating-type specific. In heterothallic fungi, each mating type expresses a single pheromone class precursor gene: strains of the MAT1-1 mating type (please refer to Dyer et al. 2016, for information on mating-type nomenclature) express the *alpha*-class gene, whereas MAT1-2 strains express the *a*-class. After secreted, each kind of pheromone is recognized by a specific cognate receptor, which is located on the cell surface of the opposite mating-type partner and that upon pheromone binding triggers the mating response. Expression of pheromone receptor-encoding genes is also mating-type specific (Jones and Bennett 2011). A notable exception for this organization is the heterothallic fungus *Hypocrea jecorina* (teleomorph of *Trichoderma reesei*), which lacks a prototypical *a*-class pheromone. Instead, this fungus possesses a hybrid pheromone that exhibits characteristics of both *alpha*- and *a*-class pheromones. Furthermore, transcription of the pheromone precursor genes in this fungus, or that of their receptors, is not strictly dependent on the mating type (Schmoll et al. 2010; Seibel et al. 2012).

In contrast to Ascomycota, a single locus for the pheromone precursor genes is present per genome in basidiomycete fungi, and the identity of this locus is mating-type specific. This locus may encompass a single pheromone precursor gene or a unique combination of genes, but its sequence is mating-type specific. Actually, the pheromone precursor genes, along with the pheromone receptor-encoding genes, constitute by themselves one of two loci that define the mating type of basidiomycete fungi (Wendland et al. 1995; Bolker et al. 1992; O'Shea et al. 1998; for review, Raudaskoski and Kothe 2010 and Freihorst et al. 2016).

The locus that codes for pheromones and pheromone receptors in fungi like *Ustilago Maydis* (here, the *MATa* locus) possesses two alternative alleles, and each allele encodes a pheromone and a pheromone receptor. In this system, the pheromone encoded by one allele is specifically recognized by the receptor encoded

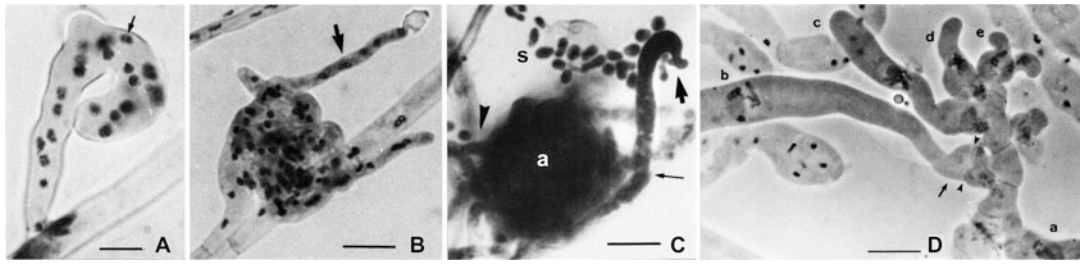


Fig. 16.3 Sexual structures of the model Sordariomycete fungus *P. anserina*. (**A**) Early female ascogonium emerging as a lateral multinucleate curved branch from a hyphal cell (*arrow points* to a nucleus). (**B**) The ascogonium coils around itself and produces a trichogenous hypha (*arrow*). (**C**) Ascogonia then become surrounded by enveloping hyphae, producing protoperithecia (note the emergence of an enveloping hypha—*arrowhead*—which grows over the expanding protoperithecium, *a*), from which the emerging trichogyne (*thin arrow*) captures a microconidium (the spermatium, *s*) to accomplish fertilization (*thick arrow*). Upon fertilization, the male nucleus is delivered into the ascogonium, and the protoperithecium develops into a perithecium. Inside perithecia, ascogenous cells differentiate from ascogonial cells and further develop into croziers. Each crozier ultimately forms two dikaryotic cells, an upper cell that produces an ascus and a basal cell that gives rise to new croziers. By this process, arborescences of croziers and asci simultaneously containing different progressive developmental stages are produced (**D**). Small lettering indicates progressive stages of ascus development. *a*–*d* are in first meiotic prophase: *a*, diplotene; *b*, pachytene; *c* and *d*, zygotene. *e* shows an early prekaryogamy differentiating ascus. Note a primordial reminiscent crozier (*arrow*) divided by septa (*arrowheads*), in which the upper (*left*) cell has differentiated into an ascus, whereas the lower cell has produced a new dikaryotic cell, which in turn successively produced new croziers and asci. Scale bar, 5 μm. Images are kind courtesy of D. Zickler (Univ. Paris Sud, France), adapted from Zickler et al. (1995) with permission

by the opposite allele and vice versa (Bolker et al. 1992). In contrast, a multi-allelic and often multigenic pheromone/receptor system has evolved in Agaricomycotina. In this system, the locus coding for pheromones and pheromone receptors may contain many subloci, each of which enclosing one receptor- and one (or multiple) pheromone-encoding gene (O'Shea et al. 1998; Wendland et al. 1995; Vaillancourt et al. 1997). Underscoring the complexity of this system, nine alleles have been identified for each of the two unlinked subloci that compose this locus in *Schizophyllum commune*, and a single allele of this locus encodes as many as two receptors and 11 pheromones (Fowler et al. 2004; Raudaskoski and Kothe 2010).

C. Mate Recognition and Attraction

The mating pheromones control mate compatibility in fungi by regulating different events of the sexual determination process. For example, sexual pheromones facilitate mating in most ascomycete fungi, but they are not essential for plasmogamy in many basidiomycetes. In these latter fungi, mating pheromones rather determine mate compatibility at postfertilization events (Raudaskoski 1998) (see also Sect. V.C).

Pioneer research in *Ascobolus stercorarius* revealed that the formation of sexual reproductive structures (ascogonia; Fig. 16.3A, B illustrates the differentiation of an ascogonium) of this heterothallic fungus does not take place in solo cultures, whatever the mating type. However, a diffusible substance produced by a cell of the opposite mating type was able to induce the differentiation of ascogonia and to further attract the growing trichogynes, the specialized female gametic cells produced by ascogonia that grow toward male cells to attain fertilization (see Fig. 16.3B, C and below) (Bistis 1956; Bistis and Raper 1963). These observations suggested a role for sexual pheromones both in the induction of sexual reproductive structures and in the chemotropic interaction between compatible mating partners. Nonetheless, further research in ascomycetes like *Neurospora crassa* and *Neurospora tetrasperma* revealed that single mating-type strains of these species were

capable of differentiating ascogonia in solitary cultures. Furthermore, these ascogonia were also able to differentiate functional trichogynes in the absence of opposite mating-type cells or pheromones. Addition of an opposite mating-type cell, however, was able to reorient the trichogyne growth toward that cell (Bistis 1981, 1996). These observations suggested that, in contrast to *A. stercorarius*, pheromones were dispensable for the differentiation of sexual reproductive structures in these fungi. Corroborating this notion, further research demonstrated that elimination of the pheromone receptors in *N. crassa* does not prevent ascogonia formation (Kim and Borkovich 2004; Kim et al. 2012). Similarly, formation of primordial sexual structures is not prevented by the absence of pheromone receptors in homothallic species like *Sordaria macrospora* and *Gibberella zeae* (teleomorph of *Fusarium graminearum*) (Mayrhofer et al. 2006; Kim et al. 2008; Lee et al. 2008b).

Mating pheromones have been implicated in the chemotropic recognition system that allows detecting and attracting mating partners in most Ascomycota fungi. Trichogynes are specialized hyphae that emerge from ascogonial cells and grow toward male gametes to subsequently fuse and capture the male fertilizing nucleus (Fig. 16.3B, C). These hyphae are morphologically similar to vegetative hyphae, but they differ from the latter in that they are able to grow when the mycelium has reached stationary growth phase—where vegetative hyphal growth normally ceases—and in that their growth exhibits positive chemotropism toward mating pheromones (Debuchy et al. 2010). Actually, in heterothallic ascomycete fungi, genetic elimination of a pheromone produces male sterility in the cognate mating type (where the corresponding pheromone would normally be produced) (Coppin et al. 2005; Kim et al. 2002; Kim and Borkovich 2006; Turina et al. 2003). In *N. crassa*, it has been shown that this sterility results from inability of male gametes to attract trichogynes (Kim and Borkovich 2006). In contrast, the capacity of trichogynes to orientate their growth toward male gametes and to be fertilized by them is not disturbed in the absence of the pheromone precursor genes. Conversely, elimination of a pheromone receptor impedes female trichogynes to reorient their growth toward pheromone-producing male cells but does not affect the capacity of male cells to attract trichogynes (Kim and Borkovich 2004; Kim et al. 2012). These data indicate that **the recognition between male and female gametic cells in heterothallic ascomycetes depends on the interaction between pheromones and their cognate receptors.**

In the ascomycete yeast *Saccharomyces cerevisiae*, mating occurs spontaneously whenever compatible cells coexist on rich medium (Fig. 16.2 illustrates the life cycle of *S. cerevisiae*). In this yeast, an addition of mating pheromone is sufficient to induce *shmoo* formation, which is the first identifiable sexual differentiation event in this fungus (Duntze et al. 1970, 1973). Therefore, akin to zygomycete trisporic acid pheromones, pheromones in this yeast are sufficient to trigger sexual development. Nevertheless, gametic cell differentiation resulting in zygophores and yeast *shmoos* is intimately associated to the cell polarization process that drives gametic cell growth toward a pheromone source.

In contrast to *S. cerevisiae*, the fission yeast *Schizosaccharomyces pombe* requires not only cells of opposite mating type to be together, but sexual differentiation also requires specific stress conditions to be induced, most notably nitrogen starvation. In this yeast, an additional level of regulation is exerted by the high-mobility group (HMG) transcription factor Ste11. This protein, whose production, nuclear localization, and activity are subject to nitrogen availability, is a master regulator of the mating program and controls the expression of many genes required to induce sexual differentiation, as well as the subsequent meiotic development (Sugimoto et al. 1991; Mata and Bahler 2006; Qin et al. 2003). Actually, MAT genes are among the targets that are positively regulated by Ste11. Thus, the mating behavior of a cell in *S. pombe* is only established after Ste11 induces the expression of the mating-type genes, a process that is promoted upon nitrogen starvation. Mating-type gene expression then stimulates the production of pheromones and pheromone receptors, which allows the establishment of the mating response between partners. Interest-

Table 16.1 Main processes regulated by mating pheromones in *Saccharomyces cerevisiae*

Process	Main target/proteins involved	References
Mate recognition and attraction		
Global transcriptional activation of mating-involved genes[a]	Fus3 MAPK cascade, Ste12	Errede and Ammerer (1989) and Ren et al. (2000)
Cell cycle arrest at G1	Fus3 MAPK cascade, Far1, Cln	Chang and Herskowitz (1990) and Peter et al. (1993)
Polarity complex wandering for mating site selection	Actin dynamics/vesicle traffic?	Dyer et al. (2013)
Polarity complex activation for mating cell polarized growth toward a partner	Far1, Cdc24, Bem1, Ste5	Butty et al. (1998), Leeuw et al. (1995), Nern and Arkowitz (1999), and Shimada et al. (2000)
Actin filament arrangement for mating cell polarized growth toward a partner	Fus1, Bni1	Matheos et al. (2004)
Plasmogamy		
Adhesion between mating partners	*a-/alpha*-agglutinins	Lipke et al. (1989) and Roy et al. (1991)
Delivery of cell wall-remodeling enzyme secretory vesicles	Fus1	McCaffrey et al. (1987), Trueheart et al. (1987), and Gammie et al. (1998)
Secretion of cell wall-remodeling enzymes	Fus2, Rvs161	Trueheart et al. (1987), Gammie et al. (1998), Elion et al. (1995), and Erdman et al. (1998)
Plasma membrane merger	Prm1, Fig1	Erdman et al. (1998), Heiman and Walter (2000), and Aguilar et al. (2007)
Karyogamy		
Anchorage of microtubules to the SPB half bridge for nuclear orientation	Spc72, Kar1	Pereira et al. (1999)
Anchorage of microtubules to the *shmoo* tip for nuclear orientation	Kar3, Cik1, Gpa1	Maddox et al. (2003), Sproul et al. (2005), and Zaichick et al. (2009)
Nuclear congression	Kar3	Meluh and Rose (1990)
Nuclear outer membrane fusion	Prm3, Kar5	Beh et al. (1997), Shen et al. (2009), and Melloy et al. (2009)

[a]Please note that this is a broad category that includes the direct or indirect activation of many factors described below in the table

ingly, Ste11 itself is activated by the pheromone signaling system (Qin et al. 2003; Kjaerulff et al. 2005); therefore, the activity of this transcriptional regulator is further amplified upon pheromone stimulation. **In this way, the induction of sexual differentiation in *S. pombe* relies on a cooperative system that integrates the pheromone signaling response with the activity of a central transcriptional regulator, Ste11, which integrates physiological and environmental stimuli.** Of note, the *P. anserina* orthologue of Ste11 plays a central role as an upstream regulator of a genetic network of HMG-domain transcriptional regulators, which include mating-type gene-encoded transcription factors, that is required for mating and sexual development in this fungus (Ait Benkhali et al. 2013). This indicates a conserved role for Ste11 as a central regulator of the sexual determination process in ascomycete fungi.

D. Mating Signaling

1. Mating Pheromone Signaling: The Yeast Paradigm

Considerable understanding of the mating signaling system has been generated through research on yeasts, most significantly on *S. cerevisiae* (Table 16.1 summarizes the major cell developmental processes regulated by mating pheromones in *S. cerevisiae*). Interestingly, in most studied ascomycete yeasts, pheromone stimulation results in the polarization of cells toward the pheromone-producing partner

(Fig. 16.2), a process referred to as *shmooing*. This process involves a reorganization of the cellular structure encompassing rearrangement of cell membrane and cytoskeletal components.

S. cerevisiae pheromones produced by *a*- and *alpha*-mating-type cells are called *a*- and *alpha*-factors, respectively. These pheromones are recognized by specific G-protein-coupled seven-transmembrane domain receptors—Ste3 and Ste2, respectively—which are expressed and decorate the cell surface in a mating-type-specific manner (Ste3 on MAT*alpha* and Ste2 MAT*a* cells) (Jenness et al. 1983; Hagen et al. 1986; Sprague et al. 1983; Hartig et al. 1986). Both receptors are coupled to a common heterotrimeric G-protein. Pheromone binding stimulates the exchange of GDP for GTP by the G-protein alpha subunit (Gpa1), which promotes the dissociation of G-alpha from a G-beta/G-gamma heterodimer that is released to activate the mating signaling response (Whiteway et al. 1989; Nomoto et al. 1990; for review, see Merlini et al. 2013). A similar system operates in yeasts like *Candida albicans*, *Kluyveromyces lactis*, and the distantly related *S. pombe*; however, the G-protein subunits implicated in the activation of the mating signaling pathway in these yeasts differ. Whereas mating signaling in *S. pombe* is activated by the G-alpha subunit, which is negatively regulated by G-beta (Obara et al. 1991; Goddard et al. 2006), G-beta activates signaling in *K. lactis* independent of G-gamma (Navarro-Olmos et al. 2010), and both G-alpha and G-beta are required in *C. albicans* (Dignard et al. 2008).

Pheromone stimulation produces three main outcomes in *S. cerevisiae*. First, pheromone signaling is transmitted by a mitogen-activated protein (MAP) kinase module to the transcription factor Ste12, which promotes the transcription of genes implicated in mating. Second, the actin cytoskeleton and the exocytotic machinery are reorganized to bring about the cell polarization that orientates the *shmoos* to grow toward each other; third, the cell cycle is arrested to allow mating and karyogamy to take place between G1 nuclei. These three outputs are mainly mediated by effectors of the G-beta/G-gamma subunit, but the G-alpha subunit also contributes to polarity establishment (Merlini et al. 2013; Jones and Bennett 2011).

One key effector of G-beta/G-gamma is the scaffold protein Ste5. This protein interacts with released G-beta/G-gamma, which remains tethered to the plasma membrane, and recruits the three mating MAP kinases (Ste11, Ste7, and Fus3) to the cell cortex (Pryciak and Huntress 1998; Whiteway et al. 1995; Choi et al. 1994; Marcus et al. 1994). This brings together the MAP kinase (MAPK) module facilitating its activation and insulation from cross talk with other MAPK signaling pathways (Lamson et al. 2006; Zalatan et al. 2012). G-beta/G-gamma also interacts with, and activates the p21-activated kinase (PAK) Ste20, the upstream kinase that phosphorylates Ste11 (not to be confused with the *S. pombe* HMG transcription factor Ste11) and promotes the activation of the Fus3 MAPK cascade (Chen and Thorner 2007; Drogen et al. 2000; Leeuw et al. 1998). Upon activation, the downstream MAPK Fus3 is released from the cell membrane and is able to reach and phosphorylate its targets, leading to Ste12 activation (van Drogen et al. 2001). In addition, Fus3 also phosphorylates the scaffold protein Far1, which associates with and inhibits the G1 cyclin (Cln)/cyclin-dependent kinase (Cdc28p) complex, triggering G1 cell cycle arrest (Chang and Herskowitz 1990; Peter et al. 1993). Far1 is a Ste5-related protein that also coordinates cellular polarity by associating with polarity establishment proteins (see Sect. II.D.3).

2. Mating Pheromone Signaling in Filamentous Fungi

Different genetic and functional conservation is observed for the components of the signaling network that drives the pheromone response in fungi. Orthologues of the pheromone receptor-encoding genes—*STE2* and *STE3* in *S. cerevisiae*—have been so far identified in all studied Ascomycota fungi (Rispail et al. 2009). However, orthologues of *STE2*, which codes for the *alpha*-class pheromone receptor, are lacking in the genome of Basidiomycota fungi, like *U. maydis*, *Cryptococcus neoformans*, *Coprinopsis cinerea* (*Coprinus cinereus*), and *S. commune*. Instead, the pheromone receptors identified in these fungi all belong to the Ste3 type, which is in agreement with only *a*-class

pheromones being present in basidiomycete fungi (Rispail et al. 2009; Raudaskoski and Kothe 2010; Bolker et al. 1992). Consistent with the different structural nature of sexual pheromones of Chytridiomycetes and Zygomycetes, no orthologues of *STE2* and *STE3* have been observed in these fungi (Rispail et al. 2009; Krishnan et al. 2012).

Pheromone receptors have been implicated in the mating signaling system of the heterothallic *N. crassa* (Kim and Borkovich 2004; Kim et al. 2012) and *H. jecorina* (Seibel et al. 2012), where their absence results in female sterility in their cognate mating types. Importantly, both Ste2- and Ste3-related pheromone receptors are also present in the homothallic ascomycetes, like *Aspergillus nidulans* (teleomorph: *Emericella nidulans*) (Seo et al. 2004), *G. zeae* (Kim et al. 2008; Lee et al. 2008b), and *S. macrospora* (Mayrhofer et al. 2006). Moreover, the absence of both receptors causes infertility in *A. nidulans* and *S. macrospora*, indicating that pheromone signaling is also necessary for homothallic mating. However, in these fungi, a single pheromone/receptor pair is sufficient to support (to some extent) mating and sexual spore formation. Interestingly, the absence of both receptors in *S. macrospora* produces infertility, whereas lack of both pheromones only results in a reduction of sexual fruiting body and spore formation. This suggests that nonspecific activation of the pheromone receptors can, to some degree, support homothallic mating in this fungus. In contrast to *A. nidulans* and *S. macrospora*, the pheromones and their receptors are not required for mating in *G. zeae*, where they rather act enhancing this process (Kim et al. 2008; Lee et al. 2008b). This indicates that in some homothallic species, pheromones and their receptors are not essential for mating. Nonetheless, downstream components of the pheromone signaling pathway are required for mating in *G. zeae*, as strains deficient for the G-protein alpha subunit Gpa1 (Yu et al. 2008) or the MAPK Gpmk1 (orthologue of *S. cerevisiae* Fus3) are sterile (Jenczmionka et al. 2003). Thus, a constitutively active pheromone signaling pathway could account for mating in this homothallic fungus. Of note, whereas pheromone function in fungi like *P. anserina* is restricted to fertilization (Coppin et al. 2005), roles in postfertilization events for pheromones and their receptors have been described in a number of ascomycetes (see below), including heterothallic (Seibel et al. 2012; Kim et al. 2012) and homothallic (Mayrhofer et al. 2006) species.

Heterotrimeric G-proteins in fungi are highly conserved. Most filamentous fungi possess three G-alpha proteins, which are classified in three major groups (I–III), whereas only one G-beta and one G-gamma protein are generally present in these fungi (Li et al. 2007). Consistent with participation in transducing the pheromone receptor signal, elimination of the group I G-alpha protein results in sterility in most studied ascomycete fungi, including both homothallic (*A. nidulans*, *G. zeae*) (Rosen et al. 1999; Yu et al. 2008) and heterothallic (*N. crassa*, *Magnaporthe oryzae*, *Cochliobolus heterostrophus*, *Gibberella fujikuroi*, *Cryphonectria parasitica*) (Ivey et al. 1996; Liu and Dean 1997; Horwitz et al. 1999; Studt et al. 2013; Gao and Nuss 1996) fungi. However, the outcome of eliminating these proteins may differ. For example, deletion of the corresponding gene in *A. nidulans* abrogates fruiting body formation (Rosen et al. 1999), while in *N. crassa* results in the formation of small aberrant fruit bodies that are sterile (Ivey et al. 1996). Similarly, the absence of the group I G-alpha protein in *G. fujikuroi* (teleomorph of *Fusarium fujikuroi*) produces female sterility; however, whereas in *N. crassa* this phenotype is observed in both mating types, in *G. fujikuroi* it is only observed in MAT1-1 strains (Studt et al. 2013). Perithecia formation is also affected in *S. macrospora* lacking the group I G-alpha protein, but these fruiting bodies still produce fertile ascospores. In this fungus, elimination of a second G-alpha protein, however, results in infertility (Kamerewerd et al. 2008); thus, some degree of functional redundancy between G-alpha proteins may exist in fungi.

Analysis of the genetic interactions between G-alpha protein and pheromone receptor components in *S. macrospora* established that the group I G-alpha protein is the predominant pheromone receptor signal transducer (Kamerewerd et al. 2008). In *N. crassa*, similar to the absence of pheromone receptors, both the directional growth of trichogynes toward male cells and their capacity to fuse with them are compro-

mised in the absence of GNA-1, the group I G-alpha protein of this fungus. Furthermore, this developmental defect is also observed in the absence of either the G-beta (GNB-1) or G-gamma (GNG-1) subunits (Krystofova and Borkovich 2005; Kim and Borkovich 2004), indicating that these three proteins are required for transducing the pheromone response. In this fungus, GNB-1 and GNG-1 interact with each other and form a complex that stabilizes GNA-1. Strains expressing constitutively activated (GTPase-deficient) alleles of *GNA-1* are fertile, but overexpression of these alleles cannot bypass the absence of GNB-1 (Yang et al. 2002; Krystofova and Borkovich 2005; Won et al. 2012; Yang and Borkovich 1999). This suggests a direct involvement for both GNA-1 and GNB-1 in mating. Furthermore, activated GNA-1 can also not bypass the absence of a pheromone receptor (Kim and Borkovich 2004); thus, additional pheromone receptor effectors might be involved in driving trichogyne directional growth and mating. Importantly, (female) sterility has also been demonstrated for a number of G-beta (Ganem et al. 2004; Kaneko et al. 2013; Nishimura et al. 2003; Rosen et al. 1999) and G-gamma (Seo et al. 2005)-deficient strains in filamentous Ascomycota, suggesting a broad participation for these proteins as positive regulators of the mating process of these fungi. A notable exception is *G. zeae*, whose G-beta protein is not required for sexual reproduction (Yu et al. 2008).

Less is known about the G-protein effectors of the pheromone signaling pathway beyond Saccharomycotina. In *S. pombe*, activation of both the pheromone-responsive MAPK module and the polarity factor Cdc42 depends on Ras1, the only Ras small GTPase present in this yeast. Actually, it has been proposed that different pools of this protein signaling from different cell compartments are distinctively implicated in regulating these pathways (Onken et al. 2006). However, whether Ras1 is implicated in linking pheromone signaling to the polarity complex during mating remains an open question. Importantly, the scaffold proteins Ste5 and Far1, which are key regulators of the mating signaling response in *S. cerevisiae*, are not present in *S. pombe* (Cote et al. 2011). Actually, homologs for Ste5 are absent in fungi beyond Saccharomycotina (Rispail et al. 2009; Cote et al. 2011). Likewise, although present in diverse filamentous fungi, deletion of the putative *far1*-orthologue in *Botrytis cinerea*—the only mycelial fungus where the gene has so far been studied—does not exert significant developmental alterations (Giesbert et al. 2014). These observations indicate significant differences in the organization of the signaling pathway transducing the mating pheromone signal in fungi.

The protein kinases that compose the MAPK mating signaling cascade are also highly conserved in fungi. Orthologues of *S. cerevisiae* Ste11, Ste7, and Fus3/Kss1 (a Fus3 paralogue controlling pseudohyphal development) have been identified in representative species of most major fungal lineages (Rispail et al. 2009). As illustrated in *N. crassa*, deficiency in either of the corresponding MAP kinases—NRC1, MEK2, and MAK2—results in female sterility due to defective protoperithecia differentiation (Kothe and Free 1998; Li et al. 2005; Pandey et al. 2004; Maerz et al. 2008). Detailed analysis has revealed that in the absence of either of these proteins, ascogonia can differentiate and recruit enveloping hyphae to produce incipient protoperithecia. However, development of these structures does not further progress, and they are unable to produce trichogynes (Lichius et al. 2012). This implication for the MAPK mating kinases in trichogyne formation has precluded analyzing their role in the subsequent fertilization process.

The three equivalent MAP kinases of *A. nidulans*—SteC, MkkB, and MpkB—are also required for fertility, and their absence blocks sexual development before fruit body formation (Wei et al. 2003; Bayram et al. 2012; Paoletti et al. 2007). These three kinases form a complex with the scaffold protein Ste50 (SteD), producing a signaling module that is required for sexual development. Interestingly, in vegetative cells this signaling module dynamically travels along the plasma membrane and crosses the cytoplasm to associate with the nuclear envelope, where MpkB (Fus3) further localizes to the nucleoplasm (Bayram et al. 2012). These observations suggest that in contrast to *S. cerevisiae*—where Ste5-anchored

Fus3 is released alone from the plasma membrane to reach the nucleus—in *A. nidulans* an entire Ste50-bound MAP signaling module migrates from the plasma membrane to the nucleus, from where MpkB (Fus3) alone is transported into the nucleus to promote sexual development (Bayram et al. 2012). Ste50 is an adaptor protein that in *S. cerevisiae* recruits Ste11 to the cell surface by association to the plasma membrane protein Opy2, as well as to the G-proteins implicated in mating, and participates in modulating the different MAPK signaling pathways (Yamamoto et al. 2010; Xu et al. 1996). Importantly, Ste50 has a broad distribution in Dikarya fungi (Rispail et al. 2009), and it has actually been implicated in mediating mating pheromone signaling in diverse species, including distantly related ascomycete yeasts (Sanchez-Paredes et al. 2011; Barr et al. 1996), filamentous ascomycetes (as illustrated by *A. nidulans*), and basidiomycetes (Jung et al. 2011; Mayorga and Gold 2001; Fu et al. 2011). This suggests that Ste50 could perform a central conserved role in organizing the mating MAPK signaling pathway in the Dikarya.

3. Directing Polarized Cell Growth to Allow Mate Encounter

The Rho GTPase Cdc42 is the main factor implicated in the establishment of cell polarization in yeasts (Martin and Arkowitz 2014). In *S. cerevisiae*, the Ste5-related scaffold protein Far1 coordinates cellular polarity by associating with polarity establishment proteins. Cdc24, the guanine nucleotide exchange factor (GEF) that activates Cdc42, is sequestered in the nucleus by association to Far1 in mitotic G1 cells. In response to mating pheromone, the Far1-Cdc24 complex is exported from the nucleus and translocated to the cell surface by associating to the G-beta/G-gamma heterodimer (Shimada et al. 2000; Nern and Arkowitz 1999; Butty et al. 1998). This promotes the interaction of Cdc24 with the scaffold protein Bem1 and with Cdc42 to form an active polarity complex that drives polarized growth. This complex coordinates the local cytoskeletal organization and vesicle trafficking by processes that include the activation of the formin Bni1, which nucleates actin cables for delivery of secretory vesicles (Evangelista et al. 1997; Chen et al. 2012). In coordination with Far1, additional interactions between mating signaling and cell polarization components participate in polarity establishment. For example, Bem1 also interacts with the MAPK scaffold Ste5 and the PAK kinase Ste20 (Leeuw et al. 1995), linking the MAPK module to the polarity complex. Also, the MAP kinase Fus3 phosphorylates the formin Bni1, further promoting its activation and correct localization (Matheos et al. 2004). Therefore, cell polarization in response to mating pheromone is established by a cooperative network of protein interactions, which allows cells to precisely orientate their growth toward a mating partner.

Cdc42 has also been implicated in establishing cell polarization in filamentous fungi; however, the role of this protein in regulating hyphal morphogenesis among different fungal species can differ. Furthermore, among different fungi, Cdc42 acts in collaboration, or partitioning roles with other Rho small GTPases, most notably with Rac1 (Arkowitz and Bassilana 2015). Both Cdc42 and Rac1, along with a third Rho GTPase—Rho4—are required for perithecia formation in *G. zeae*. Furthermore, a fourth Rho-family protein—Rho2—is dispensable for perithecia formation but required for ascospore formation (Zhang et al. 2013). Thus, overall, four of the five nonessential Rho GTPases of this fungus (which harbors an additional essential Rho protein) are implicated in sexual reproduction, stressing the importance of these proteins in this process. However, the precise molecular role of these proteins in sexual development awaits elucidation. On the other hand, some components of the polarity complex that are essential for cell polarization and mating in yeasts have been found to be dispensable in filamentous fungi, specifically the scaffold protein Bem1 in *N. crassa* (Schurg et al. 2012). **This indicates differences in the organization of the polarity complex during the mating process of filamentous fungi and yeasts and suggests that different conformations for the cell system that mediates mate attraction might have evolved in fungi with different lifestyles.**

4. Choosing the Mating Partner

In *S. pombe*, exposure to low levels of mating pheromone promote successive formation and dissipation throughout the cell surface of active Cdc42 complexes, which contain the activating GEF Scd1 (Cdc24) and the scaffold protein Scd2 (Bem1). However, these complexes are unable to trigger polarized growth due to a block in the secretion of cell wall synthases. Interestingly, an increase in pheromone concentration results in the formation of a single stable zone of active Cdc42 that promotes polar growth. These results suggest that Cdc42 explores the cell periphery in order to track the pheromone source and orientate the *shmoo* toward a mating partner. Consistent with this interpretation, cells where Cdc42 exploration is prevented mate mistakenly with sister cells (Bendezu and Martin 2013). A similar exploratory mechanism has been described in *S. cerevisiae*, but in this yeast wandering patches of polarity factors, rather than complexes that assemble and disassemble, have been implicated in tracking the pheromone gradient to orientate the *shmoo*. In this system, Myo2- and actin-dependent vesicle trafficking has been postulated to drive polarity patch wandering (Dyer et al. 2013).

III. Plasmogamy

Following recognition and attraction between mating partners, their gametic cells make contact and adhere to subsequently undergo cell fusion. This permits gathering in a same cell the nuclei that ultimately will undergo karyogamy and produce diploid zygotes. Plasmogamy accomplishment implicates a number of successive cellular events that need to be finely orchestrated in space and time in order to maintain cellular integrity and to prevent cell lysis during the cell-cell fusion process. In fungi, the generation of high intracellular turgor pressure that results from their absorptive lifestyle imposes additional constrains to this process. Notably, successful plasmogamy requires precise coordination between the processes that mediate plasma membrane fusion with those driving cell wall remodeling and fusion.

A. Fungal Cell Wall Remodeling During Plasmogamy

Cell fusion in fungi first requires the adhesion of the opposed mating cells (Table 16.1), which then merge by remodeling and fusing their cell walls. During this process, cell wall materials are removed from contact site between mating cells, resulting in the formation of a narrow channel, which allows apposition and contact between the plasma membranes of the mating partners. Fusion of the plasma membranes ensues, and further cell wall remodeling facilitates the expansion of the fusion pore between cells. This produces a widening of the fusion channel that permits nuclear migration and fusion (Gammie et al. 1998).

Cell wall removal requires the confined activity of cell wall-remodeling enzymes. In *S. cerevisiae*, the initial contact between mating cells is accompanied by a large accumulation of vesicles at the tip of the mating cell projection, adjacent to the place where cell fusion will take place (Gammie et al. 1998). Experiments where the fusion of secretory vesicles to the plasma membrane is blocked have shown that inhibition of protein secretion impedes cell wall remodeling and hampers the subsequent plasma membrane fusion (Grote 2010). This indicates that the vesicles that accumulate at the cell fusion zone actually correspond to secretory vesicles, which deliver the cell wall-remodeling enzymes to the fusion site. Furthermore, evidence also indicates that polarized actin cables provide tracks for secretory vesicle delivery in yeast, in a process dependent on formin (Bni1)-, tropomyosin-, and polarisome-dependent actin dynamics and that involves the type V myosin Myo2 (Liu and Bretscher 1992; Dorer et al. 1997; Gammie et al. 1998; Sheltzer and Rose 2009). Interestingly, *S. pombe* possesses a specific formin—Fus1—dedicated to organize the acting cytoskeleton during mating (Petersen et al. 1995, 1998). During plasmogamy, this formin organizes an actin aster that focalizes actin filaments with type V myosins near the plasma membrane fusion site. Type V myosins deliver and concentrate cell wall-degrading enzymes at the focus of this actin aster, promoting restricted cell wall degradation. In contrast,

cell wall-polymerizing enzymes are excluded from the actin focus and remain localized at the cortex of the *shmoo* tip. **These observations reveal a mechanism whereby cell wall-degrading enzymes are delivered and concentrated at the fusion site to locally replace cell wall synthases, establishing a confined zone of cell well dissolution surrounded by zones where cell wall integrity is preserved** (Dudin et al. 2015).

Polarized vesicle positioning in *S. cerevisiae* also depends on the O-glycosylated transmembrane protein Fus1 (not to be confused with the *S. pombe* formin Fus1), which itself localizes to cell fusion zone in a pheromone-dependent manner (Trueheart et al. 1987; Trueheart and Fink 1989; McCaffrey et al. 1987; Gammie et al. 1998). Fus1 localization at the cell fusion zone also depends on the polarity GTPase Cdc42 and its GEF Cdc24 (Barale et al. 2004, 2006). In addition to vesicle positioning, Fus1 promotes the localization at the cell fusion site of additional fusion-specific factors, notably of Fus2 (Paterson et al. 2008). Like Fus1, Fus2 is required for the cell wall removal process; however, unlike the former, the absence of Fus2 does not impede vesicle clustering at the fusion site, suggesting that this factor functions at a latter step of the process (Gammie et al. 1998; Elion et al. 1995). Fus2 forms a complex with the BAR-domain-containing protein Rvs161, and both proteins are transported to the fusion site also in an actin- and Myo2-dependent manner, probably by associating to a specific group of vesicles (Sheltzer and Rose 2009; Paterson et al. 2008; Brizzio et al. 1998). Although the precise role of these proteins remains undetermined, Rvs161—along with the second N-BAR protein, Rvs167—is known to induce curvature of lipid membranes in vitro (Youn et al. 2010). Therefore, the function of the Fus2/Rvs161 complex could be related to facilitating membrane deformation and/or fusion to allow exocytosis and the release of cell wall-remodeling enzymes. Of note, experimental data suggest that Fus2 is an effector of the polarity protein Cdc42; thus, this GTPase could modulate the cell wall-remodeling process through Fus2 activation (Ydenberg et al. 2012).

B. Plasma Membrane Fusion

Pheromone-regulated membrane protein 1 (Prm1) was the first identified protein required for plasma membrane fusion during mating (Heiman and Walter 2000). In *S. cerevisiae*, the absence of this protein in both mating parents results in inhibition of cell fusion, which is characterized by mating pairs in which the intervening cell wall material is successfully degraded and where plasma membranes become closely apposed; however, these membranes remain unfused. Nonetheless, around 40 % of Prm1-deficient mating pairs are still able to successfully undergo plasmogamy, suggesting the existence of additional factors controlling plasma membrane fusion. One such factor is the calcium influx system regulator protein Fig1 (Aguilar et al. 2007; Erdman et al. 1998). Lack of this protein generates a similar, but milder, cell fusion defect as the absence of Prm1. Additionally, up to 90 % of mating pairs are unable to fuse when both these proteins are absent (Aguilar et al. 2007). Notably, the tightly apposed membranes present in cells deficient for these proteins tend to invade the opposed cell cytoplasm by forming bulges that, in most cases, retain the cell integrity. However, simultaneous lysis of both membranes eventually takes place. This defect has been interpreted as a failure of a yet unidentified cell membrane fusase, which is still active in the absence of Prm1 and Fig1, but it is unable to correctly merge the plasma membranes. Under this scenario, Prm1 and Fig1 do not provide per se the fusogenic activity for plasma membrane fusion but are rather involved in regulating the process (Engel and Walter 2008; Jin et al. 2004; Aguilar et al. 2007). In addition to these two proteins, regulation of plasma membrane fusion also requires the proteolytic activities of Kex2 and Kex1, two Golgi-resident proteases (Heiman et al. 2007), and it has been proposed that protein secretion could also be involved (Grote 2010). Therefore,

delivery and/or processing of some components of the plasma membrane fusion machinery could rely on secretory pathway trafficking.

Prm1 is a conserved protein among fungi, and its involvement in cell fusion has been demonstrated in *S. pombe* (Curto et al. 2014) and in the filamentous fungus *N. crassa* (Fleissner et al. 2009). Notably, the outcome of eliminating this protein in *N. crassa* cell fusion is very similar to that observed in *S. cerevisiae*. Furthermore, Prm1 in *N. crassa* is part of the general cell fusion machinery, as it is involved in both vegetative and sexual cell fusions. In contrast, elimination of Prm1 in *S. pombe* generates a more severe defect in terms of number of mating pairs that fail to undergo cell fusion. Still, in these mating pairs, the cell wall present at the interphase between mating cells is not extensively degraded, revealing that in this yeast, Prm1 is required for cell wall remodeling. Similarly, *S. pombe* Dni1—a close relative of *S. cerevisiae* Fig1—is required for plasma membrane organization and cell wall remodeling during mating (Clemente-Ramos et al. 2009; Curto et al. 2014). These observations indicate that the organization of the molecular mechanism in which Prm1 participates might differ among fungi.

Fig1 has been implicated in the mating process of different ascomycete fungi, including *C. albicans* (Alby et al. 2010; Yang et al. 2011), *G. zeae*, *N. crassa* (Cavinder and Trail 2012), and *A. nidulans* (Zhang et al. 2014), but information about the precise function exerted by this protein in the cell-cell fusion process of these fungi is still limited. Nonetheless, *A. nidulans* Fig1 (FigA) is able to correct the mating defect of a *S. cerevisiae* Fig1-deficient strain (Zhang et al. 2014), suggesting conservation of the molecular function of this protein for, at least, these two ascomycete species. Importantly, filamentous ascomycetes (Pezizomycotina) possess an additional protein required for plasma membrane fusion during mating. This protein—LFD1—was identified in *N. crassa* and possesses functional redundancy with Prm1. Like the latter, LFD1 is required both for vegetative and sexual cell fusions at the plasma membrane merger step, and its absence results in reduced ability of trichogynes and conidia (asexual spores) to fuse (Palma-Guerrero et al. 2014).

IV. Fruit Body Formation

A. Fruiting Body Formation in Ascomycota

With the exception of Saccharomycotina and Taphrinomycotina, the Ascomycota fungi generate complex fruiting bodies (ascomata), which are formed after fertilization through highly regulated developmental processes [for review on fruit body formation in Basidiomycota, please refer to Pelkmans et al. (2016)]. These integrated structures are made of different cell types; however, two major classes of tissues can be distinguished: the envelope and the dikaryotic hyphae. The envelope is made of vegetative haploid hyphae. It shelters the dikaryotic tissue where the cells containing two unfused haploid nuclei are formed (dikaryons; see Sect. V.B). In the course of sexual development, these cells undergo karyogamy, which results in diploid cells that are immediately subjected to meiosis. The subsequent asci, mostly containing from four to eight ascospores, are then ready to be expelled from the fruiting bodies to generate new colonies.

1. Building Fruiting Bodies

Induction of fruiting body formation begins with fertilization. In ascomycetes, fertilization proceeds through two main patterns, provided that the fungal species is genetically self-compatible (homothallic) or genetically self-incompatible (heterothallic). As described above (Sect. II.A), heterothallism results in outbreeding, while homothallism results in selfing. Anyhow, the first step of sexual reproduction starts with the fusion of two compatible gametes (Fig. 16.3C). In general, the male gametes, also called antheridia or spermatia, are small conidia-like cells formed directly on the mycelium. However, in contrast to the vegetative conidia, they usually do not germinate (Esser and Prillinger 1972; Beisson-Schecroun 1962), as if their only biological fate was to deliver their haploid nucleus to the female gametes. The female gametangia, also called ascogonia, are large coiled cells (Fig. 16.3A, B). In some species (*Ascobolus immersus*, *P. anserina*, *N. crassa*, etc.), these ascogonia mature into preformed

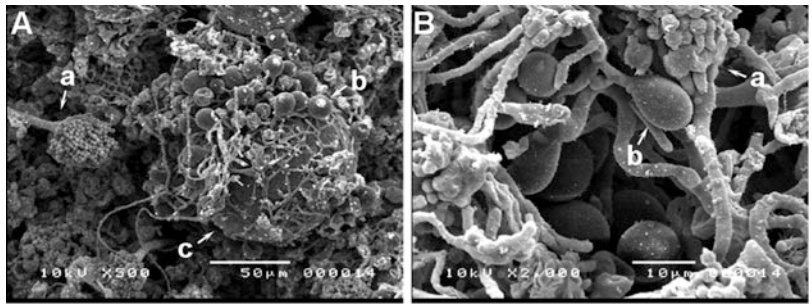


Fig. 16.4 Example of genes implicated in signaling during sexual fruit body formation in *Aspergillus nidulans*. (**A**) As in a wild-type strain, mutants defective for the MAPK SakA—the stress-activated MAPK homolog of Hog1/p38—are able to differentiate conidia-bearing conidiophores (*a*), hülle cells (*b*, specialized nursing cells for cleistothecia), and cleistothecia (*c*). However, sexual development in this mutant is derepressed (not depicted) (Kawasaki et al. 2002). In contrast, the absence of the NADPH oxidase NoxA abrogates cleistothecia formation (**B**, here a *ΔnoxAΔsakA* double mutant, in which *ΔnoxA* is epistatic on *ΔsakA*) (Lara-Ortíz et al. 2003). Images are kind gift from J. Aguirre (UNAM, México)

fruiting bodies (protoperithecia) by recruiting protective layers of vegetative hyphae (Fig. 16.3C). Wrapped up into this envelope, the mature female gamete can then await fertilization. To this end, the protoperithecia produce specialized hyphae, the trichogynes, which collect the fertilizing male haploid nucleus (Fig. 16.3C). By contrast to what happens in other eukaryotic organisms, the fusion of the two haploid nuclei (karyogamy) does not proceed immediately after fertilization. The mechanism by which this dikaryotic phase is extended in Basidiomycetes as well as in filamentous Ascomycetes remains unknown. Instead of fusing, pairs of male and female haploid nuclei are formed, which then migrate into hook-shaped ascogenous hyphae called croziers. These dikaryotic tissues grow within the haploid envelope from which they draw nutritional resources. In addition to ascogenous hyphae, the fruiting bodies contain also sterile hyphae originating from one of the haploid parental strains. Among these are the paraphyses, hyphae of female origin in most cases, which elongate from the basal part of the fruiting bodies.

Dikaryotic cells formed by heterothallic species contain one haploid nucleus of each mating type. This means that the two haploid nuclei are genetically compatible and can consequently undergo karyogamy. Again, to date, what makes genetically different nuclei to recognize each other is not completely known (see Sect. V.B.2). Finally, after several rounds of synchronized mitotic divisions, the male and female haploid nuclei fuse to form a diploid cell. Then, right after karyogamy, the diploid cells undergo meiosis and differentiate into asci. Because in many species meiosis is followed by one mitotic division, asci are formed of eight uninucleated ascospores. The layout of these asci into the fruiting body can be random or arranged in a delineate layer called the hymenium.

Ascospore discharge is dependent on the fruiting body shape. Four main types can be distinguished: apothecia, perithecia, pseudothecia, and cleistothecia. Apothecia are cup-shaped wide-open structures, where the hymenium is not enclosed within a hyphal tissue but directly exposed to the environment. Consequently, asci are discharged upward. On the contrary, perithecia are pear-shaped structures that are almost closed but form a pore at the tip of the fruiting bodies called an ostiole (Fig. 16.1A). Thus, in perithecia, discharge of unitunicate asci proceeds through the ostiole, which contains short sterile hyphae, the periphyses. Pseudothecia are alike perithecia except that asci are bitunicate and not organized into a hymenium. In such fruiting bodies, discharge of ascospores relies on the expansion of the ascus double cell wall after filling up with water. Contrary to apothecia, perithecia, and pseudothecia, cleistothecia are closed round-shaped fruiting bodies (Fig. 16.4A). The asci are found either scattered throughout the interior cavity or arising

from an enclosed hymenium. In either case, the cleistothecium wall has somehow to burst open for the ascospores to be dispersed.

Before phylogenetic analyses based on genomic data were available, the classification of fungi belonging to the Ascomycota was partly based upon fruiting body properties. Hemiascomycetes (yeasts) were fungi showing asci not enclosed in a fruiting body. Fungi that form apothecia belonged to the Discomycetes group, whereas those endowed with perithecia were Pyrenomycetes. Fungi developing pseudothecia were clustered into the Loculoascomycetes group, while fungi that form cleistothecia belonged to the Plectomycetes. Although challenged by more accurate classifications, these taxonomic groups are still present in the textbooks (Hibbett et al. 2007).

The time frame of fruiting body development and sexual cycle completion are highly variable. Most information comes from model species for which the sexual cycle can be mastered in laboratory conditions. Extensive studies performed on the heterothallic Sordariomycete *P. anserina* (Coppin et al. 2012; Espagne et al. 2011; Grognet et al. 2012; Jamet-Vierny et al. 2007; Malagnac et al. 2004; Peraza-Reyes et al. 2011; Silar 2011) (illustrated in Figs. 16.1 and 16.3) showed that male (spermatia) and female (ascogonia) gametes start to differentiate after three days of growth on minimal medium at the optimal temperature of 27 °C. Once fertilization occurs, the perithecia mature in four days. Two days after fertilization, the perithecium neck and ostiole are formed. When fully mature, two days later, each fruiting body yields hundreds of ascospores that are continuously shoot out for five days. During the course of their development, the perithecia of *P. anserina* enlarge over 100-fold, requiring considerable amounts of nutrients from the surrounding mycelium. Nutrient status of the colony by itself and also the capability of sensing this nutrient status through appropriate signaling pathways are actually two key points for fungal sexual development (Fig. 16.5 and Sect. IV.2). *N. crassa*, also a heterothallic Sordariomycetes used as a model organism, shows a sexual development pattern similar to that of *P. anserina*. However, because it does not differentiate proper spermatia, any haploid nucleus of the opposite mating type, i.e., hyphal fragments, vegetative macroconidia, and microconidia, can fertilize a female gamete. Besides, maturation of ascogonia into protoperithecia is subjected to a metabolic regulation, since it only occurs at low nitrogen concentrations. Again, nutrient status of the colony is used as a checkpoint for sexual reproduction. Sexual development proceeds slightly differently in the homothallic Eurotiomycetes *A. nidulans*. Although no proper gametes can be detected, self-fertilization results in the formation of small coiled lump of cells reminiscent of ascogonia, which are soon surrounded by a loose layer of growing hyphae that will later differentiate into the cleistothecium envelope. Around the developing fruiting bodies, a second cell type is also found: the thick-walled multinucleate hülle cells (Fig. 16.4A). The function of these very peculiar cells is to protect and provide nutrients to the

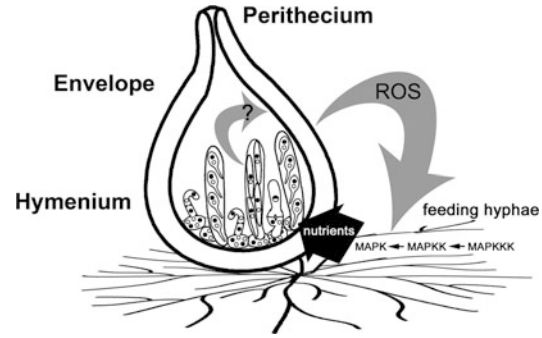


Fig. 16.5 Model for fruiting body development signaling [adapted from Jamet-Vierny et al. (2007)]. In *P. anserina*, the developing perithecium would send a signal to the surrounding mycelium possibly through diffusible reactive oxygen species (ROS) produced by the PaNox1 NADPH oxidase (Malagnac et al. 2004). This would be mediated by the Pezizomycotina-specific protein IDC1 (Jamet-Vierny et al. 2007). Indeed, both of these proteins are essential to the differentiation of the perithecium envelope. Once the oxidative signal is received by the mycelium located underneath the developing fruiting bodies, it is transduced by the PaMpk1 MAP kinase cascade (Kicka et al. 2006; Kicka and Silar 2004). Activation of this MAPK module results in the mobilization of the nutrients to feed the maturing fruiting body. The PaMpk2 MAP kinase cascade is also essential to the perithecium envelope formation, but, contrary to the PaMpk1 MAP kinase cascade, its activation is independent of PaNox1 (Lalucque et al. 2012). The nature of the putative signal emitted by the developing dikaryons to induce the ROS signalization is still unknown

developing cleistothecia. The dikaryotic ascogenous hyphae enclosed within the fruiting bodies can produce, after karyogamy and subsequent meiotic division, up to 1000 ascospores per cleistothecium (Sohn and Yoon 2002).

It is worth to note that in addition to ascospores generated though meiotic divisions, some of these fungi also produce asexual conidia. But interestingly, this aptitude to propagate through clonal divisions is irrelevant of their heterothallic or homothallic status. For instance, both *N. crassa* (self-sterile) and *A. nidulans* (self-fertile) form conidia (Fig. 16.4A), whereas *P. anserina* (self-sterile) and *S. macrospora* (self-fertile) do not.

Fruiting Body Development Requires the Coordinate Expression of Hundreds of Genes Using *N. crassa*, *P. anserina*, *S. macrospora*, *A. nidulans*, *C. heterostrophus*, *M. grisea*, etc., as model organisms, over 200 genes have been identified as essential for sexual development, mainly through classical genetic approaches. Now that large-scale screenings are available, this number will likely increase. In that respect, exploration of the *N. crassa* knockout strain collection (Park et al. 2011) will certainly be of great help. Besides, our comprehension of the genetic interactions might also improve. Originally, sterile mutants fell into three phenotypic categories: male sterile only mutants, female sterile only mutants, and male and female sterile mutants. But the opportunity to purposely form heterokaryons in *N. crassa* (Pandey et al. 2004) and *P. anserina* changed this view. Hence, when sterile mutants harboring distinct mutations are forced to form heterokaryons, it is possible to tell apart those that form the envelope of the fruiting bodies with no ascogenous hyphae inside from those that do not form fruiting bodies at all. Finally, recent high-throughput gene expression analyses will definitely help to draw even more complex genetic networks involved in sexual reproduction of fungi.

2. Reactive Oxygen Species and Fruiting Body Development

In eukaryotic cells, various metabolic pathways generate reactive oxygen species (ROS) as by-products. ROS are powerful oxidant molecules that damage macromolecules, and, as such, they are inactivated by dedicated antioxidative cellular components. But ROS are also secondary messengers involved in either intracellular or cell-to-cell signaling (Aguirre and Lambeth 2010; Scott and Eaton 2008; Tudzynski et al. 2012). The NADPH oxidase enzymes (Nox) are a major source of ROS (Lambeth 2004). These highly conserved transmembrane proteins produce superoxide by reduction of oxygen using cytoplasmic NADPH as electron donor. Three distinct families of Nox have been characterized in the Pezizomycotina, namely, NoxA, NoxB, and NoxC. NoxA and NoxB are homologs of the mammalian gp91phox, responsible for the neutrophil oxidative burst during the immune defense response. NoxC harbors calcium-binding EF-hand motifs found in the human Nox5 and plant RBOH proteins. The evidence of a link between ROS generation and induction of fruiting bodies in filamentous fungi was first established in the Eurotiomycetes, when the NoxA gene of *A. nidulans* was shown to be essential to cleistothecium development (Lara-Ortíz et al. 2003) (Fig. 16.4B). Later on, in Sordariomycetes, the PaNox1 gene of *P. anserina* and the NoxA gene of *N. crassa* were also shown to be essential to fruiting body development, i.e., perithecia (Cano-Domínguez et al. 2008; Malagnac et al. 2004). In *B. cinerea* and *Sclerotinia sclerotiorum* (Leotiomycetes), both NOXA and NOXB isoforms are required to develop sclerotia, which after fertilization with microconidia evolve into apothecia (Kim et al. 2011; Segmüller et al. 2008).

Because the intracellular concentration of NADPH is relevant for the metabolic status of a cell, it has been proposed that ROS generated by Nox could signal nutrient shortage. Indeed, in *P. anserina* PaNox1 mutants, perithecium formation can be restored if nutrients are continuously supplied (Malagnac et al. 2004). Moreover, heterokaryon experiments have shown that PaNox1 activity is not diffusible to the fungal colony but restricted to perithecia. Therefore, this enzyme is likely a cis-acting—perithecia autonomous—molecule, which is consistent with a potential signaling role in the developing fruiting body (Malagnac et al. 2004). These data also suggest that nutrient

resources could be stored in neighboring cells and mobilized through a local ROS signaling cascade to build fructifications, especially when the medium has been depleted in nutritional resources after vegetative growth of the parental mycelia.

3. Fruit Body Development Signaling

Analyses of filamentous fungus complete genome sequence show that unlike *S. cerevisiae*, only three MAPK signaling modules are present (Rispail et al. 2009; Zhao et al. 2007). The first one is orthologous to the budding yeast *FUS3* pathway involved in mating, the second one is orthologous to the *MPK1* pathway involved in the yeast cell wall integrity pathway, and the last one is orthologous to the *HOG1* yeast pathway, the osmosensing stress-related responding pathway. During *N. crassa* vegetative growth and asexual reproduction, these three MAPK modules are involved in separate functions, but a joined activity of the three of them is required to build the complex multicellular structures necessary for sexual reproduction (Maerz et al. 2008). Loss of the MAK1 pathway function (orthologous to the Mpk1 pathway) or of the OS2 pathway function (orthologous to the Hog1 pathway) both result in the absence of proper ascogonium formation. Because ascogonia are female gametes, the *Δmak-1* mutants and the *Δos-2* mutants are female sterile. In contrast, the *Δmak-2* mutants, impaired for the MAK2 pathway (orthologous to the Fus3 pathway), produced ascogonia but those do not mature into protoperithecia, leading also to female sterility.

Again, in *P. anserina*, systematic inactivation of the nine genes encoding the three different MAPKs of the three MAPK modules showed an overlapping activity restricted to female gamete differentiation (Lalucque et al. 2012). Mutants impaired in MPK1 and MPK2 pathway functions (respectively, orthologous to the *MPK1* and *FUS3* yeast pathways) are also female sterile. Although the *ΔPaMpk1* and *ΔPaMpk2* mutants can form ascogonia, these gametes do not mature into protoperithecia.

In the self-fertile ascomycete *A. nidulans*, mutants impaired in genes encoding the AnSte11 MAPKKK (Wei et al. 2003), the AnSte7 MAPKK (Bayram et al. 2012), and the AnFus3 MAPK (Paoletti et al. 2007) are sterile (see Sect. II.D.2 for further details). Moreover, null alleles of the gene encoding the SakA kinase, orthologous to Hog1p, lead to premature sexual development and increase yield of cleistothecia, the *Aspergillus* fruiting bodies (Kawasaki et al. 2002) (Fig. 16.4A).

V. The Dikaryotic Phase

In many organisms, the cell fusion process that accounts for plasmogamy during sexual reproduction is closely coupled to the subsequent nuclear fusion process. In keeping with the large diversity of reproductive systems exhibited by fungi, the timing in which these two processes take place in fungi can significantly differ.

A. The Correlation Between Plasmogamy, Karyogamy, and Meiosis in Fungi

In aquatic fungi like *A. macrogynus*, plasmogamy takes place between free-living motile gametes. These uninucleated cells fuse by their posterior ends, next to their flagella, and produce a biflagellated cell with closely apposed nuclei. These nuclei immediately fuse to produce a motile zygote (Pommerville and Fuller 1976). In zygomycetes, zygotes consist on resting spores—the zygospores—that are produced at the contact site where gametangia undergo plasmogamy. In these fungi, gametangia contribute with many nuclei to the developing zygospore, which ultimately produce recombinant haploid nuclei. However, nuclear behavior within the zygospore is not completely understood. Genetic analyses from *Phycomyces blakesleeanus* suggest that inside each zygospore, a single diploid nucleus undergoes meiosis to then divide mitotically and produce thousands of recombinant haploid nuclei (Eslava et al. 1975a, b). However, genetic data also indicate that some progeny of a single zygospore originate from more than one diploid nucleus, which could result from multiple karyogamy events. Furthermore, evidence also

indicates that the process of genetic reduction that occurs within zygospores is not a "perfect" meiosis (Chaudhary et al. 2013; Cerda-Olmedo 1975; Mehta and Cerda-Olmedo 2001). Therefore, the precise correlation between plasmogamy, karyogamy, and meiosis in these fungi is not fully understood. However, these processes are coupled to zygote development ostensibly in a concerted manner, without distinctive dikaryotic phase been perpetuated.

In contrast, in Basidiomycota and Ascomycota, which comprise most (~98 %) of the fungal species known today, plasmogamy and karyogamy are separated in space and time by the propagation of a dikaryotic phase, which is a distinctive feature of the fungi grouped in the Dikarya subkingdom (James et al. 2006). In the dikaryotic phase, the two gametic nuclei that are brought together after plasmogamy remain distinct and proliferate mitotically. These nuclei divide coordinately, in such a way that ultimately a pair of nuclei of opposed parental origin is engaged in karyogamy and undergoes meiosis. As a result, the number of karyogamies and meioses that are obtained from a single fecundation event is amplified. On the other hand, in most Dikarya fungi, karyogamy is immediately followed by meiosis, which restricts the diploid phase to a single nuclear generation.

As exemplified by Ascomycete yeasts like *S. cerevisiae* and *S. pombe*, in which karyogamy is concurrent to plasmogamy, the dikaryotic phase of some Dikarya fungi can be rather ephemeral. Furthermore, *S. cerevisiae* also exhibits an alternation of haploid and diploid phases that results from deferring meiosis from karyogamy by the propagation of a diploid phase. In this yeast, the diploid cells that result from karyogamy can proliferate mitotically by budding until they encounter nutritional limitation, which induces meiosis and sporulation (Fig. 16.2) (for review, see Neiman 2011). Unlike *S. cerevisiae*, *S. pombe* is normally haploid in nature, and meiosis and sporulation typically take place readily after karyogamy. Consequently, sexual induction in this yeast triggers all: mating, karyogamy, meiosis, and sporulation. Altogether, the above observations indicate that **the timing and relationship that karyogamy maintains with plasmogamy and meiosis can significantly vary among fungal lineages.** Our knowledge about the signals that trigger or delay the succession of these events, as well as about the mechanisms that coordinate these processes in diverse fungi, which differ in evolutionary origin and lifestyle, is still limited.

B. The Dikaryotic Phase in Ascomycota

1. Dikaryotic Development in Ascomycota

The dikaryotic phase in most mycelial ascomycetes takes place inside sexual fructifications and is restricted to specific cell types present in the fertile tissue (the hymenium) of these structures. Following plasmogamy, the trichogyne hypha delivers the male gametic nucleus into the ascogonium. Ascogonia can be naked or enclosed within an ascoma (Fig. 16.3C) and consist on syncytial cells harboring numerous female gametic nuclei. Upon entry of the male nucleus into primary ascogonial cells, both gamete nuclei multiply to produce plurinucleated cells that contain nuclei of both parental origins (Coppin et al. 1997). Within these cells, nuclei of opposite gametic origin (of different mating type in heterothallic fungi) recognize each other (Debuchy 1999) and are segregated into specialized ascogenous hyphae, where dikaryotic compartmentalization takes place. Ascogenous hyphae originate from the surface of ascogonial cells as small hyphal protrusions that, at first, are devoid of nuclei. These hyphae emerge near small groups of ascogonial nuclei, which at this time are distributed to the cell periphery (Wilson 1952). No distinctive nuclear pairing has been observed in ascogonial cells of fungi like *Pyronema confluens*, *N. crassa*, or *P. anserina* (Zickler et al. 1995; Wilson 1952; Raju 1980), but genetic analyses indicate that one nucleus from each parent migrates into ascogenous hyphae. These hyphae initially consist on plurinucleated cells. As they elongate, nuclei arrange in a single row to then become evenly spaced and divide synchronously. Subsequently, each ascogenous hypha grows around producing a hook-shaped cell called the crozier

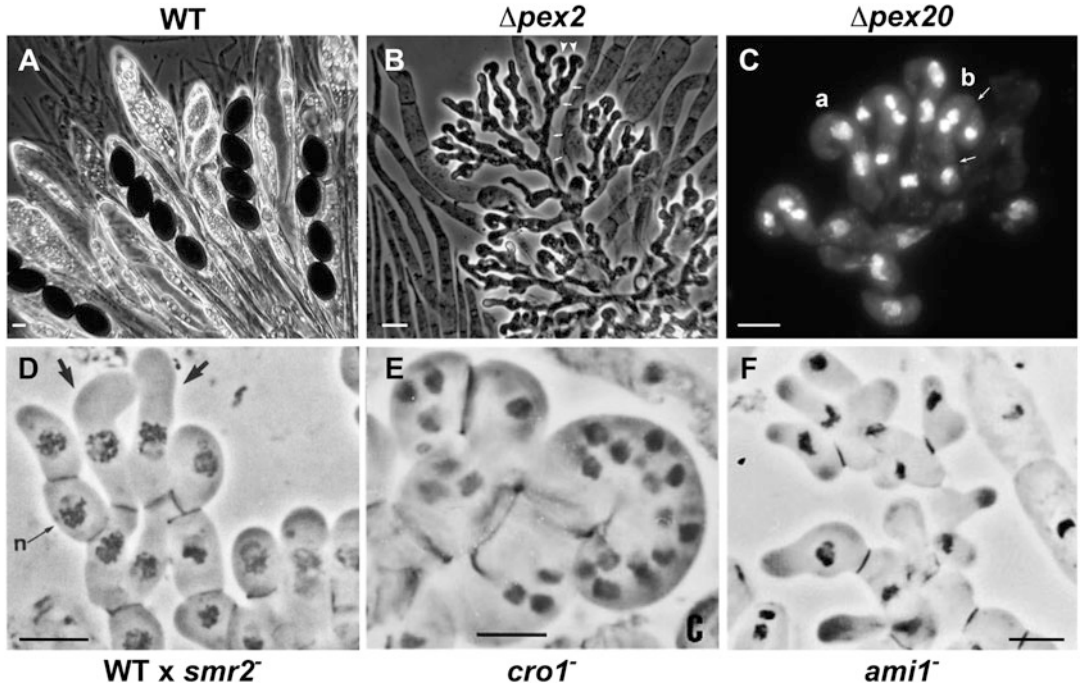


Fig. 16.6 Genes implicated in the dikaryotic stage of the fungus *P. anserina*. (**A**) Wild-type mature perithecia produce asci containing four ascospores. In contrast, *pex2* (formerly *car1*) mutant homozygous crosses produce exclusively hook-shaped dikaryotic crozier cells (**B**). In this mutant, instead of asymmetrically differentiating a meiocyte (ascus) and a new crozier, as in wild type (see Fig. 16.3**D**), each crozier gives rise to two new croziers (see the two terminal croziers—*arrowheads*—emanating from a reminiscent crozier), which, successively, result in the formation of crozier "trees" where no ascus is produced (note the arborescence bifurcations resulting from the differentiation of two croziers from a preceding crozier, *arrows*). Nonetheless, these croziers differentiate properly, and their nuclear progression up to dikaryon delimitation is normal (illustrated in **C** for a *pex20* mutant, which displays the same phenotype as *pex2* (Peraza-Reyes et al. 2011). Note the two developmental stages *highlighted*: *a*, binucleated crozier bending; *b*, tetranucleated crozier after dikaryon delimitation (*arrowheads point* to the septa separating terminal and basal uninucleate cells from a dikaryotic penultimate cell). (**D**) Uninucleate croziers from a heterozygous sexual cross involving a mating-type mutant (here an *smr2-2* by wild-type cross). Note the presence of two elongated cells (*arrows*), which might evolve into asci; *n* indicates a nucleus. (**E**) Multinucleate croziers obtained from a *cro1-1* homozygous cross. Note that *cro1* mutant croziers can be considerably large. (**F**) Abnormal uninucleate croziers produced in an *ami1* heterozygous cross. Scale bar in A and B, 10 μm; in **C**–**F**, 5 μm. Adapted from Zickler et al. (1995), Peraza-Reyes and Berteaux-Lecellier (2013), Peraza-Reyes et al. (2008, 2011), Graïa et al. (2000), Berteaux-Lecellier et al. (1998) with permission

(Fig. 16.1, but see also Fig. 16.6C). The two leading haploid nuclei of this cell (of opposite mating type) divide simultaneously with their spindles orientated in such an arrangement that two non-sister nuclei are placed together in the crook part of the crozier (Fig. 16.1; see also crozier *b* in Fig. 16.6C). A septum is then produced across the position formerly occupied by each mitotic spindle, resulting in the formation of three cells: a binucleated (dikaryotic) cell flanked by terminal and basal uninucleated cells (Raju 1980; Wilson 1952; Zickler et al. 1995). In many filamentous ascomycetes, the upper (penultimate) dikaryotic crozier cell undergoes karyogamy and differentiates into an ascus (the meiocyte), whereas the basal and terminal cells fuse to produce a new dikaryotic crozier, which propagates the dikaryotic stage (Fig. 16.3D). However, in some fungi, karyogamy can take place either between the nuclei that are isolated in the penultimate cell or between those that are reunited upon fusion of

the basal and terminal cells (Read and Beckett 1996; Wilson 1952; Zickler et al. 1995; Raju 1980). By this process, ascogenous hyphae successively produce a cell that will be engaged in meiosis and ascospore formation and a second cell that will proliferate mitotically and perpetuate the dikaryotic phase. This results in the formation of arborescences in which croziers and asci with a gradual developmental stage arrangement are simultaneously present (Fig. 16.3C). Like this, one fertilization event produces numerous asci (up to two hundred in species like *P. anserina*, Zickler et al. 1995), which are all issued with independent meioses.

2. The Mating-Type Gene-Dependent Internuclear Recognition Allows Biparental Dikaryotic Cell Formation

In heterothallic fungi, genetic analyses of meiotic segregation of individual asci indicate that each ascus differentiates from a dikaryotic cell always containing nuclei of opposite parental origin (e.g., Shear and Dodge 1927; Zickler et al. 1995). This indicates the existence of a stringent internuclear recognition system that selectively distributes gametic nuclei during ascogenous hyphae differentiation. The process whereby opposite gametic nuclei recognize each other in order to produce biparental dikaryotic cells remains not completely understood. However, the mating-type genes are known to be implicated in this process. In the heterothallic *P. anserina*, it has been demonstrated that heterozygous sexual crosses involving mating-type mutant strains yield uniparental progeny. Genetic and cytological analyses show that this progeny mainly arises from monokaryotic ascogenous hyphae, in which a single haploid nucleus is compartmentalized in a crozier cell (Fig. 16.6D) and undergoes meiosis without a previous karyogamy (haploid meiosis) (Zickler et al. 1995). Moreover, the progeny of these heterozygous crosses is predominantly derived from the MAT mutant nuclei, which indicates that when the mating-type genes are defective, the nuclear identity is altered and produces "selfish" nuclei that enter into ascogenous hyphae ignoring the nuclei of the opposite parental origin (Zickler et al. 1995; Arnaise et al. 1997). **This indicates that the mating-type genes establish the nuclear identity that allows recognition between nuclei of opposite parental origin and ensure biparental progeny during sexual reproduction.** During ascogenous hypha differentiation, both gametic nuclei coexist in a common cytoplasm; therefore, the system governing nuclear identity likely resides on gene products that do not diffuse to neighbor nuclei. In *P. anserina*, the MAT genes *SMR2* and *FPR1* [please refer to Dyer et al. (2016) for further information on MAT gene function] code for transcription factors that have a nucleus-limited expression (Arnaise et al. 1997), consistent with a function for these factors in manifesting by themselves the nuclear identity. On the other hand, it has been postulated that interactions between nuclear DNA and the spindle pole bodies (SPBs, the fungal functional equivalents of centrosomes) could participate in mediating recognition between opposite mating-type nuclei (Thompson-Coffe and Zickler 1994). Identification of the actual signals defining nuclear identity and how these signals are transmitted to the cell machinery driving nuclear movement and distribution remains a challenging task.

3. Dikaryotic Cell Differentiation

Few genes implicated in dikaryotic cell differentiation have been so far identified, and among them only a couple have been characterized. In *N. crassa*, the banana (*ban*) mutant defined a dominant mutation in which early ascogenous cells produce asci that differentiate a single giant banana-like ascospore. However, late ascogenous cells arrest from going into karyogamy and meiosis, and their nuclei proliferate mitotically instead. These nuclear divisions occur without corresponding cell divisions, resulting in multinucleate croziers (Raju and Newmeyer 1977). Similarly, a second dominant mutation producing giant ascospores alters the development of late ascogenous hyphae yielding multinucleate croziers (Raju 1987). This second mutation—*prf*—is genetically unlinked to *ban* and differs from the latter

in also exhibiting defective ascus apical ostiole differentiation. Also, defects in SPB structure during ascus development have been described for *ban* mutants. Two additional mutants—*Cwl1* and *Cwl2*—producing multinucleated crozier cells have been identified in *N. crassa*. In these mutants, the formation of multinucleate cells has been associated to deficient septa formation in ascogenous hypha. However, since these mutants also exhibit defective septation in vegetative cells, they could represent factors implicated in the general cell septation process, a process that also involves the Rho GTPase Rho-4 (Raju 1992; Rasmussen and Glass 2005).

Elimination of the UCS protein Cro1 in *P. anserina* produces a multinucleated crozier phenotype similar to that of *N. crassa Ban* and *Prf* mutants. In the absence of Cro1, however, most ascogenous hyphae become multinucleated (Fig. 16.6E) independently of the perithecium developmental progression. Furthermore, in spite of their nuclear condition, a number of these croziers are able to differentiate asci and to undergo karyogamy, meiosis, and ascospore formation, although these processes occur abnormally (Berteaux-Lecellier et al. 1998; Simonet and Zickler 1978). *Cro1* mutants display defects in septa formation in ascogenous cells but not in vegetative hyphae. Also, microtubule and actin cytoskeletal organization is defective in *cro1* mutant croziers, including impaired assembly of actin rings involved in septa formation. USC (UNC-45/Cro1/She4) proteins are myosin-interacting chaperones that assist actomyosin-dependent processes, such as actin cable-based transport and cytokinetic actomyosin ring assembly (for review, see Hellerschmied and Clausen 2014). **These observations suggest that Cro1 could participate in dikaryotic cell differentiation by establishing the actomyosin interaction implicated in contractile ring assembly during septa formation.**

The *P. anserina* pleckstrin homology (PH) domain-containing protein Ami1 is a protein that is also implicated in controlling nuclear distribution in crozier cells. However, in contrast to the above-described proteins, the absence of Ami1 affects nuclear migration, resulting in the formation of some uninucleated croziers (Fig. 16.6F) (Graïa et al. 2000). Like its orthologues in *S. cerevisiae* (Num1) (Kormanec et al. 1991) and *A. nidulans* (ApsA) (Fischer and Timberlake 1995), Ami1 is implicated in nuclear migration, and the distribution of nuclei in different cell types is compromised when this protein is defective. During mitosis, *S. cerevisiae* Num1 is asymmetrically distributed to the cell cortex by association to the ER, where it interacts with microtubules and dynein to facilitate the migration of nuclei. Moreover, this protein also regulates the partitioning during cell division of other organelles, specifically of mitochondria (Farkasovsky and Kuntzel 1995, 2001; Lackner et al. 2013; Chao et al. 2014). Interestingly, in addition to nuclear defects, the croziers produced in the absence of Ami1 are also abnormal in shape, suggesting that ascogenous hypha morphogenesis also relies on this protein.

4. The Mitosis-Meiosis Transition

In *P. anserina*, the *CAR1* gene (hereafter referred to as *PEX2*, as it was subsequently renamed; see below) was initially discovered as a gene required for karyogamy (Simonet and Zickler 1972, 1978). The sexual development of mutants defective for this gene does not progress further than the dikaryotic stage. However, *pex2* mutants are able to differentiate normal croziers. Actually, the coordinated mitoses and septa formation that drive dikaryotic compartmentalization in these croziers are unaffected. As illustrated in Fig. 16.6C, this process results in the formation of a dikaryotic cell flanked by two uninucleate cells (note the dikaryotic cell delimited by two septa—indicated by arrows—on crozier *b*), as in wild-type development. In *P. anserina*, the dikaryotic penultimate cell undergoes karyogamy and ascus formation, while the two uninucleate flanking cells fuse and produce a new crozier, which perpetuates the dikaryotic stage. This latter process is also not affected in *pex2* mutants, but the nuclei present in the dikaryotic penultimate cell never fuse and instead divide mitotically. However, in contrast to *P. anserina cro1* or *N. crassa Ban* and *Prf* mutants, these nuclei do not accumulate within croziers

but are rather engaged in the formation of a new crozier. Consequently, *pex2* mutants produce profuse crozier arborescences (Fig. 16.6B), where no ascus is produced or diploid nucleus can be detected (Fig. 16.6C) (Simonet and Zickler 1972, 1978).

A number of observations suggest that this developmental defect results from failure to enter meiosis rather than from a defect in karyogamy per se. First, it has been demonstrated that the fusion of haploid nuclei in vegetative hyphae of *pex2* mutants is not affected, suggesting that the process of nuclear fusion per se is not impaired in these mutants (Berteaux-Lecellier et al. 1995). Second, mutants with altered internuclear recognition (i.e., deficient for mating-type genes; see Sect. V.B.2) (Zickler et al. 1995) or defective for nuclear fusion (see Sect. VI.A.2) (Vasnier et al. 2014) are still able to undergo haploid meiosis when karyogamy is prevented, suggesting that karyogamy is not strictly required for meiosis to proceed. Still, meiosis is never observed in *pex2* mutants.

In filamentous Ascomycetes, the premeiotic replication of DNA occurs before karyogamy (Zickler 2006). The nuclei present in *pex2* mutant croziers can divide mitotically, indicating that DNA replication is not impaired in these nuclei. However, there are fundamental differences between the meiotic and mitotic S-phases, and those driving chromosome morphogenesis are critical. Many proteins implicated in chromosome assembly and division are common during meiosis and mitosis; however, a number of these proteins are specific to meiosis. Some of these proteins are incorporated into chromosomes during premeiotic DNA replication (e.g., Storlazzi et al. 2008), which indicates that in filamentous ascomycetes, the commitment to undergo meiosis is taken before karyogamy. **These observations indicate that meiotic induction takes place in dikaryotic cells in a *PEX2*-dependent process**. They also suggest that in the absence of this gene, the mitotic division "program" is preserved.

Notably, further research demonstrated that *CAR1* actually encodes for a peroxisome biogenesis factor—PEX2—that is required for the import of proteins into the peroxisome matrix (Berteaux-Lecellier et al. 1995). These unexpected findings revealed a role for peroxisomes in the induction of meiotic development. However, the precise role that this organelle performs during meiocyte differentiation is still unknown. Nevertheless, along with PEX2, a number of additional specific peroxisome biogenesis factors (peroxins) have also been shown to be required for meiotic induction in *P. anserina* (Bonnet et al. 2006; Peraza-Reyes et al. 2008, 2011). It has been postulated that these peroxins could constitute an alternative peroxisome import pathway, which drives the import into peroxisomes of a yet unidentified protein necessary to initiate meiotic development. Alternatively, the induction of meiotic development could depend on a signaling pathway that signals from peroxisomes by association to a specific group of peroxins. Of note, the developmental defects produced by the elimination of PEX2 (or other meiosis-required peroxins) in *P. anserina* are not observed upon elimination of the corresponding proteins in *A. nidulans* (Hynes et al. 2008, 2010). This indicates that the factors regulating entry into meiosis, or their subcellular organization, can significantly vary between different fungi, even when closely related.

C. The Dikaryotic Phase in Basidiomycota

In contrast to Ascomycota, the dikaryotic phase of Basidiomycota fungi is not restricted to the fertile tissues of sexual fructifications and is extended to the somatic phase of the life cycle. As illustrated by *U. maydis* (Ustilaginomycotina), the dikaryotic stage of most basidiomycetes can commence when haploid vegetative cells undergo plasmogamy to produce a dikaryotic mycelium. In this plant pathogen, in addition, each stage is associated to a specific phase of the life cycle. While haploid monokaryotic yeast cells proliferate saprophytically by budding, the dikaryotic stage is associated with the infective phase of the fungus, as only dikaryotic hyphae are able to infect and induce disease in the host (maize). Actually, sexual reproduction of this fungus is absolutely dependent on the presence of the plant. This virulence-related development commences by the formation of conjugation tubes by the yeast-like haploid

cells, which is regulated by the pheromone signaling system. These cells undergo plasmogamy by fusing their tips and generate a dikaryotic hypha, which can infect the plant and proliferate within to ultimately differentiate diploid teliospores. Teliospores are resting spores that are produced inside of fungus-induced plant tumors and that upon germination undergo meiosis to produce haploid meiospores (basidiospores) (Vollmeister et al. 2012).

In Agaricomycotina, the dikaryotic phase frequently initiates by the fusion of hyphae from vegetative monokaryotic haploid mycelia. Cell anastomosis is followed by the reciprocal migration of haploid nuclei from one parental mycelium into the opposed preexisting mycelium, giving rise to haploid dikaryotic mycelia (also referred to as the secondary mycelium) (Kues 2000 and references therein). However, homokaryotic mycelia can also be dikaryotized by nuclei arising from hyphal fragments or spores (Vilgalys and Sun Bao 1994; Williams et al. 1984; Buller 1931). In these fungi, the mating-type genes (which include the pheromone and pheromone receptor-encoding genes) are dispensable for the initial cell fusion process, but the nuclear migration allowing dikaryotization and dikaryon growth is controlled by these genes. Importantly, the homokaryotic and heterokaryotic (dikaryotic) vegetative mycelia of these fungi exhibit different mating behavior, as homokaryotic mycelium can both accept and donate nuclei in a mating reaction, whereas in many fungi, dikaryotic mycelium cannot stably incorporate a third type of nucleus but still can donate nuclei and fertilize a homokaryotic mycelium. In this process, which is referred to as the Buller phenomenon (described initially by Buller 1931; for review, see Kues 2000; Anderson and Kohn 2007), both types of nuclei from the dikaryotic mycelium can initially migrate into the opposed homokaryotic mycelium. This can produce a dikaryotic mycelium where only one type of incoming nuclei is accepted, a dikaryotic mosaic mycelium containing sectors with different combinations of nuclei, or even a relatively stable trikaryotic mycelium. Still, in some cases, the two incoming nuclei from the fertilizing dikaryon can replace the original monokaryotic mycelium nuclei (May 1988; James et al. 2009). Furthermore, the segregation of monokaryotic sectors from a dikaryon and their subsequent reassociation with a different type of nucleus (Nieuwenhuis et al. 2013a; James et al. 2009), as well as nuclear exchange between heterokaryotic mycelia (Johannesson and Stenlid 2004), can dynamically transform the nuclear associations of heterokaryotic mycelia in nature. **This remarkable genetic flexibility permits to establish recombinant genotypes in nature without the occurrence of meiosis and reflects that mating in basidiomycetes is a rather complex and asymmetrical process in nature** (Anderson and Kohn 2007; Nieuwenhuis et al. 2013b).

Early pioneer studies on *Coprinopsis* (*Coprinus*) *lagopus* established that the dikaryotization of a monokaryotic mycelium depended on the migration of the incoming fertilizing nucleus throughout the established accepting mycelium. In this process, immigrant nuclei from one genotype undergo rapid intercellular migration—with velocities up to ten times as great as the rate of hyphal growth—over a preexisting mycelium containing resident nuclei of another genotype (Buller 1931). These nuclei migrate through microtubule tracks (Raudaskoski 1998), likely propelled by microtubule-dependent molecular motors; however, the process whereby distinct type of nuclei get differentially engaged in intercellular displacements is currently not known. Furthermore, it is also known that the migration of these nuclei depends on the B mating-type locus, which encodes for the mating pheromones and pheromone receptors (Wendland et al. 1995; Papazian 1950), but the process by which nuclear migration is induced also remains unclear.

Different cellular processes have evolved in basidiomycetes to maintain dikaryotic cells. Whereas dikaryotic hyphae of species like *Heterobasidion annosum* and *Agaricus bisporus* consist on multinucleate hyphae possessing as many as 30 nuclei per cell (Chase and Ullrich 1983; Evans 1959), regular pairing of complementary nuclei is observed in hyphal cells of species like *C. cinerea* and *S. commune* (Kamada et al. 1993; Niederpruem et al. 1971).

Nonetheless, balanced association of the two types of gametic nuclei exists in the heterokaryotic hyphae of these fungi. Central to dikaryon establishment is the process of formation of clamp connections. Upon its arrival to the apical cells of the mycelium, the incoming migrating nucleus is paired with a compatible resident nucleus. Subsequently, a clamp (or hook) cell emerges as a lateral branch close to these nuclei and next to the site where a septum will be produced. Clamp cells then grow around backward toward its parent hypha, and one of the nuclei (the one located closer to the tip) migrates into the developing clamp cell, whereas the second one remains in the hyphal cell beneath the site where the septum will be formed. Both nuclei then divide synchronously with their spindles oriented in such an arrangement that a pair of complementary nuclei (one of them leaving the clamp cell) is laid together above the future septum location. A pair of septa is then produced across the previous location of the spindles, and one of them separates the clamp cell from the hypha, and the second one divides the hypha into apical and subapical cells. As a result, two complementary nuclei are isolated in the apical cell delimited from uninucleate clamp and subapical cells. Further, the clamp cell continues to grow backward and fuses with a peg produced beneath the newly formed hyphal septum. The fusion between the clamp cell and the peg delivers the clamp cell nucleus back into the subapical cell, which regains the dikaryotic state [extensively reviewed in Kues (2000)]. In this way, the coordinated nuclear division and septa formation associated to the formation of clamp connections ensure the isolation of a pair of compatible (of opposite mating type) nuclei in each hyphal compartment along hyphal growth. Still, while clamp cells have not been observed in *A. bisporus* (Evans 1959), clamp connections are also produced in compatible matings of *H. annosum*, even when possessing highly multinucleate cells (Chase and Ullrich 1983). Importantly, as shown in *U. maydis* (Scherer et al. 2006), clamp connections are present in basidiomycetes beyond Agaricomycotina.

While the nuclear migration accounting for the initial dikaryotization of a homokaryon is controlled by the B mating-type locus, clamp cell formation is governed by both mating-type loci (Raudaskoski and Kothe 2010). The A mating-type genes encode for homeodomain proteins, which when synthetized from compatible nuclei heterodimerize to produce an active transcription factor that activates specific target genes. The A mating-type genes are required for clamp cell formation, whereas the induction of the subapical peg, with which clamp cells fuse, depends on the B mating-type genes (Badalyan et al. 2004) [please refer to Freihorst et al. (2016) for further information on basidiomycete mating-type genes].

The resemblance of the dikaryotic cell compartmentalization processes occurring in croziers in ascomycetes and clamp connections in basidiomycetes—the two fungal lineages exhibiting a distinctive dikaryotic stage—has led to propose a common evolutionary origin for these two structures. However, analysis of the functional conservation of the homeodomain transcription factors—which control clamp cell formation in basidiomycetes—during dikaryotic cell development in the ascomycete *P. anserina* has provided no support for such hypothesis (Coppin et al. 2012).

Ultimately, the two compatible nuclei of dikaryotic cells undergo karyogamy and meiosis to subsequently produce sexual spores containing the nuclear products of meiosis, the basidiospores. This process takes place in the basidia (the basidiomycete meiocyte) that develop in the hymenium of complex sexual fructifications (reviewed in Pelkmans et al. 2016).

VI. Karyogamy

Karyogamy is the process whereby nuclei fuse to produce diploid cells. In fungi, this process normally takes place in specialized sexual cycle cells, which compartmentalize the gametic-derived nuclei, and is coordinated with the progression of the steps of the sexual cycle, like plasmogamy and meiosis. However, in many fungal species, haploid nuclei of vegetative cells can eventually fuse to produce diploid nuclei. This process usually takes place with a low frequency, and the generated diploid nuclei are relatively stable, as they can spontaneously

go back to the haploid state by successive segregation and loss of whole chromosomes. This process, which is referred to as the parasexual cycle, also differs from typical sexual reproduction in that the steps encompassing it occur independently of one another. Nonetheless, this process can contribute to genetic variability and promote fitness (Schoustra et al. 2007). Whether the same molecular process produces somatic and germinal (those produced during sexual reproduction) diploid nuclei is still unknown. However, in some fungi, epigenetic marks can define the identity and fate of the diploid nuclei that are produced at different stages of the life cycle.

In the homothallic *A. nidulans*, where the parasexual cycle was first described (Pontecorvo et al. 1953), vegetative diploid nuclei can divide indefinitely, whereas germinal diploid nuclei are committed to meiosis and never divide mitotically. Furthermore, if diploid mycelium is induced to reproduce sexually, a single—likely diploid—nucleus is compartmentalized into the ascogenous cells produced within sexual fructifications. However, this nucleus undergoes aberrant meiosis, and ascospores are produced in irregular numbers (Scazzocchio 2006; Pontecorvo et al. 1953). Nonetheless, diploid nuclei produced in somatic cells of some fungi can successfully be engaged in sexual reproduction. This is actually the case in the human pathogen *C. neoformans*, a causal agent of fungal meningoencephalitis (Lin and Heitman 2006). This basidiomycete is able to undergo heterothallic bisexual reproduction by a process involving yeast to hypha dimorphic transition. Haploid yeast cells of opposite mating type (denoted as *a* and *alpha*) recognize each other and mate to produce dikaryotic hyphae. Aerial hyphae then develop from these hyphae and differentiate round basidia at their tips, where karyogamy, meiosis, and basidiospore formation take place. Importantly, nuclear fusion can also take place in vegetative yeast cells immediately after mating. These diploid cells can propagate mitotically as yeasts or produce diploid hyphae, which eventually differentiate basidia and undergo meiosis and basidiospore formation (Sia et al. 2000). Interestingly, in this fungus, the proportion of each mating type in nature seems to be rather unequal, and about 99 % of environmental and clinical isolates belong to the *alpha*-mating type (Fu et al. 2014). Furthermore, *C. neoformans* is able to undergo unisexual reproduction by mating between same-sex cells, predominantly by the *alpha*-mating-type cells (Lin et al. 2005). During this process, *alpha-alpha* diploid nuclei that can resume the sexual cycle by undergoing meiosis are produced. These diploid nuclei can originate by nuclear fusion either early in vegetative cells or in the sexually differentiated basidia, or they can be produced by nuclear endoreplication.

In the Dikarya fungi, karyogamy normally takes place in the specialized cell type, which will further develop into a meiocyte. This cell is called an ascus, in the Ascomycota, and a basidium, in the Basidiomycota, and also corresponds to the cell that ultimately produces the sexual spores.

A. The Molecular Mechanisms of Karyogamy

The molecular mechanisms underlying karyogamy have been most intensively studied in *S. cerevisiae*. In this yeast, karyogamy implicates the migration of the two haploid nuclei toward each other, which is referred to as nuclear congression, and the subsequent fusion of the nuclear envelopes. Pioneer work involving the isolation and characterization of nuclear fusion mutations in this yeast led to the identification of most genes known to govern the progression of karyogamy (referred to as *kar* genes, for karyogamy defective). These mutations resulted in the formation of aberrant zygotes that were unable to fuse their haploid nuclei and could broadly be grouped in two main classes depending on the position of their nuclei. In the so-called type I mutants, which defined the nuclear congression genes, nuclei fail to pair close to one another and remain separated by about 3 microns. In contrast, nuclei in type II mutants become closely apposed but remain unfused. These mutants defined the nuclear envelope fusion genes (Rose 1996).

1. Nuclear Congression

In *S. cerevisiae*, nuclear congression is initiated shortly after mating. Actually, critical developmental processes promoting karyogamy are regulated by the pheromone signaling system that controls mating. Early experiments showed that heterokaryotic cells obtained by fusion of protoplasts derived from mitotic cells were unable to fuse their nuclei; however, if the cells producing the protoplasts were pretreated with mating pheromone their nuclei could effectively fuse and produce diploid cells even when bearing same mating type (Rose et al. 1986). These experiments demonstrated that the competency of nuclei to undergo karyogamy was regulated by the mating pheromone response.

Upon stimulation of a haploid *S. cerevisiae* cell with the pheromone of the opposite mating type, the cell cycle is arrested at G1, and the cell forms the *shmoo* projection toward the source of the pheromone. Cell fusion takes place at the tip of the *shmoo* projection (Fig. 16.2). Actually, the initial step required to facilitate karyogamy takes place in *shmoos* before mating and consists on the orientation of nuclei toward the *shmoo* projection. This process initiates by the reorganization of cytoplasmic microtubules, which are relocated from the outer plaque of the SPB to the half bridge, a lateral extension of the SPB. This process depends on the transfer of the gamma-tubulin receptor Spc72p from the SPB outer plaque to the half bridge. This process is stimulated by mating pheromone exposure and depends on anchorage of Spc72 to the half bridge component Kar1 (Pereira et al. 1999). Subsequently, the plus ends of microtubules are transported along polarized actin cables into the cell mating projections. The protein Kar9, in association with the microtubule end-binding protein Bim1 and the type V myosin Myo2, facilitates this transport (Hwang et al. 2003). Mating pheromones also stimulate the capture of cytoplasmic microtubules at the cortical site of the *shmoo* tip by promoting both the localization of the kinesin-14 family protein Kar3 and its non-motor-associated protein Cik1 to the microtubules plus ends (Maddox et al. 2003; Sproul et al. 2005) and the interaction of Kar3 with the G-alpha protein Gpa1, which localizes at the cortex of the *shmoo* tip and provides an anchor for this kinesin at this site (Zaichick et al. 2009). These processes orientate the nucleus and direct the cytoplasmic microtubules toward the mating projection. Actually, nuclear movement toward this site commences before plasmogamy takes place.

Following cell fusion, the cytoplasmic microtubules of a cell protrude into the cytoplasm of its mating partner and contact the opposite nucleus. These microtubules emanate from the half bridge of a SPB, where they are anchored by their minus ends to the Spc72/gamma-tubulin complex, and interact with Spc72 located on the half bridge of the opposite SPB (on the mating partner nucleus). This second interaction between microtubules and Spc72 is mediated by the minus-end-directed kinesin motor Kar3, which from this SPB provides the force that pulls the opposite nucleus and allows for nuclear congression (Gibeaux et al. 2013). As a result, nuclei converge and their SPBs get closely apposed. The fusion of the nuclear envelopes normally occurs in the vicinity of these SPBs.

Very little is known about the processes that mediate nuclear congression beyond yeasts; however, as illustrated in *C. neoformans*, this process might be rather divergent in other fungi (Lee and Heitman 2012). This basidiomycete lacks orthologues of *KAR1* and *KAR9*, and those for *KAR3* and *KAR4* are not required for karyogamy (Lee and Heitman 2012). *S. cerevisiae KAR4* encodes a transcription factor that promotes the induction of genes implicated in karyogamy (Lahav et al. 2007; Kurihara et al. 1996). Therefore, these observations could indicate that nuclear congression is dispensable for karyogamy in some fungal lineages, or they could reflect fundamental differences in the molecular mechanisms accounting for nuclear congression.

2. Nuclear Envelope Fusion

Nuclear envelope fusion is a process that takes place in two steps, where outer membrane fusion precedes inner membrane fusion and is

followed by the fusion of the SPBs (Melloy et al. 2007). The fusion of the outer membranes is dependent on ER-resident SNARE proteins—namely, the Q-SNAREs Sec20, Bos1, Ufe1, and Use1, which conceivably provide the membrane fusogenic activity (Rogers et al. 2013). In addition, nuclear outer membrane fusion also requires the proteins Prm3 and Kar5 (Melloy et al. 2009; Shen et al. 2009). These two proteins are induced upon pheromone stimulation and interact with each other at the nuclear membrane, where they are enriched at the site where membrane fusion commences, adjacent to the SPBs (Shen et al. 2009; Beh et al. 1997). In addition, Kar5 seems to also coordinate the processes of fusion of the outer and inner nuclear membranes. Ultimately, inner membrane takes place, in a process that depends on the ER-resident chaperon proteins Kar8 (Jem1) and Kar2 (Bip) (Melloy et al. 2009; Nishikawa and Endo 1997; Rose et al. 1989). Notably, Kar2 is involved in protein import into the ER, and strains deficient for Kar7 (Sec66), a nonessential component of the Sec63 ER protein import complex, or for Sec63 per se also display nuclear fusion defects. The precise connection between ER protein import and nuclear fusion is not clear, but genetic data suggest that these proteins could have separate roles in these processes (Ng and Walter 1996). Interestingly, among the *C. neoformans* orthologues of genes implicated in karyogamy in *S. cerevisiae*, the only one required for karyogamy is *KAR7*. Furthermore, this gene is equally required for bisexual (opposite sex) and unisexual (same-sex) reproduction in *C. neoformans* (Lee and Heitman 2012). These observations suggest not only a relevant conserved function for this protein, but they also indicate that both vegetative and germinal nuclear fusions depend on this protein.

Recently, an additional protein required for karyogamy was identified in *S. macrospora*. This protein—Slp1—is a SUN-domain-containing ER protein whose absence blocks nuclear envelope fusion. Interestingly, karyogamy impairment in mutants devoid for this protein does not abrogate the following meiosis, and instead parallel haploid meioses take place. Furthermore, the meiotic recombination and synapsis program in these meioses is switched, such that it now occurs between sister chromatids instead than between homologous chromosomes, allowing the production of a significant number of ascospores (Vasnier et al. 2014). These data reveal a tight connection between karyogamy and meiotic development, which permits to adjust meiosis in response to specific clues on karyogamy status.

As illustrated in *S. cerevisiae*, the pheromone response pathway stimulates many of the processes driving sexual development (Table 16.1). These processes must be precisely orchestrated in time in order for successful mating to take place. For instance, commencing the cell wall-remodeling processes required plasmogamy before encountering a mating partner could have significant detrimental consequences. Consistent with this notion, the expression of a number of genes that are induced upon pheromone stimulation shows a temporal delay during the mating process. It has been shown that the transcription factor Kar4, which itself is induced upon pheromone stimulation by Ste12, provides specificity and allows for the delayed temporal induction for a subset of pheromone-inducible genes that are required for karyogamy, such as Kar3 and Cik1 (Lahav et al. 2007; Kurihara et al. 1996). As exemplified by this gene, sequential activation of key regulatory factors governing different developmental steps could account for the efficient temporal control of the mating process. Additional research is required to further elucidate how the precise temporal orchestration of sexual development is achieved in the diverse developmental programs of fungi.

VII. Premeiotic Genome Scrutiny and Defense Mechanisms

For some filamentous ascomycetes (Pezizomycotina), sexual reproduction is also a time to check for genome integrity. Interspersed repeats account for a significant fraction of eukaryotic genomes and can range from a few percent to more than 50 % of genomic contents.

As these selfish elements do not participate in cellular development and maintenance of their host, they are mainly considered as a genomic burden. To protect genomes against these invasive DNA repeats and to minimize their transcriptional noise homology-based epigenetic defense systems have been selected through evolution. This is the purpose of the premeiotic fungal transcriptional gene silencing systems related to the repeat-induced point mutation (RIP) process that was originally described in *N. crassa* (Selker et al. 1987).

A. RIP and RIP-Like Defense Systems Are Premeiotic Processes

Genetic evidences showed that RIP takes place at the dikaryotic stage of the sexual cycle. As a consequence, RIP events occur independently in each of the individual haploid nucleus contained in the dikaryotic cells. RIP targets are DNA repeats longer than 400 bp (Watters et al. 1999) that share over 80 % of nucleotide identity. Once subjected to RIP, repeats are mutagenized, mostly via C–G to T–A transitions. As a result, the *N. crassa* genome shows a complete absence of intact and active mobile elements (Galagan et al. 2003), and natural repeats are AT rich. In addition, most of the cytosines of RIPed loci are de novo methylated. When genes are embedded in RIPed loci, their expression is silenced because cytosine methylation inhibits transcription elongation (Rountree and Selker 1997). If the RIP mutagenic effect results in introduction of Mendelian mutations, the silencing of RIPed alleles is reversible.

To date, only one gene essential to RIP has been described. The *rid* gene (RIP defective, Freitag et al. 2002) encodes a putative DNA methyltransferase. When inactivated, it prevents the two outcomes of RIP, i.e., cytosine de novo methylation and nucleotide mutagenesis of newly formed repeats, while methylation patterns of previously RIPed alleles are maintained. Despite its canonical structure of eukaryotic DNA methyltransferase, the *N. crassa* RID protein failed to show any in vitro enzymatic activity (Freitag et al. 2002). Besides, whether RID plays any direct role in nucleotide mutagenesis remains unknown. The molecular mechanism that allows recognition of repeats has also remained poorly understood for long. It has first been established that none of the proteins involved in *N. crassa* RNA interference pathways were essential to RIP (Lee et al. 2010). This meant that homology recognition of repeats dispersed in the genome do not proceed through siRNA signaling. Recent evidences point toward direct DNA-DNA interactions of homologous segments, provided that they share triplets of base pairs displayed with a matching periodicity of 11 or 12 base pairs (Gladyshev and Kleckner 2014). This original homology-dependent pairing system does not rely upon the canonical homology recognition pathway because it can proceed in the absence of MEI3, the only RAD51/DMC1 protein in *N. crassa* (Gladyshev and Kleckner 2014).

But, if the core of the RIP mechanism is far from being understood, its impact upon chromatin plasticity and gene expression has been extensively described in *N. crassa*. Heavily methylated and AT-rich RIPed sequences are converted into heterochromatin through a succession of steps that can be summarized as follows. After the phosphatase PP1 removes phosphorylation of the histone H3 serine 10 (H3S10p) (Honda and Selker 2008), the DIM-5 histone methyltransferase trimethylates the histone H3 lysine 9 residues (H3K9me3) (Tamaru and Selker 2001). This enzyme is one of the core components of a complex that also contains DIM-7, DIM-8 (a DNA repair protein), DIM-9 (a histone deacetylase), and CUL4 (a cullin protein): the DCDC complex (Lewis et al. 2010a, b). This complex is essential to direct histone methylation of RIPed sequences. Specifically, DIM-7 targets DIM-5 to heterochromatin. Once trimethylated, the H3K9me3 residues recruit HP1 proteins. Then, the DNA methyltransferase DIM-2 is brought to the RIPed loci by HP1 (Honda and Selker 2008) to methylate any cytosine residues. By inhibiting trimethylation of H3K9, the DMM complex prevents the heterochromatin to spread into unRIPed neighboring sequences (Honda et al. 2010). Thus, as a consequence, RIPed regions are highly heterochromatic, and this feature is coextensive with the RIP-induced DNA methylation.

RIP is not restricted to *N. crassa*. This process is widely conserved among the Pezizomycotina and has important physiological and genomic consequences. Typical C to T RIP transitions have been observed in numbers of species belonging to this subphylum, mostly using in silico analyses (see Clutterbuck 2011 for the most recent compilation), but also by experimental evidences (Coleman et al. 2009; Coppin and Silar 2007; Cuomo et al. 2007; Fudal et al. 2009; Graïa et al. 2001; Idnurm and Howlett 2003; Ikeda et al. 2002; Nakayashiki et al. 1999). To our knowledge, none of those species show DNA methylation. Thus, *N. crassa* might be an exception with respect to the RIP features. Interestingly, the Pezizomycete *A. immersus* presents an RIP-related mechanism named MIP for methylation induced premeiotically, where repeats are subjected to DNA methylation but not to mutagenesis during the dikaryotic stage of sexual reproduction. The *masc1* gene of *A. immersus*, which encodes a putative DNA methyltransferase homologous to the *N. crassa* RID protein, is essential to de novo methylation of DNA repeats through MIP, while methylation is fully maintained on previously MIPed alleles (Malagnac et al. 1997). But when the *rid* mutants of *N. crassa* are fertile, crosses involving *masc1* mutant strains are arrested at an early stage of sexual reproduction and therefore sterile. Similarly, DmtA, the *Masc1* and RID orthologue of *A. nidulans*, a homothallic fungus showing no DNA methylation but RIP signatures in its genome, is also essential for early sexual development (Lee et al. 2008a). Whether some of the RIP-related genome defense systems are also required for completion of sexual reproduction in Pezizomycotina remains to be investigated.

B. Meiotic Silencing by Unpaired DNA in *N. crassa*

Besides the vegetative quelling pathway, *N. crassa* presents a second RNAi pathway, specifically active during meiosis (Aramayo and Metzenberg 1996). The MSUD (meiotic silencing by unpaired DNA) identifies unpaired genomic regions during prophase homolog pairing (Shiu et al. 2001). Once identified, the genes included within the unpaired DNA segments are silenced, as well as all homologous copies that may be found in the genome, whether or not they are themselves paired. This meiotic silencing is temporary since the expression of the unpaired alleles gradually resumed shortly after spore formation (Shiu et al. 2006). A working model proposes that after an unpaired segment is detected, an aberrant RNA (aRNA) is transcribed from the unpaired locus. Once addressed to the perinuclear region, the aRNA is turned into a double-stranded RNA (dsRNA) by SAD-1, an RNA-directed RNA polymerase homologous to QDE-1 (Shiu and Metzenberg 2002), probably along with SAD-3, a helicase that may contribute to form dsRNA (Hammond et al. 2011). dsRNA is processed into small interfering RNAs (siRNAs) by DCL-1, a Dicer protein (Alexander et al. 2008). Once converted into single strand by the exonuclease QIP (Lee et al. 2010; Xiao et al. 2010), the siRNAs are loaded onto the Argonaute SMS-2 protein that uses siRNA to target the homologous mRNA (Lee et al. 2003). All the proteins of this meiotic silencing complex are also essential to sexual reproduction.

Meiotic silencing relies upon trans-acting small RNA intermediates to signal unpaired DNA. Indeed, the *sad-1* gene encoding an RNA-directed RNA polymerase homologous to QDE-1 (Shiu et al. 2001) and the *sms-2* and *sms-3* genes encoding paralogs of the Argonaute protein QDE-2 and of Dicer protein DCL-2, respectively, are all essential to MSUD (Lee et al. 2003). SAD-1, SMS-2, and SMS-3 are also required for sexual reproduction. Two more proteins of unknown function, SAD-4 and SAD-5 (restricted to Sordariales), are important for the formation of siRNA involved in meiotic silencing (masiRNAs) but are not essential to sexual reproduction (Hammond et al. 2013).

As for RIP, while the silencing process of MSUD is well deciphered, the process that detects unpaired DNA is poorly understood. But it has been recently demonstrated that SAD-6, a putative SNF2-family protein closely related to Rad54, is required for efficient MSUD (Samarajeewa et al. 2014). Because the Rad54 protein plays a part in the repair of double-strand breaks by homologous recombination,

the unpaired DNA recognition step of MSUD may be achieved through homologous recombination, on the contrary of the RIP searching mechanism.

Interestingly, the *sad-1* mutant strains can perform interspecific crosses. Such crosses are otherwise barren when done with wild-type strains, suggesting that meiotic silencing, by targeting unpaired DNA due to chromosomal polymorphism, could be part of the genetic barrier that prevents interspecific crosses. But if genes encoding SAD-1-like protein can be found in most of fungal genomes, to date, MSUD has been described in *N. crassa* only.

VIII. Meiosis

The purpose of meiotic process is to reduce by half any diploid genome such that the genetic material of gametes will be suitable for fecundation and subsequent development of new diploid zygotes, genetically different from their parents. Meiosis has been studied in fungal model organism for a long time. Indeed, sexual development in fungi is a rather quick process, but more importantly, unlike in plants and animals, **the fungal meiotic spores are grouped together (tetrads) in a single structure, the ascus of the Ascomycetes (illustrated in Figs. 16.1B, 16.2 and 16.6A) and the basidium of the Basidiomycetes**. Thus, each single event of meiosis can be monitored. This is a quite peculiar asset, since such tetrad analysis can be performed only in fungal model organisms or single-celled algae. Moreover, when ascospores are linearly arranged in asci, as those of *N. crassa* or *S. macrospora*, it gives a straightforward picture of the former nuclear divisions. Hence, if two haploid strains harboring polymorphic alleles of a given gene are crossed, the alleles will either segregate during the first meiotic division or during the second meiotic division.

An important corpus of data on meiosis mechanistic was gathered thanks to fungal model species. Among those, one can name *A. immersus*, *A. nidulans*, *N. crassa*, *P. anserina*, *S. cerevisiae*, *S. macrospora*, *S. pombe*, and *C. cinerea*. However, apart from this laboratory well-acclimated species, completion of a sexual cycle onto petri dish is impossible for most fungi. On the other hand, the full set of genes required to perform meiosis is found in most, if not all the available complete fungal genome sequences, even though their sexual cycle is not documented in nature. Consequently, dispersion through sexual reproduction, which is the only way to ensure genetic variability among offspring, might be more widely used than expected based on functional evidences.

Meiotic division is performed once diploid cells are formed, thus after karyogamy. It also requires DNA synthesis prior division. While premeiotic replication is mechanistically analogous to its mitosis counterpart, it spans over a longer time frame. As shown in Saccharomycotina, this delay might be related to the formation of meiotic double-strand breaks (DSBs) (Borde et al. 2000). DSBs initiate the meiotic recombination, which is an essential step for completion of the sexual cycle. After the extended G2 phase, the meiotic prophase I (chronologically divided into leptotene, zygotene, pachytene, and diplotene) is devoted to recognition, pairing, and subsequent recombination of homologous maternal and paternal chromosomes (homologs). The initial step of the juxtaposition of the homologs, which happens early during prophase (leptotene), involves long-distance interaction to recognize homology. How homologs locate each other into the nucleus is not yet understood. This spatial disposition of homologs, unique to the meiotic prophase I, has peculiar genetic consequences. By comparison with the parental genotypes, it creates novel allele combinations. Indeed, during mitotic division, when some of the infrequent sporadic DSBs are repaired through the homologous recombination pathway, sister chromatids are used as template. By contrast, during meiosis, all of the frequent programmed DSBs are repaired through the recombination pathway, and because of the co-alignment of bivalents, non-sister chromatids of homologs are used as template.

DSBs are induced at early leptotene, thus before pairing, by evolutionary conserved Spo11p protein. Initially identified in *S. cerevisiae* (Bergerat et al. 1997), this meiosis-specific topoisomerase II-like catalyzes DSB formation by the way of its endodeoxyribonuclease activity. This cleavage of the DNA molecule is

followed by the resection of the 5' ends of the fragments, leading to the formation of protuberant 3' ends soon to be protected by a protein complex that includes the single-strand binding proteins Rad51 and eventually Dmc1 when present (Keeney 2001). By zygotene, the assembly of a scaffold-like structure called the synaptonemal complex (SC) is initiated. Being the most universally conserved structure of meiosis, the SC mediates the co-alignment of homologs along their entire length. The SC is made of three distinct elements: two lateral elements bridged by transverse filaments on which lays a central element, the latter being parallel and equidistant to the lateral elements. Like poles, the two lateral elements assemble along the pair of bivalents, each of them in the vicinity of the two sister chromatids. The SC first forms foci of nucleation in between the homologs, co-aligned at ~400-nm distance. This is concomitant with generation of single-strand ends by resection. Once formed, these protuberant 3' ends invade their respective homologous duplexes to give single-end invasion (SEI) intermediates. At early to mid-pachytene, after repair synthesis, double Holliday junctions (DHJs) are formed. By then, the SC is fully assembled, and upon SC completion, synapsis brings the chromosome axes of homologs at ~100-nm distance. This close vicinity promotes recombination at late pachytene. It has been long considered that resolution of DHJs would give two different outcomes. If the DHJs are resolved with reciprocal exchange of DNA, this would lead to inter-homolog crossing-overs (COs). In heterozygous crosses of eight-spore ascomycetes, this will typically result in a 4:4 segregation pattern. On the other hand, if DHJs are resolved without reciprocal exchange of DNA, this would lead to noncrossovers (NCOs), also called gene conversion. In that case, heterozygous crosses of eight-spore ascomycetes will typically yield asci showing a 6:2 segregation pattern.

Most of the effective DSBs will turn into NCOs, while the rest of them will mature into COs. The signal that determines if DSB will give NCOs or COs is still unknown. However, in *S. cerevisiae*, NCOs versus COs determination occurs before the resolution of DHJs (Allers and Lichten 2001; Börner et al. 2004). In fact, in the budding yeast, all of the DHJs are resolved into COs. The decision to engage into COs pathway might be concomitant with the SEI formation. Moreover, in fungi, as in most eukaryotes, localization of COs is not random. Hot spots of recombination have been well defined in *S. cerevisiae* genome. Many of them are located into gene promoters, which are genomic regions exhibiting euchromatic features (Brachet et al. 2012). In addition, in a defined genetic interval, the presence of a first CO decreases the probability that a second CO takes place in its close vicinity, which is known as interference. By contrast, NCOs do not exhibit interference. The molecular basis of interference is unknown. Moreover, to ensure accurate segregation of homologs at meiotic division I, each pair of chromosomes has to undergo at least one CO. But because fungal chromosomes have quite different lengths, without regulation, probability would be rather high that the smallest chromosomes would not undergo any CO. Interestingly, *A. nidulans* and *S. pombe* that do not form SC show a high number of COs per bivalent when compared to fungal species endowed with SC. Conversely, if SC is impaired resulting in an absence of synapsis, recombination is abolished (Espagne et al. 2011).

In between end of leptotene and early pachytene, the telomeres of aligned bivalents are attached to the inner surface of the nuclear envelope. This conserved meiotic feature is called the bouquet stage. The bouquet forms independently of DSBs and is also seen in haploid meiosis. To date, the function of the bouquet remains unclear. After completion of recombination, the SC is lost, and subsequently, at diplotene, homologs are not anymore co-aligned along their entire length but only connected at sites of crossovers (referred as chiasmata in cytological studies). This is called the diffuse stage. To allow the segregation of each homolog into daughter cells, chiasmata are released at metaphase I. Successful execution of meiotic division II is linked to the release of centromeric cohesion. Indeed, if sister chromatids do not part at metaphase II, this leads to imbalance chromosome numbers into gametes. Aneuploid gametes harboring to few or too many chromosomes are the most prominent cause of sterility in fungi as in all of the organisms that rely on sexual reproduction.

Sporulation is dependent on spindles position, both meiotic spindles and postmeiotic mitosis spindles. When postmeiotic mitosis occurs, asci are composed of eight ascospores instead of the four meiotic products. In pseudohomothallic species as in *P. anserina*, after postmeiotic mitosis, two nuclei of opposite mating types are packed into one ascospore (Fig. 16.1B). This requires identification of nuclei genetically different, which mechanism is still unknown. Interestingly, mating-type-deficient strains of *P. anserina* are unable to form heterokaryotic biparental ascospores. This leads rather to monokaryotic ascospores or to dikaryotic uniparental ascospores. Packing nuclei of opposite mating types into *P. anserina* ascospores requires also correct position of spindles. Migration of nuclei is primary dependent on actin filaments as well as on actin-myosin interactions and to a lesser extent on microtubules (Thompson-Coffe and Zickler 1994).

IX. Concluding Remarks

A. Is Sexual Development an Uncommon Way to Reproduce?

When most eukaryotes reproduced sexually, a fifth of fungal species were thought to reproduce through clonal divisions. This has long been considered as a surprisingly high and unmatched proportion of asexual species for an entire kingdom. Indeed, even if sexual reproduction is associated with some immediate costs, it seems essential for the long-term persistence of species. Fungal species that reproduce clonally are not subjected to meiotic recombination that breaks down advantageous allele combinations built by past selection. Their genome might also escape to contamination by parasitic genetic elements. Sex provides some additional positive outcomes that outbalance these costs. Sexual reproduction reduces the accumulation of deleterious mutations. It leads also to a more rapid adaptive response by building new beneficial combinations of alleles, which is clearly important in changing environments.

The scattered distribution of species endowed with sexual reproduction among the fungal kingdom suggests that sex has been lost independently several times. However, population genetics shows that many of these allegedly asexual species have finally been found to reproduce sexually in nature. For others, it was recently possible to master successfully their sexual cycle under laboratory conditions. From a genomic perspective, other signs of sexual reproduction have been uncovered. First, the vast majority of the fungal complete genome sequences currently available show clear footprints of RIP, which is indicative that sexual reproduction was effective at some point during the evolutionary history of these species. Second, the mating-type loci and the set of genes required for sex can be identified from complete genome sequences of most species considered to be asexual. Moreover, these genes are often found to evolve under purifying selection, as expected for functional genes. Because, in asexual fungal species, the evolutionary constraints on genes involved in sexual reproduction would be relaxed, these evidences suggest that sexual reproduction is still occurring in nature or that it occurred until recently.

Finding a partner to mate is one of the sex costs to consider. To do so, fungi depend on pheromone-based systems. These communication systems have evolved differently depending on fungal lifestyles and genetic compatibility. As a consequence, molecules of different chemical nature are used for recognition and attraction of mating partners. Moreover, because finding the compatible partner can be somehow challenging for heterothallic fungi, some of them have evolved into pseudohomothallic species, thanks to sophisticated molecular mechanisms that are still not well understood. As this bypass makes them particularly efficient in terms of meiospore dissemination, it will be of great interest to get further knowledge on these mechanisms.

B. Multiple Fruiting Body Architecture but a Common Dikaryotic Phase

Sexual reproduction in fungi involves both isogamy and anisogamy. In the case of anisogamy, although male and female gametes exhibit a

variety of shapes depending on fungal species, the smaller gamete is considered to be the male gamete, whereas the larger gamete is considered to be the female one. In dikaryotic fungi, fertilization results in cells where the two parental nuclei remain unfused. The dikaryotic phase is a peculiar hallmark of fungal sexual development. But, although well characterized from cytological prospects, numerous questions about the dikaryotic cell ontogeny are still awaiting. How the two parental nuclei recognize each other is one of the most fundamental questions that should be addressed in the near future. Moreover, the dikaryotic stage is associated with specific defense mechanisms that scrutinize the fungal genome integrity and prevent repeated elements to spread.

Fungal fruiting bodies display a variety of shapes, which impact the dispersion of the offspring. To date, among the filamentous ascomycetes, four main types have been described, but further exploration of the fungal diversity might eventually allow the discovery of more fruiting bodies. Although the environmental stimuli inducing fruit body development might be quite diverse, the molecular pathways leading to their assembly are well conserved. Furthermore, many of the factors that govern the progression of sexual development in fungi are conserved. However, significant differences in their organization and regulation could account for the diversity of sexual strategies evolved in fungi. Further comparative and inventive research should lead to a comprehensive understanding on the key developmental processes that have shaped sexual reproduction over fungal evolution.

Acknowledgments We are grateful to Jesús Aguirre and Denise Zickler for providing pictures. L. Peraza is supported by DGAPA, UNAM (PAPIIT IA201815).

References

Aanen DK, Hoekstra RF (2007) Why sex is good: on fungi and beyond. In: Heitman J, Kronstad JW, Taylor JW, Casselton LA (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 527–534

Aguilar PS, Engel A, Walter P (2007) The plasma membrane proteins Prm1 and Fig1 ascertain fidelity of membrane fusion during yeast mating. Mol Biol Cell 18(2):547–556

Aguirre J, Lambeth JD (2010) Nox enzymes from fungus to fly to fish and what they tell us about Nox function in mammals. Free Radic Biol Med 49(9): 1342–1353

Ait Benkhali J, Coppin E, Brun S, Peraza-Reyes L, Martin T, Dixelius C, Lazar N, van Tilbeurgh H, Debuchy R (2013) A network of HMG-box transcription factors regulates sexual cycle in the fungus Podospora anserina. PLoS Genet 9(7), e1003642

Alby K, Schaefer D, Sherwood RK, Jones SK Jr, Bennett RJ (2010) Identification of a cell death pathway in *Candida albicans* during the response to pheromone. Eukaryot Cell 9(11):1690–1701

Alexander WG, Raju NB, Xiao H, Hammond TM, Perdue TD, Metzenberg RL, Pukkila PJ, Shiu PK (2008) DCL-1 colocalizes with other components of the MSUD machinery and is required for silencing. Fungal Genet Biol 45(5):719–727

Allers T, Lichten M (2001) Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106(1):47–57

Anderson JB, Kohn LM (2007) Dikaryons, diploids, and evolution. In: Heitman J, Kronstad JW, Taylor JW, Casselton L (eds) Sex in fungi: molecular determination and evolutionary implications. American Society of Microbiology, Washington, DC, pp 333–348

Aramayo R, Metzenberg RL (1996) Meiotic transvection in fungi. Cell 86(1):103–113

Arkowitz RA, Bassilana M (2015) Regulation of hyphal morphogenesis by Ras and Rho small GTPases. Fungal Biol Rev 29(1):7–19

Arnaise S, Debuchy R, Picard M (1997) What is a bona fide mating-type gene? Internuclear complementation of mat mutants in *Podospora anserina*. Mol Gen Genet 256(2):169–178

Austin DJ, Bu'Lock JD, Gooday GW (1969) Trisporic acids: sexual hormones from *Mucor mucedo* and *Blakeslea trispora*. Nature 223(5211):1178–1179

Badalyan SM, Polak E, Hermann R, Aebi M, Kues U (2004) Role of peg formation in clamp cell fusion of homobasidiomycete fungi. J Basic Microbiol 44(3):167–177

Barale S, McCusker D, Arkowitz RA (2004) The exchange factor Cdc24 is required for cell fusion during yeast mating. Eukaryot Cell 3(4):1049–1061

Barale S, McCusker D, Arkowitz RA (2006) Cdc42p GDP/GTP cycling is necessary for efficient cell fusion during yeast mating. Mol Biol Cell 17(6): 2824–2838

Barr MM, Tu H, Van Aelst L, Wigler M (1996) Identification of Ste4 as a potential regulator of Byr2 in the sexual response pathway of *Schizosaccharomyces pombe*. Mol Cell Biol 16(10):5597–5603

Bayram Ö, Bayram ÖS, Ahmed YL, J-i M, Valerius O, Rizzoli SO, Ficner R, Irniger S, Braus GH (2012) The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. PLoS Genet 8(7)

Beh CT, Brizzio V, Rose MD (1997) KAR5 encodes a novel pheromone-inducible protein required for homotypic nuclear fusion. J Cell Biol 139(5): 1063–1076

Beisson-Schecroun J (1962) Cellular incompatibility and nucleo-cytoplasmic interactions in the "barrage" phenomena in *Podospora anserina*. Ann Genet 4:4–50

Bendezu FO, Martin SG (2013) Cdc42 explores the cell periphery for mate selection in fission yeast. Curr Biol 23(1):42–47

Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P (1997) An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature 386(6623):414–417

Berteaux-Lecellier V, Picard M, Thompson-Coffe C, Zickler D, Panvier-Adoutte A, Simonet JM (1995) A nonmammalian homolog of the PAF1 gene (Zellweger syndrome) discovered as a gene involved in caryogamy in the fungus *Podospora anserina*. Cell 81(7):1043–1051

Berteaux-Lecellier V, Zickler D, Debuchy R, Panvier-Adoutte A, Thompson-Coffe C, Picard M (1998) A homologue of the yeast SHE4 gene is essential for the transition between the syncytial and cellular stages during sexual reproduction of the fungus *Podospora anserina*. EMBO J 17(5):1248–1258

Billiard S, Lopez-Villavicencio M, Hood ME, Giraud T (2012) Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J Evol Biol 25(6):1020–1038

Bistis G (1956) Sexuality in ascobolus-stercorarius.1. Morphology of the ascogonium—plasmogamy—evidence for a sexual hormonal mechanism. Am J Bot 43(6):389–394

Bistis GN (1981) Chemotrophic interactions between trichogynes and macroconidia of opposite mating-type in *Neurospora crassa*. Mycologia 73: 959–975

Bistis GN (1996) Trichogynes and fertilization in Uni- and bimating type colonies of *Neurospora tetrasperma*. Fungal Genet Biol 20(1):93–98

Bistis GN, Raper JP (1963) Heterothallism and sexuality in *Ascobolus stercorarius*. Am J Bot 50(9):880–891

Bolker M, Urban M, Kahmann R (1992) The a mating type locus of *U. maydis* specifies cell signaling components. Cell 68(3):441–450

Bonnet C, Espagne E, Zickler D, Boisnard S, Bourdais A, Berteaux-Lecellier V (2006) The peroxisomal import proteins PEX2, PEX5 and PEX7 are differently involved in *Podospora anserina* sexual cycle. Mol Microbiol 62(1):157–169

Borde V, Goldman AS, Lichten M (2000) Direct coupling between meiotic DNA replication and recombination initiation. Science (New York, NY) 290(5492):806–809

Börner GV, Kleckner N, Hunter N (2004) Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117(1):29–45

Brachet E, Sommermeyer V, Borde V (2012) Interplay between modifications of chromatin and meiotic recombination hotspots. Biol Cell 104(2):51–69

Brizzio V, Gammie AE, Rose MD (1998) Rvs161p interacts with Fus2p to promote cell fusion in *Saccharomyces cerevisiae*. J Cell Biol 141(3):567–584

Bruggeman J, Debets AJ, Wijngaarden PJ, deVisser JA, Hoekstra RF (2003) Sex slows down the accumulation of deleterious mutations in the homothallic fungus *Aspergillus nidulans*. Genetics 164(2): 479–485

Buller AHR (1931) Researches on fungi ... by A. H. Reginald Buller, vol 4. Longmans, Green and Co., London, New York

Butty AC, Pryciak PM, Huang LS, Herskowitz I, Peter M (1998) The role of Far1p in linking the heterotrimeric G protein to polarity establishment proteins during yeast mating. Science 282(5393):1511–1516

Cano-Domínguez N, Alvarez-Delfín K, Hansberg W, Aguirre J (2008) NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in *Neurospora crassa*. Eukaryot Cell 7(8):1352–1361

Carlile MJ (1996) The discovery of fungal sex hormones: I. Sirenin. Mycologist 10(1):3–5

Cavinder B, Trail F (2012) Role of Fig1, a component of the low-affinity calcium uptake system, in growth and sexual development of filamentous fungi. Eukaryot Cell 11(8):978–988

Cerda-Olmedo E (1975) The genetics of *Phycomyces blakesleeanus*. Genet Res 25(3):285–296

Chang F, Herskowitz I (1990) Identification of a gene necessary for cell cycle arrest by a negative growth factor of yeast: FAR1 is an inhibitor of a G1 cyclin, CLN2. Cell 63(5):999–1011

Chao JT, Wong AK, Tavassoli S, Young BP, Chruscicki A, Fang NN, Howe LJ, Mayor T, Foster LJ, Loewen CJ (2014) Polarization of the endoplasmic reticulum by ER-septin tethering. Cell 158(3):620–632

Chase TE, Ullrich RC (1983) Sexuality, distribution, and dispersal of *Heterobasidion annosum* in pine plantations of Vermont. Mycologia 75(5):825–831

Chaudhary S, Polaino S, Shakya VP, Idnurm A (2013) A new genetic linkage map of the zygomycete fungus *Phycomyces blakesleeanus*. PLoS One 8(3), e58931

Chen RE, Thorner J (2007) Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1773(8):1311–1340

Chen H, Kuo CC, Kang H, Howell AS, Zyla TR, Jin M, Lew DJ (2012) Cdc42p regulation of the yeast formin Bni1p mediated by the effector Gic2p. Mol Biol Cell 23(19):3814–3826

Choi KY, Satterberg B, Lyons DM, Elion EA (1994) Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in *S. cerevisiae*. Cell 78(3):499–512

Clemente-Ramos JA, Martin-Garcia R, Sharifmoghadam MR, Konomi M, Osumi M, Valdivieso MH (2009) The tetraspan protein Dni1p is required for correct membrane organization and cell wall remodelling during mating in *Schizosaccharomyces pombe*. Mol Microbiol 73(4):695–709

Clutterbuck AJ (2011) Genomic evidence of repeat-induced point mutation (RIP) in filamentous ascomycetes. Fungal Genet Biol 48(3):306–326

Coleman JJ, Rounsley SD, Rodriguez-Carres M, Kuo A, Wasmann CC, Grimwood J, Schmutz J, Taga M, White GJ, Zhou S, Schwartz DC, Freitag M, Ma L-J, Danchin EGJ, Henrissat B, Coutinho PM, Nelson DR, Straney D, Napoli CA, Barker BM, Gribskov M, Rep M, Kroken S, Molnár I, Rensing C, Kennell JC, Zamora J, Farman ML, Selker EU, Salamov A, Shapiro H, Pangilinan J, Lindquist E, Lamers C, Grigoriev IV, Geiser DM, Covert SF, Temporini E, Vanetten HD (2009) The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expansion. PLoS Genet 5(8)

Coluccio AE, Rodriguez RK, Kernan MJ, Neiman AM (2008) The yeast spore wall enables spores to survive passage through the digestive tract of Drosophila. PLoS One 3(8), e2873

Coppin E, Silar P (2007) Identification of PaPKS1, a polyketide synthase involved in melanin formation and its use as a genetic tool in *Podospora anserina*. Mycol Res 111(Pt 8):901–908

Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61(4): 411–428

Coppin E, de Renty C, Debuchy R (2005) The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Eukaryot Cell 4(2):407–420

Coppin E, Berteaux-Lecellier V, Bidard F, Brun S, Ruprich-Robert G, Espagne E, Aït-Benkhali J, Goarin A, Nesseir A, Planamente S, Debuchy R, Silar P (2012) Systematic deletion of homeobox genes in *Podospora anserina* uncovers their roles in shaping the fruiting body. PLoS One 7(5)

Cote P, Sulea T, Dignard D, Wu C, Whiteway M (2011) Evolutionary reshaping of fungal mating pathway scaffold proteins. mBio 2(1):e00230-00210.

Cuomo CA, Güldener U, Xu J-R, Trail F, Turgeon BG, Di Pietro A, Walton JD, Ma L-J, Baker SE, Rep M, Adam G, Antoniw J, Baldwin T, Calvo S, Chang Y-L, Decaprio D, Gale LR, Gnerre S, Goswami RS, Hammond-Kosack K, Harris LJ, Hilburn K, Kennell JC, Kroken S, Magnuson JK, Mannhaupt G, Mauceli E, Mewes H-W, Mitterbauer R, Muehlbauer G, Münsterkötter M, Nelson D, O'Donnell K, Ouellet T, Qi W, Quesneville H, Roncero MIG, Seong K-Y, Tetko IV, Urban M, Waalwijk C, Ward TJ, Yao J, Birren BW, Kistler HC (2007) The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science (New York, NY) 317(5843): 1400–1402

Curto MA, Sharifmoghadam MR, Calpena E, De Leon N, Hoya M, Doncel C, Leatherwood J, Valdivieso MH (2014) Membrane organization and cell fusion during mating in fission yeast requires multipass membrane protein Prm1. Genetics 196(4): 1059–1076

Czempinski K, Kruft V, Wostemeyer J, Burmester A (1996) 4-Dihydromethyltrisporate dehydrogenase from *Mucor mucedo*, an enzyme of the sexual hormone pathway: purification, and cloning of the corresponding gene. Microbiology 142(Pt 9): 2647–2654

Debuchy R (1999) Internuclear recognition: a possible connection between euascomycetes and homobasidiomycetes. Fungal Genet Biol 27(2-3):218–223

Debuchy R, Berteaux-Lecellier V, Silar P (2010) Mating systems and sexual morphogenesis in Ascomycetes. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Washington, DC, pp 501–535

Dignard D, Andre D, Whiteway M (2008) Heterotrimeric G-protein subunit function in *Candida albicans*: both the alpha and beta subunits of the pheromone response G protein are required for mating. Eukaryot Cell 7(9):1591–1599

Doll K, Chatterjee S, Scheu S, Karlovsky P, Rohlfs M (2013) Fungal metabolic plasticity and sexual development mediate induced resistance to arthropod fungivory. Proc Biol Sci 280(1771):20131219

Dorer R, Boone C, Kimbrough T, Kim J, Hartwell LH (1997) Genetic analysis of default mating behavior in *Saccharomyces cerevisiae*. Genetics 146(1):39–55

Drogen F, O'Rourke SM, Stucke VM, Jaquenoud M, Neiman AM, Peter M (2000) Phosphorylation of the MEKK Ste11p by the PAK-like kinase Ste20p is required for MAP kinase signaling in vivo. Curr Biol 10(11):630–639

Dudin O, Bendezu FO, Groux R, Laroche T, Seitz A, Martin SG (2015) A formin-nucleated actin aster concentrates cell wall hydrolases for cell fusion in fission yeast. J Cell Biol 208(7):897–911

Duntze W, MacKay V, Manney TR (1970) *Saccharomyces cerevisiae*: a diffusible sex factor. Science 168(3938):1472–1473

Duntze W, Stotzler D, Bucking-Throm E, Kalbitzer S (1973) Purification and partial characterization of -factor, a mating-type specific inhibitor of cell reproduction from *Saccharomyces cerevisiae*. Eur J Biochem 35(2):357–365

Dyer JM, Savage NS, Jin M, Zyla TR, Elston TC, Lew DJ (2013) Tracking shallow chemical gradients by actin-driven wandering of the polarization site. Curr Biol 23(1):32–41

Dyer PS, Inderbitzin P, Debuchy R (2016) Mating-type structure, function, regulation and evolution in the pezizomycotina. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 351–385

Elion EA, Trueheart J, Fink GR (1995) Fus2 localizes near the site of cell fusion and is required for both cell fusion and nuclear alignment during zygote formation. J Cell Biol 130(6):1283–1296

Engel A, Walter P (2008) Membrane lysis during biological membrane fusion: collateral damage by misregulated fusion machines. J Cell Biol 183(2): 181–186

Erdman S, Lin L, Malczynski M, Snyder M (1998) Pheromone-regulated genes required for yeast mating differentiation. J Cell Biol 140(3):461–483

Errede B, Ammerer G (1989) STE12, a protein involved in cell-type-specific transcription and signal transduction in yeast, is part of protein-DNA complexes. Genes Dev 3(9):1349–1361

Eslava AP, Alvarez MI, Burke PV, Delbruck M (1975a) Genetic recombination in sexual crosses of phycomyces. Genetics 80(3):445–462

Eslava AP, Alvarez MI, Delbruck M (1975b) Meiosis in Phycomyces. Proc Natl Acad Sci USA 72(10):4076–4080

Espagne E, Vasnier C, Storlazzi A, Kleckner NE, Silar P, Zickler D, Malagnac F (2011) Sme4 coiled-coil protein mediates synaptonemal complex assembly, recombinosome relocalization, and spindle pole body morphogenesis. Proc Natl Acad Sci USA 108(26):10614–10619

Esser K, Prillinger H (1972) A new technique for using spermatia in the production of mutants in Podospora. Mutat Res 16(4):417–419

Evangelista M, Blundell K, Longtine MS, Chow CJ, Adames N, Pringle JR, Peter M, Boone C (1997) Bni1p, a yeast formin linking cdc42p and the actin cytoskeleton during polarized morphogenesis. Science 276(5309):118–122

Evans HJ (1959) Nuclear behaviour in the cultivated mushroom. Chromosoma 10(1-6):115–135

Farkasovsky M, Kuntzel H (1995) Yeast Num1p associates with the mother cell cortex during S/G2 phase and affects microtubular functions. J Cell Biol 131 (4):1003–1014

Farkasovsky M, Kuntzel H (2001) Cortical Num1p interacts with the dynein intermediate chain Pac11p and cytoplasmic microtubules in budding yeast. J Cell Biol 152(2):251–262

Fischer R, Timberlake WE (1995) *Aspergillus nidulans* apsA (anucleate primary sterigmata) encodes a coiled-coil protein required for nuclear positioning and completion of asexual development. J Cell Biol 128(4):485–498

Fleissner A, Diamond S, Glass NL (2009) The *Saccharomyces cerevisiae* PRM1 homolog in Neurospora crassa is involved in vegetative and sexual cell fusion events but also has postfertilization functions. Genetics 181(2):497–510

Fowler TJ, Mitton MF, Rees EI, Raper CA (2004) Crossing the boundary between the Balpha and Bbeta mating-type loci in *Schizophyllum commune*. Fungal Genet Biol 41(1):89–101

Freihorst D, Fowler TJ, Bartholomew K, Marjatta R, Stephen Horton J, Kothe E (2016) The mating type genes of the basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 329–349

Freitag M, Williams RL, Kothe GO, Selker EU (2002) A cytosine methyltransferase homologue is essential for repeat-induced point mutation in Neurospora crassa. Proc Natl Acad Sci USA 99(13):8802–8807

Fu J, Mares C, Lizcano A, Liu Y, Wickes BL (2011) Insertional mutagenesis combined with an inducible filamentation phenotype reveals a conserved STE50 homologue in *Cryptococcus neoformans* that is required for monokaryotic fruiting and sexual reproduction. Mol Microbiol 79(4):990–1007

Fu C, Sun S, Billmyre RB, Roach KC, Heitman J (2015) Unisexual versus bisexual mating in *Cryptococcus neoformans*: consequences and biological impacts. Fungal Genet Biol 78:65–75

Fudal I, Ross S, Brun H, Besnard A-L, Ermel M, Kuhn M-L, Balesdent M-H, Rouxel T (2009) Repeat-induced point mutation (RIP) as an alternative mechanism of evolution toward virulence in *Leptosphaeria maculans*. Mol Plant Microbe Interact 22(8):932–941

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang S, Nielsen CB, Butler J, Endrizzi M, Qui D, Ianakiev P, Bell-Pedersen D, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stange-Thomann N, Barrett R, Gnerre S, Kamal M, Kamvysselis M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metzenberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li W, Pratt RJ, Osmani SA, DeSouza CP, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seiler S, Dunlap J, Radford A, Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebbole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422(6934):859–868

Gammie AE, Brizzio V, Rose MD (1998) Distinct morphological phenotypes of cell fusion mutants. Mol Biol Cell 9(6):1395–1410

Ganem S, Lu SW, Lee BN, Chou DY, Hadar R, Turgeon BG, Horwitz BA (2004) G-protein beta subunit of *Cochliobolus heterostrophus* involved in virulence, asexual and sexual reproductive ability, and morphogenesis. Eukaryot Cell 3(6):1653–1663

Gao S, Nuss DL (1996) Distinct roles for two G protein alpha subunits in fungal virulence, morphology, and reproduction revealed by targeted gene disruption. Proc Natl Acad Sci USA 93(24):14122–14127

Gibeaux R, Politi AZ, Nedelec F, Antony C, Knop M (2013) Spindle pole body-anchored Kar3 drives

the nucleus along microtubules from another nucleus in preparation for nuclear fusion during yeast karyogamy. Genes Dev 27(3):335–349

Giesbert S, Siegmund U, Schumacher J, Kokkelink L, Tudzynski P (2014) Functional analysis of BcBem1 and its interaction partners in *Botrytis cinerea*: impact on differentiation and virulence. PLoS One 9(5), e95172

Gladyshev E, Kleckner N (2014) Direct recognition of homology between double helices of DNA in *Neurospora crassa*. Nat Commun 5

Goddard A, Ladds G, Forfar R, Davey J (2006) Identification of Gnr1p, a negative regulator of G alpha signalling in *Schizosaccharomyces pombe*, and its complementation by human G beta subunits. Fungal Genet Biol 43(12):840–851

Gooday GW (1968) Hormonal control of sexual reproduction in *Mucor mucedo*. New Phytol 67:815–821

Gooday GW, Carlile MJ (1997) The discovery of fungal sex hormones: III. Trisporic acid and its precursors. Mycologist 11(3):126–130

Graïa F, Berteaux-Lecellier V, Zickler D, Picard M (2000) ami1, an orthologue of the Aspergillus nidulans apsA gene, is involved in nuclear migration events throughout the life cycle of Podospora anserina. Genetics 155(2):633–646

Graïa F, Lespinet O, Rimbault B, Dequard-Chablat M, Coppin E, Picard M (2001) Genome quality control: RIP (repeat-induced point mutation) comes to Podospora. Mol Microbiol 40(3):586–595

Grognet P, Lalucque H, Silar P (2012) The PaAlr1 magnesium transporter is required for ascospore development in *Podospora anserina*. Fungal Biol 116(10):1111–1118

Grote E (2010) Secretion is required for late events in the cell-fusion pathway of mating yeast. J Cell Sci 123(Pt 11):1902–1912

Hagen DC, McCaffrey G, Sprague GF Jr (1986) Evidence the yeast STE3 gene encodes a receptor for the peptide pheromone a factor: gene sequence and implications for the structure of the presumed receptor. Proc Natl Acad Sci USA 83(5):1418–1422

Hammond TM, Xiao H, Boone EC, Perdue TD, Pukkila PJ, Shiu PK (2011) SAD-3, a putative helicase required for meiotic silencing by unpaired DNA, interacts with other components of the silencing machinery. G3 1(5):369–376

Hammond TM, Xiao H, Boone EC, Decker LM, Lee SA, Perdue TD, Pukkila PJ, Shiu PK (2013) Novel proteins required for meiotic silencing by unpaired DNA and siRNA generation in Neurospora crassa. Genetics 194(1):91–100

Hartig A, Holly J, Saari G, MacKay VL (1986) Multiple regulation of STE2, a mating-type-specific gene of *Saccharomyces cerevisiae*. Mol Cell Biol 6(6): 2106–2114

Heiman MG, Walter P (2000) Prm1p, a pheromone-regulated multispanning membrane protein, facilitates plasma membrane fusion during yeast mating. J Cell Biol 151(3):719–730

Heiman MG, Engel A, Walter P (2007) The Golgi-resident protease Kex2 acts in conjunction with Prm1 to facilitate cell fusion during yeast mating. J Cell Biol 176(2):209–222

Hellerschmied D, Clausen T (2014) Myosin chaperones. Curr Opin Struct Biol 25:9–15

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai Y-C, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson K-H, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo J-M, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüssler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao Y-J, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111(Pt 5):509–547

Honda S, Selker EU (2008) Direct interaction between DNA methyltransferase DIM-2 and HP1 is required for DNA methylation in *Neurospora crassa*. Mol Cell Biol 28(19):6044–6055

Honda S, Lewis ZA, Huarte M, Cho LY, David LL, Shi Y, Selker EU (2010) The DMM complex prevents spreading of DNA methylation from transposons to nearby genes in *Neurospora crassa*. Genes Dev 24(5):443–454

Horwitz BA, Sharon A, Lu SW, Ritter V, Sandrock TM, Yoder OC, Turgeon BG (1999) A G protein alpha subunit from *Cochliobolus heterostrophus* involved in mating and appressorium formation. Fungal Genet Biol 26(1):19–32

Hwang E, Kusch J, Barral Y, Huffaker TC (2003) Spindle orientation in *Saccharomyces cerevisiae* depends on the transport of microtubule ends along polarized actin cables. J Cell Biol 161(3):483–488

Hynes MJ, Murray SL, Khew GS, Davis MA (2008) Genetic analysis of the role of peroxisomes in the utilization of acetate and fatty acids in *Aspergillus nidulans*. Genetics 178(3):1355–1369

Hynes MJ, Murray SL, Kahn FK (2010) Deletion of the RING-finger peroxin 2 gene in *Aspergillus nidulans* does not affect meiotic development. FEMS Microbiol Lett 306(1):67–71

Idnurm A, Howlett BJ (2003) Analysis of loss of pathogenicity mutants reveals that repeat-induced point mutations can occur in the Dothideomycete *Leptosphaeria maculans*. Fungal Genet Biol 39(1):31–37

Idnurm A, James TY, Vilgalys R (2007) Sex in the rest: mysterious mating in the Chytridiomycota and Zygomycota. In: Heitman J, Kronstad J, Taylor J, Casselton L (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 407–418

Ikeda K-i, Nakayashiki H, Kataoka T, Tamba H, Hashimoto Y, Tosa Y, Mayama S (2002) Repeat-induced point mutation (RIP) in *Magnaporthe grisea*: implications for its sexual cycle in the natural field context. Mol Microbiol 45(5):1355–1364

Ivey FD, Hodge PN, Turner GE, Borkovich KA (1996) The G alpha i homologue gna-1 controls multiple differentiation pathways in *Neurospora crassa*. Mol Biol Cell 7(8):1283–1297

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443(7113):818–822

James TY, Johansson SB, Johannesson H (2009) Trikaryon formation and nuclear selection in pairings between heterokaryons and homokaryons of the root rot pathogen *Heterobasidion parviporum*. Mycol Res 113(5):583–590

Jamet-Vierny C, Debuchy R, Prigent M, Silar P (2007) IDC1, a pezizomycotina-specific gene that belongs to the PaMpk1 MAP kinase transduction cascade of the filamentous fungus *Podospora anserina*. Fungal Genet Biol 44(12):1219–1230

Jenczmionka NJ, Maier FJ, Losch AP, Schafer W (2003) Mating, conidiation and pathogenicity of *Fusarium graminearum*, the main causal agent of the head-blight disease of wheat, are regulated by the MAP kinase gpmk1. Curr Genet 43(2):87–95

Jenness DD, Burkholder AC, Hartwell LH (1983) Binding of alpha-factor pheromone to yeast a cells: chemical and genetic evidence for an alpha-factor receptor. Cell 35(2 Pt 1):521–529

Jin H, Carlile C, Nolan S, Grote E (2004) Prm1 prevents contact-dependent lysis of yeast mating pairs. Eukaryot Cell 3(6):1664–1673

Johannesson H, Stenlid J (2004) Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. Fungal Genet Biol 41(5):563–570

Jones SK Jr, Bennett RJ (2011) Fungal mating pheromones: choreographing the dating game. Fungal Genet Biol 48(7):668–676

Jung KW, Kim SY, Okagaki LH, Nielsen K, Bahn YS (2011) Ste50 adaptor protein governs sexual differentiation of *Cryptococcus neoformans* via the pheromone-response MAPK signaling pathway. Fungal Genet Biol 48(2):154–165

Kamada T, Hirai K, Fujii M (1993) The role of the cytoskeleton in the pairing and positioning of the two nuclei in the apical cell of the dikaryon of the basidiomycete *Coprinus cinereus*. Exp Mycol 17(4):338–344

Kamerewerd J, Jansson M, Nowrousian M, Poggeler S, Kuck U (2008) Three alpha-subunits of heterotrimeric G proteins and an adenylyl cyclase have distinct roles in fruiting body development in the homothallic fungus *Sordaria macrospora*. Genetics 180(1):191–206

Kaneko I, Iyama-Kadono M, Togashi-Nishigata K, Yamaguchi I, Teraoka T, Arie T (2013) Heterotrimeric G protein β subunit GPB1 and MAP kinase MPK1 regulate hyphal growth and female fertility in *Fusarium sacchari*. Mycoscience 54(2):148–157

Kawasaki L, Sánchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45(4): 1153–1163

Keeney S (2001) Mechanism and control of meiotic recombination initiation. Curr Top Dev Biol 52: 1–53

Kicka S, Silar P (2004) PaASK1, a mitogen-activated protein kinase kinase kinase that controls cell degeneration and cell differentiation in *Podospora anserina*. Genetics 166(3):1241–1252

Kicka S, Bonnet C, Sobering AK, Ganesan LP, Silar P (2006) A mitotically inheritable unit containing a MAP kinase module. Proc Natl Acad Sci USA 103(36):13445–13450

Kim H, Borkovich KA (2004) A pheromone receptor gene, pre-1, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52(6):1781–1798

Kim H, Borkovich KA (2006) Pheromones are essential for male fertility and sufficient to direct chemotropic polarized growth of trichogynes during mating in *Neurospora crassa*. Eukaryot Cell 5(3): 544–554

Kim H, Metzenberg RL, Nelson MA (2002) Multiple functions of mfa-1, a putative pheromone precursor gene of *Neurospora crassa*. Eukaryot Cell 1(6):987–999

Kim HK, Lee T, Yun SH (2008) A putative pheromone signaling pathway is dispensable for self-fertility in the homothallic ascomycete *Gibberella zeae*. Fungal Genet Biol 45(8):1188–1196

Kim H-j, Chen C, Kabbage M, Dickman MB (2011) Identification and characterization of *Sclerotinia sclerotiorum* NADPH oxidases. Appl Environ Microbiol 77(21):7721–7729

Kim H, Wright SJ, Park G, Ouyang S, Krystofova S, Borkovich KA (2012) Roles for receptors, pheromones, G proteins, and mating type genes during sexual reproduction in *Neurospora crassa*. Genetics 190(4):1389–1404

Kjaerulff S, Lautrup-Larsen I, Truelsen S, Pedersen M, Nielsen O (2005) Constitutive activation of the fission yeast pheromone-responsive pathway induces ectopic meiosis and reveals ste11 as a mitogen-activated protein kinase target. Mol Cell Biol 25(5):2045–2059

Kormanec J, Schaaff-Gerstenschlager I, Zimmermann FK, Perecko D, Kuntzel H (1991) Nuclear migration in *Saccharomyces cerevisiae* is controlled by the highly repetitive 313 kDa NUM1 protein. Mol Gen Genet 230(1-2):277–287

Kothe E (1996) Tetrapolar fungal mating types: sexes by the thousands. FEMS Microbiol Rev 18(1):65–87

Kothe GO, Free SJ (1998) The isolation and characterization of nrc-1 and nrc-2, two genes encoding protein kinases that control growth and development in *Neurospora crassa*. Genetics 149(1):117–130

Krishnan A, Almen MS, Fredriksson R, Schioth HB (2012) The origin of GPCRs: identification of mammalian like rhodopsin, adhesion, glutamate and frizzled GPCRs in fungi. PLoS One 7(1), e29817

Krystofova S, Borkovich KA (2005) The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a Gbetagamma dimer required for normal female fertility, asexual development, and galpha protein levels in *Neurospora crassa*. Eukaryot Cell 4(2):365–378

Kues U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64(2):316–353

Kurihara LJ, Stewart BG, Gammie AE, Rose MD (1996) Kar4p, a karyogamy-specific component of the yeast pheromone response pathway. Mol Cell Biol 16(8):3990–4002

Lackner LL, Ping H, Graef M, Murley A, Nunnari J (2013) Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria. Proc Natl Acad Sci USA 110(6):E458–467

Lahav R, Gammie A, Tavazoie S, Rose MD (2007) Role of transcription factor Kar4 in regulating downstream events in the *Saccharomyces cerevisiae* pheromone response pathway. Mol Cell Biol 27(3):818–829

Lalucque H, Malagnac F, Brun S, Kicka S, Silar P (2012) A non-Mendelian MAPK-generated hereditary unit controlled by a second MAPK pathway in *Podospora anserina*. Genetics 191(2):419–433

Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4(3):181–189

Lamson RE, Takahashi S, Winters MJ, Pryciak PM (2006) Dual role for membrane localization in yeast MAP kinase cascade activation and its contribution to signaling fidelity. Curr Biol 16(6):618–623

Lara-Ortíz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol Microbiol 50(4):1241–1255

Lee SC, Heitman J (2012) Function of Cryptococcus neoformans KAR7 (SEC66) in karyogamy during unisexual and opposite-sex mating. Eukaryot Cell 11(6):783–794

Lee SC, Heitman J (2014) Sex in the Mucoralean fungi. Mycoses 57(Suppl 3):18–24

Lee DW, Pratt RJ, McLaughlin M, Aramayo R (2003) An argonaute-like protein is required for meiotic silencing. Genetics 164(2):821–828

Lee DW, Freitag M, Selker EU, Aramayo R (2008a) A cytosine methyltransferase homologue is essential for sexual development in *Aspergillus nidulans*. PLoS One 3(6)

Lee J, Leslie JF, Bowden RL (2008b) Expression and function of sex pheromones and receptors in the homothallic ascomycete *Gibberella zeae*. Eukaryot Cell 7(7):1211–1221

Lee H-C, Li L, Gu W, Xue Z, Crosthwaite SK, Pertsemlidis A, Lewis ZA, Freitag M, Selker EU, Mello CC, Liu Y (2010) Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. Mol Cell 38(6):803–814

Leeuw T, Fourest-Lieuvin A, Wu C, Chenevert J, Clark K, Whiteway M, Thomas DY, Leberer E (1995) Pheromone response in yeast: association of Bem1p with proteins of the MAP kinase cascade and actin. Science 270(5239):1210–1213

Leeuw T, Wu C, Schrag JD, Whiteway M, Thomas DY, Leberer E (1998) Interaction of a G-protein betasubunit with a conserved sequence in Ste20/PAK family protein kinases. Nature 391(6663):191–195

Lewis ZA, Adhvaryu KK, Honda S, Shiver AL, Knip M, Sack R, Selker EU (2010a) DNA methylation and normal chromosome behavior in Neurospora depend on five components of a histone methyltransferase complex, DCDC. PLoS Genet 6(11)

Lewis ZA, Adhvaryu KK, Honda S, Shiver AL, Selker EU (2010b) Identification of DIM-7, a protein required to target the DIM-5 H3 methyltransferase to chromatin. Proc Natl Acad Sci USA 107(18): 8310–8315

Li D, Bobrowicz P, Wilkinson HH, Ebbole DJ (2005) A mitogen-activated protein kinase pathway essential for mating and contributing to vegetative growth in Neurospora crassa. Genetics 170(3):1091–1104

Li L, Wright SJ, Krystofova S, Park G, Borkovich KA (2007) Heterotrimeric G protein signaling in filamentous fungi. Annu Rev Microbiol 61:423–452

Lichius A, Lord KM, Jeffree CE, Oborny R, Boonyarungsrit P, Read ND (2012) Importance of MAP kinases during protoperithecial morphogenesis in *Neurospora crassa*. PLoS One 7(8), e42565

Lin X, Heitman J (2006) The biology of the *Cryptococcus neoformans* species complex. Annu Rev Microbiol 60:69–105

Lin X, Hull CM, Heitman J (2005) Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature 434(7036): 1017–1021

Lipke PN, Wojciechowicz D, Kurjan J (1989) AG alpha 1 is the structural gene for the *Saccharomyces cerevisiae* alpha-agglutinin, a cell surface glycoprotein involved in cell-cell interactions during mating. Mol Cell Biol 9(8):3155–3165

Liu H, Bretscher A (1992) Characterization of TPM1 disrupted yeast cells indicates an involvement of tropomyosin in directed vesicular transport. J Cell Biol 118(2):285–299

Liu S, Dean RA (1997) G protein alpha subunit genes control growth, development, and pathogenicity of *Magnaporthe grisea*. Mol Plant Microbe Interact 10(9):1075–1086

Machlis L (1958) Evidence for a sexual hormone in allomyces. Physiol Plant 11(1):181–192

Machlis L (1968) The response of wild type male gametes of allomyces to sirenin. Plant Physiol 43 (8):1319–1320

Machlis L, Nutting WH, Williams MW, Rapoport H (1966) Production, isolation, and characterization of sirenin. Biochemistry (Mosc) 5(7):2147–2152

Maddox PS, Stemple JK, Satterwhite L, Salmon ED, Bloom K (2003) The minus end-directed motor Kar3 is required for coupling dynamic microtubule plus ends to the cortical shmoo tip in budding yeast. Curr Biol 13(16):1423–1428

Maerz S, Ziv C, Vogt N, Helmstaedt K, Cohen N, Gorovits R, Yarden O, Seiler S (2008) The nuclear Dbf2-related kinase COT1 and the mitogen-activated protein kinases MAK1 and MAK2 genetically interact to regulate filamentous growth, hyphal fusion and sexual development in Neurospora crassa. Genetics 179(3):1313–1325

Malagnac F, Wendel B, Goyon C, Faugeron G, Zickler D, Rossignol JL, Noyer-Weidner M, Vollmayr P, Trautner TA, Walter J (1997) A gene essential for de novo methylation and development in Ascobolus reveals a novel type of eukaryotic DNA methyltransferase structure. Cell 91(2):281–290

Malagnac F, Lalucque H, Lepere G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserina*. Fungal Genet Biol 41(11):982–997

Marcus S, Polverino A, Barr M, Wigler M (1994) Complexes between STE5 and components of the pheromone-responsive mitogen-activated protein kinase module. Proc Natl Acad Sci USA 91(16): 7762–7766

Martin SG, Arkowitz RA (2014) Cell polarization in budding and fission yeasts. FEMS Microbiol Rev 38(2):228–253

Mata J, Bahler J (2006) Global roles of Ste11p, cell type, and pheromone in the control of gene expression during early sexual differentiation in fission yeast. Proc Natl Acad Sci USA 103(42):15517–15522

Matheos D, Metodiev M, Muller E, Stone D, Rose MD (2004) Pheromone-induced polarization is dependent on the Fus3p MAPK acting through the formin Bni1p. J Cell Biol 165(1):99–109

May G (1988) Somatic incompatibility and individualism in the coprophilous Basidiomycete, *Coprinus cinereus*. Trans Br Mycol Soc 91(3):443–451

Mayorga ME, Gold SE (2001) The ubc2 gene of Ustilago maydis encodes a putative novel adaptor protein required for filamentous growth, pheromone response and virulence. Mol Microbiol 41(6): 1365–1379

Mayrhofer S, Weber JM, Poggeler S (2006) Pheromones and pheromone receptors are required for proper sexual development in the homothallic ascomycete *Sordaria macrospora*. Genetics 172(3):1521–1533

McCaffrey G, Clay FJ, Kelsay K, Sprague GF Jr (1987) Identification and regulation of a gene required for cell fusion during mating of the yeast *Saccharomyces cerevisiae*. Mol Cell Biol 7(8):2680–2690

Mehta BJ, Cerda-Olmedo E (2001) Intersexual partial diploids of phycomyces. Genetics 158(2):635–641

Melloy P, Shen S, White E, McIntosh JR, Rose MD (2007) Nuclear fusion during yeast mating occurs by a three-step pathway. J Cell Biol 179(4):659–670

Melloy P, Shen S, White E, Rose MD (2009) Distinct roles for key karyogamy proteins during yeast nuclear fusion. Mol Biol Cell 20(17):3773–3782

Meluh PB, Rose MD (1990) *KAR3*, a kinesin-related gene required for yeast nuclear fusion. Cell 60: 1029–1041

Merlini L, Dudin O, Martin SG (2013) Mate and fuse: how yeast cells do it. Open Biol 3(3):130008

Nakayashiki H, Nishimoto N, Ikeda K, Tosa Y, Mayama S (1999) Degenerate MAGGY elements in a subgroup of *Pyricularia grisea*: a possible example of successful capture of a genetic invader by a fungal genome. Mol Gen Genet 261(6):958–966

Navarro-Olmos R, Kawasaki L, Dominguez-Ramirez L, Ongay-Larios L, Perez-Molina R, Coria R (2010) The beta subunit of the heterotrimeric G protein triggers the *Kluyveromyces lactis* pheromone response pathway in the absence of the gamma subunit. Mol Biol Cell 21(3):489–498

Neiman AM (2011) Sporulation in the budding yeast *Saccharomyces cerevisiae*. Genetics 189(3): 737–765

Nern A, Arkowitz RA (1999) A Cdc24p-Far1p-Gbetagamma protein complex required for yeast orientation during mating. J Cell Biol 144(6):1187–1202

Ng DT, Walter P (1996) ER membrane protein complex required for nuclear fusion. J Cell Biol 132(4):499–509

Ni M, Feretzaki M, Sun S, Wang X, Heitman J (2011) Sex in fungi. Annu Rev Genet 45:405–430

Niederpruem DJ, Jersild RA, Lane PL (1971) Direct microscopic studies of clamp connection formation in growing hyphae of *Schizophyllum commune*—I. The dikaryon. Arch Mikrobiol 78(3):268–280

Nieuwenhuis BP, Debets AJ, Aanen DK (2013a) Fungal fidelity: nuclear divorce from a dikaryon by mating or monokaryon regeneration. Fungal Biol 117(4):261–267

Nieuwenhuis BP, Nieuwhof S, Aanen DK (2013b) On the asymmetry of mating in natural populations of the mushroom fungus *Schizophyllum commune*. Fungal Genet Biol 56:25–32

Nishikawa S, Endo T (1997) The yeast JEM1p is a DnaJ-like protein of the endoplasmic reticulum membrane required for nuclear fusion. J Biol Chem 272(20):12889–12892

Nishimura M, Park G, Xu JR (2003) The G-beta subunit MGB1 is involved in regulating multiple steps of infection-related morphogenesis in *Magnaporthe grisea*. Mol Microbiol 50(1):231–243

Nomoto S, Nakayama N, Arai K, Matsumoto K (1990) Regulation of the yeast pheromone response pathway by G protein subunits. EMBO J 9(3):691–696

Nutting WH, Rapoport H, Machlis L (1968) The structure of sirenin. J Am Chem Soc 90(23):6434–6438

Obara T, Nakafuku M, Yamamoto M, Kaziro Y (1991) Isolation and characterization of a gene encoding a G-protein alpha subunit from *Schizosaccharomyces pombe*: involvement in mating and sporulation pathways. Proc Natl Acad Sci USA 88(13): 5877–5881

Onken B, Wiener H, Philips MR, Chang EC (2006) Compartmentalized signaling of Ras in fission yeast. Proc Natl Acad Sci USA 103(24):9045–9050

O'Shea SF, Chaure PT, Halsall JR, Olesnicky NS, Leibbrandt A, Connerton IF, Casselton LA (1998) A large pheromone and receptor gene complex determines multiple B mating type specificities in *Coprinus cinereus*. Genetics 148(3):1081–1090

Palma-Guerrero J, Leeder AC, Welch J, Glass NL (2014) Identification and characterization of LFD1, a novel protein involved in membrane merger during cell fusion in *Neurospora crassa*. Mol Microbiol 92(1):164–182

Pandey A, Roca MG, Read ND, Glass NL (2004) Role of a mitogen-activated protein kinase pathway during conidial germination and hyphal fusion in *Neurospora crassa*. Eukaryot Cell 3(2):348–358

Paoletti M, Seymour FA, Alcocer MJC, Kaur N, Calvo AM, Archer DB, Dyer PS (2007) Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. Curr Biol 17(16): 1384–1389

Papazian HP (1950) Physiology of the incompatibility factors in *Schizophyllum commune*. Bot Gaz 112(2):143–163

Park G, Colot HV, Collopy PD, Krystofova S, Crew C, Ringelberg C, Litvinkova L, Altamirano L, Li L, Curilla S, Wang W, Gorrochotegui-Escalante N, Dunlap JC, Borkovich KA (2011) High-throughput production of gene replacement mutants in *Neurospora crassa*. Methods Mol Biol 722:179–189

Paterson JM, Ydenberg CA, Rose MD (2008) Dynamic localization of yeast Fus2p to an expanding ring at the cell fusion junction during mating. J Cell Biol 181(4):697–709

Pelkmans JF, Lugones LG, Wösten HAB (2016) Fruiting body formation in basidiomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. Springer, Cham, pp 387–405

Peraza-Reyes L, Berteaux-Lecellier V (2013) Peroxisomes and sexual development in fungi. Front Physiol 4:244

Peraza-Reyes L, Zickler D, Berteaux-Lecellier V (2008) The peroxisome RING-Finger complex is required for meiocyte formation in the fungus *Podospora anserina*. Traffic 9(11):1998–2009

Peraza-Reyes L, Arnaise S, Zickler D, Coppin E, Debuchy R, Berteaux-Lecellier V (2011) The importomer peroxins are differentially required for peroxisome assembly and meiotic development in *Podospora anserina*: insights into a new peroxisome import pathway. Mol Microbiol 82(2):365–377

Pereira G, Grueneberg U, Knop M, Schiebel E (1999) Interaction of the yeast gamma-tubulin complex-binding protein Spc72p with Kar1p is essential for microtubule function during karyogamy. EMBO J 18(15):4180–4195

Peter M, Gartner A, Horecka J, Ammerer G, Herskowitz I (1993) FAR1 links the signal transduction pathway to the cell cycle machinery in yeast. Cell 73(4):747–760

Petersen J, Weilguny D, Egel R, Nielsen O (1995) Characterization of fus1 of *Schizosaccharomyces pombe*: a developmentally controlled function needed for conjugation. Mol Cell Biol 15(7): 3697–3707

Petersen J, Nielsen O, Egel R, Hagan IM (1998) FH3, a domain found in formins, targets the fission yeast formin Fus1 to the projection tip during conjugation. J Cell Biol 141(5):1217–1228

Pommerville J, Fuller MS (1976) The cytology of the gametes and fertilization of *Allomyces macrogynus*. Arch Microbiol 109(1-2):21–30

Pommerville J, Olson LW (1987) Evidence for a male-produced pheromone in *Allomyces macrogynus*. Exp Mycol 11(3):245–248

Pontecorvo G, Roper JA, Hemmons LM, Macdonald KD, Bufton AW (1953) The genetics of *Aspergillus nidulans*. Adv Genet 5:141–238

Pryciak PM, Huntress FA (1998) Membrane recruitment of the kinase cascade scaffold protein Ste5 by the Gbetagamma complex underlies activation of the yeast pheromone response pathway. Genes Dev 12(17):2684–2697

Qin J, Kang W, Leung B, McLeod M (2003) Ste11p, a high-mobility-group box DNA-binding protein, undergoes pheromone- and nutrient-regulated nuclear-cytoplasmic shuttling. Mol Cell Biol 23(9):3253–3264

Raju NB (1980) Meiosis and ascospore genesis in Neurospora. Eur J Cell Biol 23(1):208–223

Raju NB (1987) A *Neurospora* mutant with abnormal croziers, giant ascospores, and asci having multiple apical pores. Mycologia 79(5):696–706

Raju NB (1992) Genetic control of the sexual cycle in *Neurospora*. Mycol Res 96:241–262

Raju NB, Newmeyer D (1977) Giant ascospores and abnormal croziers in a mutant of *Neurospora crassa*. Exp Mycol 1:152–165

Rasmussen CG, Glass NL (2005) A Rho-type GTPase, rho-4, is required for septation in *Neurospora crassa*. Eukaryot Cell 4(11):1913–1925

Raudaskoski M (1998) The relationship between B-mating-type genes and nuclear migration in *Schizophyllum commune*. Fungal Genet Biol 24(1-2):207–227

Raudaskoski M, Kothe E (2010) Basidiomycete mating type genes and pheromone signaling. Eukaryot Cell 9(6):847–859

Read ND, Beckett A (1996) Ascus and ascospore morphogenesis. Mycol Res 100(11):1281–1314

Ren B, Robert F, Wyrick JJ, Aparicio O, Jennings EG, Simon I, Zeitlinger J, Schreiber J, Hannett N, Kanin E, Volkert TL, Wilson CJ, Bell SP, Young RA (2000) Genome-wide location and function of DNA binding proteins. Science 290(5500): 2306–2309

Rispail N, Soanes DM, Ant C, Czajkowski R, Grunler A, Huguet R, Perez-Nadales E, Poli A, Sartorel E, Valiante V, Yang M, Beffa R, Brakhage AA, Gow NA, Kahmann R, Lebrun MH, Lenasi H, Perez-Martin J, Talbot NJ, Wendland J, Di Pietro A (2009) Comparative genomics of MAP kinase and calcium-calcineurin signalling components in plant and human pathogenic fungi. Fungal Genet Biol 46(4):287–298

Rogers JV, Arlow T, Inkellis ER, Koo TS, Rose MD (2013) ER-associated SNAREs and Sey1p mediate nuclear fusion at two distinct steps during yeast mating. Mol Biol Cell 24(24):3896–3908

Rose MD (1996) Nuclear fusion in the yeast *Saccharomyces cerevisiae*. Annu Rev Cell Dev Biol 12: 663–695

Rose MD, Price BR, Fink GR (1986) *Saccharomyces cerevisiae* nuclear fusion requires prior activation by alpha factor. Mol Cell Biol 6(10):3490–3497

Rose MD, Misra LM, Vogel JP (1989) KAR2, a karyogamy gene, is the yeast homolog of the mammalian BiP/GRP78 gene. Cell 57(7):1211–1221

Rosen S, Yu JH, Adams TH (1999) The Aspergillus nidulans sfaD gene encodes a G protein beta subunit that is required for normal growth and repression of sporulation. EMBO J 18(20): 5592–5600

Rountree MR, Selker EU (1997) DNA methylation inhibits elongation but not initiation of transcription in *Neurospora crassa*. Genes Dev 11(18): 2383–2395

Roy A, Lu CF, Marykwas DL, Lipke PN, Kurjan J (1991) The AGA1 product is involved in cell surface attachment of the *Saccharomyces cerevisiae* cell adhesion glycoprotein a-agglutinin. Mol Cell Biol 11(8):4196–4206

Sahadevan Y, Richter-Fecken M, Kaerger K, Voigt K, Boland W (2013) Early and late trisporoids differentially regulate beta-carotene production and gene transcript Levels in the mucoralean fungi *Blakeslea trispora* and *Mucor mucedo*. Appl Environ Microbiol 79(23):7466–7475

Samarajeewa DA, Sauls PA, Sharp KJ, Smith ZJ, Xiao H, Groskreutz KM, Malone TL, Boone EC, Edwards KA, Shiu PK, Larson ED, Hammond TM (2014) Efficient detection of unpaired DNA requires a member of the rad54-like family of homologous recombination proteins. Genetics 198(3):895–904

Sanchez-Paredes E, Kawasaki L, Ongay-Larios L, Coria R (2011) The Galpha subunit signals through the Ste50 protein during the mating pheromone response in the yeast *Kluyveromyces lactis*. Eukaryot Cell 10(4):540–546

Scazzocchio C (2006) Aspergillus genomes: secret sex and the secrets of sex. Trends Genet 22(10): 521–525

Scherer M, Heimel K, Starke V, Kamper J (2006) The Clp1 protein is required for clamp formation and pathogenic development of *Ustilago maydis*. Plant Cell 18(9):2388–2401

Schimek C, Kleppe K, Saleem AR, Voigt K, Burmester A, Wostemeyer J (2003) Sexual reactions in Mortierellales are mediated by the trisporic acid system. Mycol Res 107(Pt 6):736–747

Schimek C, Petzold A, Schultze K, Wetzel J, Wolschendorf F, Burmester A, Wostemeyer J (2005) 4-Dihydromethyltrisporate dehydrogenase, an enzyme of the sex hormone pathway in *Mucor mucedo*, is constitutively transcribed but its activity is differently regulated in (+) and (-) mating types. Fungal Genet Biol 42(9):804–812

Schmoll M, Seibel C, Tisch D, Dorrer M, Kubicek CP (2010) A novel class of peptide pheromone precursors in ascomycetous fungi. Mol Microbiol 77(6):1483–1501

Schoustra SE, Debets AJ, Slakhorst M, Hoekstra RF (2007) Mitotic recombination accelerates adaptation in the fungus *Aspergillus nidulans*. PLoS Genet 3(4), e68

Schurg T, Brandt U, Adis C, Fleissner A (2012) The *Saccharomyces cerevisiae* BEM1 homologue in *Neurospora crassa* promotes co-ordinated cell behaviour resulting in cell fusion. Mol Microbiol 86(2):349–366

Scott B, Eaton CJ (2008) Role of reactive oxygen species in fungal cellular differentiations. Curr Opin Microbiol 11(6):488–493

Segmüller N, Kokkelink L, Giesbert S, Odinius D, van Kan J, Tudzynski P (2008) NADPH oxidases are involved in differentiation and pathogenicity in

Botrytis cinerea. Mol Plant Microbe Interact 21(6): 808–819

Seibel C, Tisch D, Kubicek CP, Schmoll M (2012) The role of pheromone receptors for communication and mating in *Hypocrea jecorina* (*Trichoderma reesei*). Fungal Genet Biol 49(10):814–824

Selker EU, Jensen BC, Richardson GA (1987) A portable signal causing faithful DNA methylation de novo in *Neurospora crassa*. Science (New York, NY) 238(4823):48–53

Seo JA, Han KH, Yu JH (2004) The gprA and gprB genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol Microbiol 53(6):1611–1623

Seo JA, Han KH, Yu JH (2005) Multiple roles of a heterotrimeric G-protein gamma-subunit in governing growth and development of *Aspergillus nidulans*. Genetics 171(1):81–89

Shear CL, Dodge BO (1927) Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. J Agric Res 34:1019–1042

Sheltzer JM, Rose MD (2009) The class V myosin Myo2p is required for Fus2p transport and actin polarization during the yeast mating response. Mol Biol Cell 20(12):2909–2919

Shen S, Tobery CE, Rose MD (2009) Prm3p is a pheromone-induced peripheral nuclear envelope protein required for yeast nuclear fusion. Mol Biol Cell 20(9):2438–2450

Sherwood RK, Bennett RJ (2009) Fungal meiosis and parasexual reproduction–lessons from pathogenic yeast. Curr Opin Microbiol 12(6):599–607

Shimada Y, Gulli MP, Peter M (2000) Nuclear sequestration of the exchange factor Cdc24 by Far1 regulates cell polarity during yeast mating. Nat Cell Biol 2(2):117–124

Shiu PK, Metzenberg RL (2002) Meiotic silencing by unpaired DNA: properties, regulation and suppression. Genetics 161(4):1483–1495

Shiu PK, Raju NB, Zickler D, Metzenberg RL (2001) Meiotic silencing by unpaired DNA. Cell 107(7): 905–916

Shiu PK, Zickler D, Raju NB, Ruprich-Robert G, Metzenberg RL (2006) SAD-2 is required for meiotic silencing by unpaired DNA and perinuclear localization of SAD-1 RNA-directed RNA polymerase. Proc Natl Acad Sci USA 103(7):2243–2248

Sia RA, Lengeler KB, Heitman J (2000) Diploid strains of the pathogenic basidiomycete *Cryptococcus neoformans* are thermally dimorphic. Fungal Genet Biol 29(3):153–163

Silar P (2011) Grafting as a method for studying development in the filamentous fungus *Podospora anserina*. Fungal Biol 115(8):793–802

Simonet JM, Zickler D (1972) Mutations affecting meiosis in *Podospora anserina*. I. Cytological studies. Chromosoma 37(3):327–351

Simonet JM, Zickler D (1978) Genes involved in caryogamy and meiosis in *Podospora anserina*. Mol Gen Genet 162:237–242

Sohn KT, Yoon KS (2002) Ultrastructural study on the cleistothecium development in *Aspergillus nidulans*. Mycobiology 30(3):117–127

Sprague GF Jr, Jensen R, Herskowitz I (1983) Control of yeast cell type by the mating type locus: positive regulation of the alpha-specific STE3 gene by the MAT alpha 1 product. Cell 32(2):409–415

Sproul LR, Anderson DJ, Mackey AT, Saunders WS, Gilbert SP (2005) Cik1 targets the minus-end kinesin depolymerase kar3 to microtubule plus ends. Curr Biol 15(15):1420–1427

Storlazzi A, Tesse S, Ruprich-Robert G, Gargano S, Poggeler S, Kleckner N, Zickler D (2008) Coupling meiotic chromosome axis integrity to recombination. Genes Dev 22(6):796–809

Studt L, Humpf HU, Tudzynski B (2013) Signaling governed by G proteins and cAMP is crucial for growth, secondary metabolism and sexual development in *Fusarium fujikuroi*. PLoS One 8(2), e58185

Sugimoto A, Iino Y, Maeda T, Watanabe Y, Yamamoto M (1991) *Schizosaccharomyces pombe* ste11+ encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. Genes Dev 5(11):1990–1999

Tamaru H, Selker EU (2001) A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. Nature 414(6861):277–283

Thompson-Coffe C, Zickler D (1994) How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. Dev Biol 165(1):257–271

Trueheart J, Fink GR (1989) The yeast cell fusion protein FUS1 is O-glycosylated and spans the plasma membrane. Proc Natl Acad Sci USA 86(24):9916–9920

Trueheart J, Boeke JD, Fink GR (1987) Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. Mol Cell Biol 7(7):2316–2328

Tudzynski P, Heller J, Siegmund U (2012) Reactive oxygen species generation in fungal development and pathogenesis. Curr Opin Microbiol 15(6): 653–659

Turina M, Prodi A, Alfen NK (2003) Role of the Mf1-1 pheromone precursor gene of the filamentous ascomycete *Cryphonectria parasitica*. Fungal Genet Biol 40(3):242–251

Vaillancourt LJ, Raudaskoski M, Specht CA, Raper CA (1997) Multiple genes encoding pheromones and a pheromone receptor define the B beta 1 mating-type specificity in *Schizophyllum commune*. Genetics 146(2):541–551

Van Den Ende H (1967) Sexual factor of the mucorales [62]. Nature 215(5097):211–212

Van den Ende H, Wiechmann AH, Reyngoud DJ, Hendriks T (1970) Hormonal interactions in *Mucor*

mucedo and *Blakeslea trispora*. J Bacteriol 101(2): 423–428

van Drogen F, Stucke VM, Jorritsma G, Peter M (2001) MAP kinase dynamics in response to pheromones in budding yeast. Nat Cell Biol 3(12):1051–1059

Vasnier C, de Muyt A, Zhang L, Tesse S, Kleckner NE, Zickler D, Espagne E (2014) Absence of SUN-domain protein Slp1 blocks karyogamy and switches meiotic recombination and synapsis from homologs to sister chromatids. Proc Natl Acad Sci USA 111(38):E4015–4023

Vilgalys R, Sun Bao L (1994) Assessment of species distributions in Pleurotus based on trapping of airborne basidiospores. Mycologia 86(2):270–274

Vollmeister E, Schipper K, Baumann S, Haag C, Pohlmann T, Stock J, Feldbrugge M (2012) Fungal development of the plant pathogen *Ustilago maydis*. FEMS Microbiol Rev 36(1):59–77

Watters MK, Randall TA, Margolin BS, Selker EU, Stadler DR (1999) Action of repeat-induced point mutation on both strands of a duplex and on tandem duplications of various sizes in Neurospora. Genetics 153(2):705–714

Wei H, Requena N, Fischer R (2003) The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol Microbiol 47(6):1577–1588

Wendland J, Vaillancourt LJ, Hegner J, Lengeler KB, Laddison KJ, Specht CA, Raper CA, Kothe E (1995) The mating-type locus B alpha 1 of *Schizophyllum commune* contains a pheromone receptor gene and putative pheromone genes. EMBO J 14(21): 5271–5278

Wetzel J, Scheibner O, Burmester A, Schimek C, Wostemeyer J (2009) 4-dihydrotrisporin-dehydrogenase, an enzyme of the sex hormone pathway of *Mucor mucedo*: purification, cloning of the corresponding gene, and developmental expression. Eukaryot Cell 8(1):88–95

Wetzel J, Burmester A, Kolbe M, Wostemeyer J (2012) The mating-related loci sexM and sexP of the zygomycetous fungus *Mucor mucedo* and their transcriptional regulation by trisporoid pheromones. Microbiology 158(Pt 4):1016–1023

Whiteway M, Hougan L, Dignard D, Thomas DY, Bell L, Saari GC, Grant FJ, O'Hara P, MacKay VL (1989) The STE4 and STE18 genes of yeast encode potential beta and gamma subunits of the mating factor receptor-coupled G protein. Cell 56(3):467–477

Whiteway MS, Wu C, Leeuw T, Clark K, Fourest-Lieuvin A, Thomas DY, Leberer E (1995) Association of the yeast pheromone response G protein beta gamma subunits with the MAP kinase scaffold Ste5p. Science 269(5230):1572–1575

Williams END, Todd NK, Rayner ADM (1984) Characterization of the spore rain of *Coriolus versicolor* and its ecological significance. Trans Br Mycol Soc 82(2):323–326

Wilson IM (1952) The ascogenous hyphae of *Pyronema confluens*. Ann Bot 16(3):321–339

Won S, Michkov AV, Krystofova S, Garud AV, Borkovich KA (2012) Genetic and physical interactions between Galpha subunits and components of the Gbetagamma dimer of heterotrimeric G proteins in *Neurospora crassa*. Eukaryot Cell 11(10): 1239–1248

Wostemeyer J, Schimek C (2007) Trisporic acid and mating in zygomycetes. In: Heitman J, Kronstad J, Taylor J, Casselton L (eds) Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC, pp 431–443

Xiao H, Alexander WG, Hammond TM, Boone EC, Perdue TD, Pukkila PJ, Shiu PK (2010) QIP, a protein that converts duplex siRNA into single strands, is required for meiotic silencing by unpaired DNA. Genetics 186(1):119–126

Xu G, Jansen G, Thomas DY, Hollenberg CP, Ramezani Rad M (1996) Ste50p sustains mating pheromone-induced signal transduction in the yeast *Saccharomyces cerevisiae*. Mol Microbiol 20(4):773–783

Yamamoto K, Tatebayashi K, Tanaka K, Saito H (2010) Dynamic control of yeast MAP kinase network by induced association and dissociation between the Ste50 scaffold and the Opy2 membrane anchor. Mol Cell 40(1):87–98

Yang Q, Borkovich KA (1999) Mutational activation of a Galphai causes uncontrolled proliferation of aerial hyphae and increased sensitivity to heat and oxidative stress in *Neurospora crassa*. Genetics 151(1):107–117

Yang Q, Poole SI, Borkovich KA (2002) A G-protein beta subunit required for sexual and vegetative development and maintenance of normal G alpha protein levels in Neurospora crassa. Eukaryot Cell 1(3):378–390

Yang M, Brand A, Srikantha T, Daniels KJ, Soll DR, Gow NA (2011) Fig1 facilitates calcium influx and localizes to membranes destined to undergo fusion during mating in *Candida albicans*. Eukaryot Cell 10(3):435–444

Ydenberg CA, Stein RA, Rose MD (2012) Cdc42p and Fus2p act together late in yeast cell fusion. Mol Biol Cell 23(7):1208–1218

Youn JY, Friesen H, Kishimoto T, Henne WM, Kurat CF, Ye W, Ceccarelli DF, Sicheri F, Kohlwein SD, McMahon HT, Andrews BJ (2010) Dissecting BAR domain function in the yeast Amphiphysins Rvs161 and Rvs167 during endocytosis. Mol Biol Cell 21(17):3054–3069

Yu HY, Seo JA, Kim JE, Han KH, Shim WB, Yun SH, Lee YW (2008) Functional analyses of heterotrimeric G protein G alpha and G beta subunits in *Gibberella zeae*. Microbiology 154(Pt 2):392–401

Zaichick SV, Metodiev MV, Nelson SA, Durbrovskyi O, Draper E, Cooper JA, Stone DE (2009) The mating-specific Galpha interacts with a kinesin-14 and regulates pheromone-induced nuclear migration in budding yeast. Mol Biol Cell 20(12):2820–2830

Zalatan JG, Coyle SM, Rajan S, Sidhu SS, Lim WA (2012) Conformational control of the Ste5 scaffold protein insulates against MAP kinase misactivation. Science 337(6099):1218–1222

Zhang C, Wang Y, Wang J, Zhai Z, Zhang L, Zheng W, Zheng W, Yu W, Zhou J, Lu G, Shim WB, Wang Z (2013) Functional characterization of Rho family small GTPases in *Fusarium graminearum*. Fungal Genet Biol 61:90–99

Zhang S, Zheng H, Long N, Carbo N, Chen P, Aguilar PS, Lu L (2014) FigA, a putative homolog oflow-affinity calcium system member Fig1 in *Saccharomyces cerevisiae*, is involved in growth and asexual and sexual development in Aspergillus nidulans. Eukaryot Cell 13(2):295–303

Zhao X, Mehrabi R, Xu J-R (2007) Mitogen-activated protein kinase pathways and fungal pathogenesis. Eukaryot Cell 6(10):1701–1714

Zickler D (2006) Meiosis in mycelial fungi. In: Kues U, Fisher R (eds) The mycota I. Growth, differentiation and sexuality. Springer, Berlin, Heidelberg, pp 415–438

Zickler D, Arnaise S, Coppin E, Debuchy R, Picard M (1995) Altered mating-type identity in the fungus *Podospora anserina* leads to selfish nuclei, uniparental progeny, and haploid meiosis. Genetics 140(2):493–503

17 Sexual Development in *Trichoderma*

Monika Schmoll[1], Ting-Fang Wang[2]

CONTENTS

I. Introduction

Fungi of the genus *Trichoderma* are known for their diverse applications producers of enzymes, as secondary metabolites, and as potent biocontrol organisms (Schuster and Schmoll 2010). Therefore, tools for strain improvement are among the major subjects of research with *Trichoderma* in academia and industry (Kubicek et al. 2009; Mukherjee et al. 2013; Schmoll et al. 2014).

[1]Department Health and Environment, Bioresources, Austrian Institute of Technology GmbH, Konrad-Lorenz Strasse 24, 3430 Tulln, Austria; e-mail: monika.schmoll@ait.ac.at
[2]Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan; e-mail: tfwang@gate.sinica.edu.tw

The genus *Trichoderma* was mentioned already about 200 years ago in literature (Persoon 1794), and also a potential anamorph–teleomorph relationship was suspected very early (Tulasne and Tulasne 1865). However, it took more than 100 years until this relationship could be confirmed for *Trichoderma reesei* by molecular (Kuhls et al. 1996) and biological (Seidl et al. 2009) data. *T. reesei* is one of the most productive organisms used in biotechnology for enzyme production. Due to its efficient production of cellulolytic enzymes, *T. reesei* became a model organism for plant cell wall degradation (Glass et al. 2013; Schmoll et al. 2014).

The strain used in research and industry nowadays along with a sizable number of derivatives, QM6a, was isolated from the Solomon Islands during WWII (Reese 1976). Prior to the availability of molecular methods, reliable species identification was problematic with *Trichoderma* (Samuels 2006) and may be one reason for the considerable delay in the achievement of sexual development under laboratory conditions: Initially, the strain isolated at the Solomon Islands was named *T. viride* (Reese et al. 1950) but later on recognized as a new species, *T. reesei* (Simmons 1977). Shortly thereafter it was synonymized with *T. longibrachiatum* (Bissett 1984), and the name *T. reesei* was no longer used for some time until molecular data confirmed species identity and an anamorph–teleomorph relationship with *Hypocrea jecorina* was proposed (Kuhls et al. 1996). At this time, sexual crossing has already been attempted, but without determination of mating types and lack of knowledge on suitable conditions, these efforts failed and *T. reesei* QM6a was considered asexual. Subsequently,

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

the name of the teleomorph, *H. jecorina*, was used to describe the whole species. However, after a recent decision of the International Code of Nomenclature for algae, fungi, and plants (ICN) and a community vote, *T. reesei* became the only name to be used for this species [reviewed in Atanasova et al. (2013a)]. When identifying novel nature isolates at the molecular level using published sequences and selection of strains to be crossed, these issues with nomenclature need careful consideration. In recent years, the relationship between *T. reesei* and *H. jecorina* was reevaluated in detail, and the species was found to in fact comprise additional species, including the novel *T. parareesei*, which contains strains isolated from soil and is reported to be a clonal asexual species (Druzhinina et al. 2010).

Actually, it is difficult to safely confirm that a species is truly asexual (Taylor et al. 1999). Indications of recombination in the population genetic structure have been observed frequently, even though for many of those fungi, a sexual cycle is not known. Additionally, it has to be kept in mind that optimal conditions for sexual development vary considerably from one species to another (Moore-Landecker 1992; Pöggeler et al. 2006). Hence, if mating has never been achieved in culture, the issue remains that the reasons for that might be suboptimal conditions, incorrectly or not precisely determined species, or wrong mating types. Asexual, clonal populations of species are known to succeed in competition for some time but ultimately are assumed to reach an evolutionary end point. Therefore, failed attempts for crossing of even high numbers of potential mating partners are not sufficient proofs for asexuality (Taylor et al. 1999).

Trichoderma spp. are frequently isolated from soil (Klein and Eveleigh 1998), but they are also often found growing on decaying plant material and bark, where isolation from fruiting bodies is not uncommon. Consequently, a sexual cycle is obvious for diverse species of the genus *Trichoderma* in nature (Jaklitsch 2009, 2011) but has only achieved under artificial conditions for *T. reesei* so far. With the now-precise molecular species delimitation (Atanasova et al. 2013a) along with knowledge on mating types and culture conditions, new isolates can additionally be checked for species assignment by sexual crossing, as is commonly done with other fungi (Perkins et al. 1976).

Sexual development means the recombination between two individual genomes that increases genetic diversity, eliminates deleterious mutations, and ideally results in an organism with improved competence to survive and succeed in a changing environment (Debuchy et al. 2010). Genome recombination, however, can also result in decreased abilities to cope with a challenging situation. For mating to happen, these organisms first need to sense each other, i.e., communicate and decide whether they are suitable partners.

In fungi, two types of sexual systems have evolved. Homothallic fungi are self-fertile, and a single individual can reproduce sexually even when cultured alone. For heterothallic fungi, two compatible partners of opposite mating types are necessary for successful sexual development (Ni et al. 2011).

Mating requires a higher investment of resources compared to asexual development. Additionally, formation of female reproductive structures that might never be fertilized due to the absence of a suitable partner makes preparation for sexual development a risky effort in terms of resource allocation.

When sexual development with *T. reesei* was achieved under laboratory conditions (Seidl et al. 2009), it was the first filamentous ascomycete of industrial importance, for which this tool became available. Thereafter, many other important species, such as *Penicillium chrysogenum*, *P. roqueforti*, or *Aspergillus niger*, followed (Böhm et al. 2013; Frisvad et al. 2014; Ropars et al. 2014).

Availability of this tool opened up new perspectives for strain improvement, and leverage of the benefits of sexual development is only in its beginnings. Classical genetic tools, which have been applied in other fungi for decades, are now also available for *T. reesei*, and conventional breeding is gaining interest as a method to improve nature isolates without using genetic modification. For industrial applications, strains with different characteristics can be combined, with the major advantage being that it is not necessary to know the precise molecular basics for the properties of a given strain. Progeny of a cross will have in part acquired characteristics of both mating partners—provided these charac-

teristics do not exclude each other—and can be selected for combinations. Moreover, outcrossing of unwanted mutations or deletions, such as, for example, in case of nonhomologous end-joining deficient strains, is now possible (Schuster et al. 2012b).

II. Discovery of Sexual Development in *T. reesei*

Since its application in industry, the ability to improve strain performance by crossing was subject to research with *T. reesei*. Actually, only few of the strains of *Trichoderma/Hypocrea* identified in nature came from sources other than perithecia. Additionally, no indication was found that populations of this fungus would show reproductive isolation in nature, and it was suggested that *H. jecorina/T. reesei* reproduces sexually (Lieckfeldt et al. 2000).

Consequently, there was no evidence that sexual development would not be possible with *T. reesei*. Nevertheless, after a large-scale attempt to cross various isolates without success, it was concluded that *T. reesei* QM6a was a clonal, asexual derivative of *H. jecorina*, which had lost its ability to undergo sexual development (*Kuhls et al.* 1996). This study more or less buried the hope to achieve mating under laboratory conditions for some time.

Several years later, a small RNA was found in a screening for cellulase-regulating factors (Schmoll et al. 2004). This RNA turned out to encode the peptide pheromone precursor HPP1 (Schmoll et al. 2010b). In searching the genome sequence of *T. reesei*, which became available shortly after detection of *hpp1*, we also found a mating-type locus indicating *T. reesei* to be heterothallic, which confirmed earlier studies, another pheromone precursor gene, and both pheromone receptor genes. In order to test if sexual development might indeed be possible, we used a strain that had already produced fruiting bodies in axenic culture (Lieckfeldt et al. 2000) and for the first time obtained fruiting bodies and ascospores with QM6a (Seidl et al. 2009; Fig. 17.1). Crossing of *T. reesei* was found to be most efficient at low temperatures around 20 °C in ambient daylight upon growth on complex media such as malt extract or PDA but is also possible on minimal media (Lieckfeldt et al. 2000; Seidl et al. 2009). While in many fungi rather scarce nutritional conditions are required for sexual development (Moore-Landecker 1992), mating in *T. reesei* is even improved on higher concentrations of complex media and as in other fungi is dependent on the nature of the carbon source (Li and Schmoll unpublished).

III. Mating-Type Structure in *Trichoderma*

As bipolar heterothallic fungi, *T. reesei* strains can have mating type MAT1-1 or MAT1-2, which is determined by a genomic locus containing one of two possible sequences (idiomorphs) (Debuchy and Turgeon 2006; Metzenberg and Glass 1990). The transcriptional regulators encoded in these genomic regions are responsible for mating-type-specific gene regulation and consequently in part also the phenotype of a strain. The progeny of a sexual cross then theoretically shows an equal frequency of MAT1-1 strains and MAT1-2 strains.

In *T. reesei* the mating-type locus is flanked by a highly conserved DNA lyase and a less-conserved hypothetical protein. *T. reesei* QM6a was found to be a MAT1-2 strain by screening of the genome sequence (http://genome.jgi-psf.org/Trire2/), and the MAT1-1 idiomorph was revealed by investigation of the MAT1-1 strain of CBS999.97 (Seidl et al. 2009). The MAT1-1 idiomorph contains three open reading frames with *mat1-1-1*, comprising a characteristic MAT alpha1 domain (Pfam 04769); *mat1-1-2*, showing an A2-domain; and *mat1-1-3*, encoding a member of the class I of HMG-box proteins (cd01389). While the MAT1-1 sequence is 5.5 kb long, the MAT1-2 idiomorph comprises the open reading frame of *mat1-2-1*, which encodes the characteristic HMG domain protein found in MAT1-2 loci and only spans 4.3 kb. Intriguingly, the *mat1-1-1* open reading frame extends beyond the mating-type locus boundary and is therefore in part also present

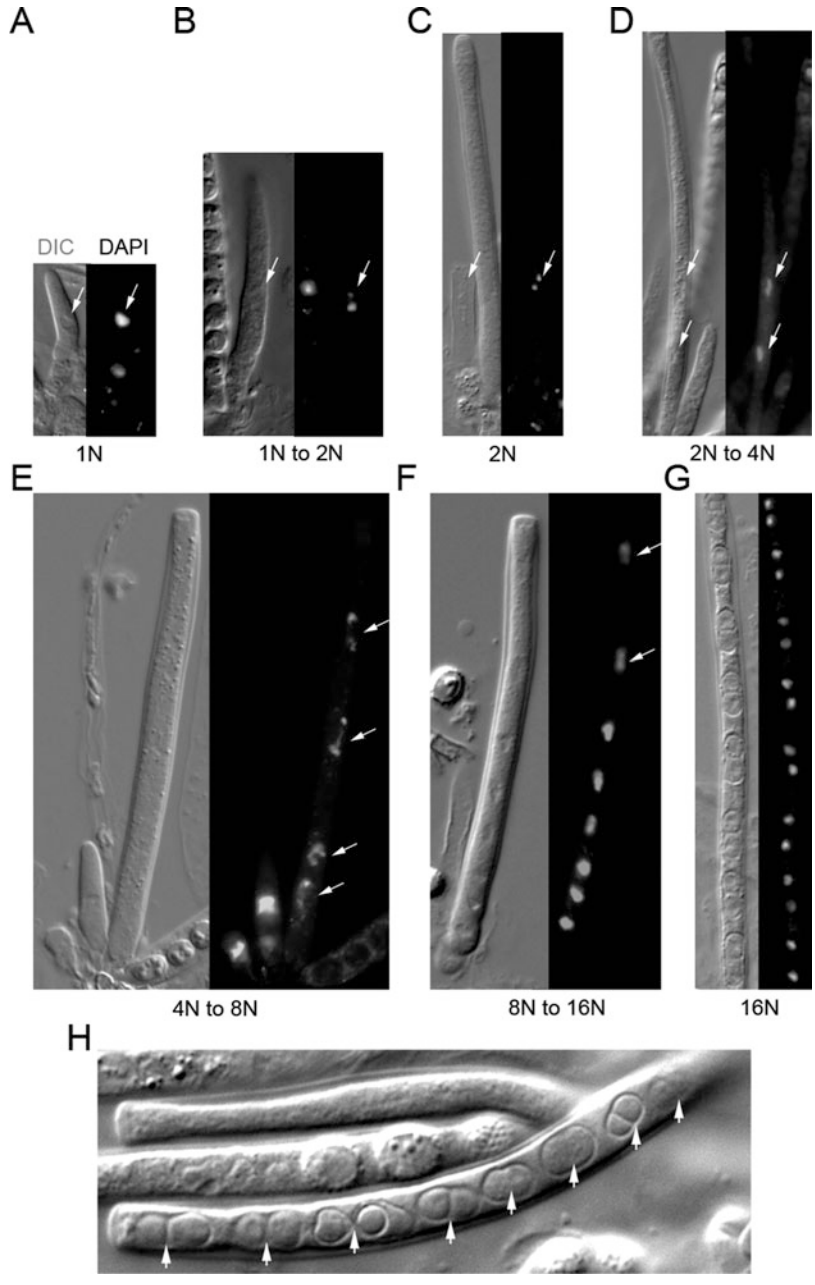


Fig. 17.1 Visualization of four rounds of nuclear division during *T. reesei* ascospore formation/maturation. (**a–g**) Developing asci were manually dissected, stained with DAPI, and then visualized by fluorescence microscopy. DIC and DAPI fluorescence images are shown. Nuclei (N) are marked by *white arrows*. (**h**) A DIC image of developing asci showing synchronous division of eight nuclei (8N) into 16 nuclei (16N). Reprinted with permission from Chuang et al. (2015)

in MAT1-2 strains. Although it is unlikely to be functionally expressed (Seidl et al. 2009), the relevance of this finding remains to be determined.

Mating-type loci in the other so far sequenced *Trichoderma* species (http://genome.jgi-psf.org/programs/fungi/) resemble that of *T. reesei*, and the sequenced strains are MAT1-2 in case of *T. atroviride*, *T. virens*, *T. harzianum*, and *T. longibrachiatum*, and only the sequenced *T. asperellum* strain is MAT1-1 (Schmoll 2013).

IV. Female Fertility

Successful mating requires one of the partners to assume the male role and one to assume the female role, which is under artificial conditions normally determined by the cultivation medium and inoculation technique. In general, filamentous fungi are hermaphroditic, which means they can assume the male or female role in a cross, independent of their mating type (Debuchy et al. 2010; Nieuwenhuis and Aanen 2012). In recent years, however, this view was debated, and it is likely that preferences exist (Heitman et al. 2013).

After the finding that QM6a was indeed able to undergo sexual development with a nature isolate, the aim was to exchange its mating type to create a blueprint strategy for industrial strain improvement with derivatives of QM6a. Unfortunately, this strategy was only successful for the nature isolate CBS999.97 but not for QM6a, which did not form fruiting bodies despite the compatible mating types of partner strains. This phenomenon was interpreted as a defect of QM6a in female fertility (Seidl et al. 2009). Due to the extensive and practically exclusive application of QM6a and its progeny in research and industry, this strain has become the workhorse of the genus. Decades of strain improvement caused the strains nowadays used in industry to be substantially different to the wild-type strain isolated more than 60 years ago. Hence, crossing of industrial high-performance strains with fully fertile nature isolates of *T. reesei* is not a useful option, and solutions for the problem need to be found.

Female sterility is not uncommon in nature and even can have some advantages in natural populations (Hornok et al. 2007). Considering that production of female reproductive structures that might never be fertilized due to lack of a compatible mating partner means a substantial loss of resources, the ability to reproduce sexually may not pay off. Consequently it is possible that partially or exclusively asexual production provides a competitive advantage, at least for some time. Ultimately, asexual reproduction due to such a defect, if gradually increasing in a population, might lead to a change in population structure if environmental conditions deteriorate (Leslie and Klein 1996; Taylor et al. 1999) or even in an evolutionary end point (Geiser et al. 1996; Lobuglio et al. 1993).

For *N. crassa*, it is known that prolonged cultivation under laboratory conditions can result in female fertility. As QM6a has been handled in the lab for decades, it could not be excluded that such a defect might also have been acquired artificially. However, in the meantime, the genomic region containing the genes responsible for the defect of QM6a could be narrowed down to one locus with several genes clearly correlating with female fertility/sterility in female fertile or sterile progeny from crosses of QM6a with female fertile strains (Schmoll and Freitag unpublished). Hence it can be concluded that a natural phenomenon rather than an acquired defect is the reason for female sterility of QM6a.

Female sterility varies substantially in different populations, and mutations in numerous loci can lead to female sterility. Among the genes in such loci, functions in amino acid transport or a nitrilase were found, which would not be expected to be relevant for development (Klittich and Leslie 1988; Xu and Leslie 1996), but the mechanism governing female fertility is not well known. Also transcription factors and kinases can be responsible for female fertility (Deng et al. 2007; Kim et al. 2002; Sun et al. 2009), and pheromone receptors are responsible for female fertility in their cognate mating type, also in *T. reesei* (Kim and Borkovich 2004; Kim et al. 2012; Seibel et al. 2012a). However, none of these genes are located within the female fertility locus detected in QM6a as mentioned above.

V. Analyzing Sexual Competence in *T. reesei*

Achieving sexual crossing between compatible partners is surprisingly simple with *T. reesei* [for details, see Schmoll (2013)]. When investigating regulation of sexual development, diverse defects have to be considered. The most obvious analysis concerns the environmental conditions under which sexual development is preferred compared to asexual development, which includes nutritional conditions such as carbon source, nitrogen source, or sulfur source but also light/darkness, temperature, CO2 content, or humidity (Pöggeler et al. 2006). Unfortunately, up to now, no conditions have been found that would induce formation of protoperithecia or trichogynes in *T. reesei*. Hence, determination of female and male fertility requires a different approach than in other fungi.

Usually mating assays with *T. reesei* strains are set up by inoculating the two compatible partners on opposite sides of a plate ("confrontation assay"), where they will form fruiting bodies at the site of their encounter. Using this method, it would be difficult to compare fruiting body formation of strains with different growth rates, as a delay in fruiting body formation could well be the result of slower growth. In such a case, a mixture of equal amounts of spores of both partners can be used for inoculation at one spot, which leads to fruiting body formation after roughly the same time than with the confrontation assay.

A certain genetic defect may only occur in one but not in another condition, and careful investigation may enable crossing despite such a defect. One example for such a defect in *T. reesei* is lack of functional ENV1, which results in deregulation of the pheromone system and female sterility in light, but not in darkness (Seibel et al. 2012b).

A further, very important criterion is male or female fertility. In a cross between two compatible partners, one has to assume the male role and the other one the female role for mating to happen. Therefore, even if both partners would in principle be able to undergo sexual development with a fully competent mating partner, it is just as impossible to cross two male sterile strains as it is to cross two female sterile strains. This problem has major implications for strain development in industry and especially for *T. reesei*.

As outlined above, QM6a, the parental strain of all strains nowadays used in research and industry, was found to be female sterile (Seidl et al. 2009). Since the industrial strains were adjusted to high-production characteristics under fermentation conditions by multiple round of random mutation, it was important that the crossing partner would not introduce too extensive alterations in the genome (e.g., from a nature isolate), which would likely abolish production capacity. While this finding poses serious problems with application of sexual development in industry, it can be used to determine the relevance of certain genes, mutations, or conditions for male and female fertility in *T. reesei*.

Importantly, the defect of QM6a in female fertility renders attempts to analyse sexual development using already available mutants derived from this strain (including those in QM9414 and RUT C30) problematic. No effects on female fertility can be studied, because all these strains are female sterile already. Moreover, if a mutation is found to abolish the ability to undergo sexual development with a compatible partner, there is no way of knowing, if this is due to a general defect in sexual development or merely a problem with male fertility. Additionally, the mating partner would have to be a fully sexually competent nature isolate, because with all derivatives of QM6a, even if the mating type would have been exchanged, mating is impossible, except for the unlikely case that by chance the mutation has restored female fertility in this strain.

Until the precise defects of QM6a that cause its female sterility are known, backcrossing of QM6a derivatives with a fertile nature isolate can restore sexual competence as shown previously (Schuster et al. 2012b). Thereby, the female sterile strain is crossed with the fertile nature isolate, which leads to progeny of diverse phenotypes (including such showing segmental aneuploidy) due to the combined genomes of two different strains. Afterwards, fertile progeny of suitable mating type are again used for crossing with the female sterile

strain, and after some repetitions (ideally 10 or more) of this process, the phenotype of the previously female sterile strain is largely restored while sexual competence as acquired from the nature isolate is retained.

This very strategy has also been used to obtain information on the genomic loci responsible for the defect in female fertility of QM6a. Comparison of three independent backcrossed lines led to identification of three genomic loci that were retained after 10 (back)crosses, and one of these loci significantly correlated with female fertility. Statistics of distribution of female fertility and female sterility in progeny of the obtained fertile strains with female sterile QM6a further indicate that in accordance with the correlation found, only one locus is responsible for the defect (Schmoll et al. unpublished results).

Female fertility of QM6a bears the advantage that this strain can serve as a testing strain for mutations introduced in fully fertile strains constructed by backcrossing or in the background of CBS999.97 (Seibel et al. 2012a, b). If a MAT1-1 mutant can undergo sexual development with female sterile QM6a, then it must be female fertile, and the mutation has no effect on female fertility. The same mutation, when introduced into QM6a, provides information on its influence on male fertility: If then the mutation abolishes sexual development with a fertile partner, it is responsible for male fertility, because a male and female sterile strain cannot mate anymore.

VI. Segmental Aneuploidy

To gain insight into the molecular mechanism of *T. reesei* sexual development, the yeast tetrad dissection technique has been applied to sequentially separate the 16 ascospores of each hexadecad asci. All 16 ascospores were germinated individually to form isolated spore colonies, and then their phenotypes, such as spore viability, colony morphology, and colony color, were determined. The genotypes of these ascospores were also compared using array-based comparative genomic hybridization (aCGH) and genomic PCR. The results of these experiments showed that the 16 ascospores in each

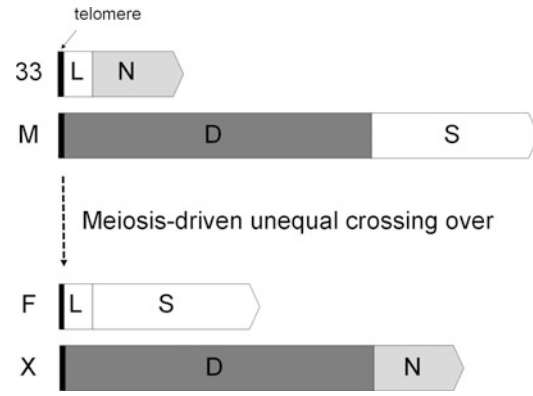


Fig. 17.2 Reciprocal exchange between scaffold M and scaffold 33. Scaffold M and scaffold 33 in wild-type CBS999.97(1-2), and scaffold F and scaffold X in wild-type CBS999.97(1-1). Organization and length of the four segments (L, N, D, and S) in these four scaffolds are indicated

hexadecad could be classified into four linearly arranged groups and each group contained four genetically nearly identical ascospores, indicating that the 16 ascospores are likely generated via meiosis followed by two rounds of post-meiotic mitosis (Chuang et al. 2015).

Surprisingly, sexually crossing of CBS999.97(1-1) to CBS999.97(1-2) or QM6a often (>90 %) generated two types of SAN (segmentally aneuploid) ascospores (Chuang et al. 2015). The haploid genomes of the CBS999.97(1-2) and QM6a are similar to that of ancestral *T. reesei* strain(s). In contrast, the CBS999.97(1-1) haploid genome contains a reciprocal arrangement between scaffold 33 and scaffold M of the CBS999.97(1-2) genome. Scaffold 33 consists of a 5′ "L" segment (~33 kb) and a 3′ "N" segment (~170 kb), whereas scaffold M has a 5′ "D" segment (~523 kb) and a 3′ "S" segment (~452 kb). In the CBS999.97(1-1) genome, the L segment and the S segment form scaffold X, and the D segment and the N segment form scaffold F (Fig. 17.2). The first type of SAN ascospores could germinate to form mycelia but these strains produce white conidia. In contrast, CBS999.97(1-1), CBS999.97(1-2), and QM6a produce green conidia. The second type of SAN ascospores were unable to germinate. The genomes of the viable SAN progeny con-

tain two D segments but lack the L segment (Chuang et al. 2015).

Notably, the *T. reesei* polyketide synthase 4 gene (*tpks4*) is located within the L segment that is lost in viable SAN progeny. The PKS4 protein products are responsible for the green conidial pigmentation in the wild-type strains (Atanasova et al. 2013b). Loss of the L segment (comprising *tpks4*) in these SAN progeny is the main cause for the white-conidia phenotype. Furthermore, the two D segments resulted in an increase of xylanase (but not cellulase) production in these viable SAN progeny. Intriguingly, there is a carbohydrate-active enzyme (CAZy) gene cluster in the D segment. This CAZy gene cluster contains a set of hemicellulases or xylanases (*abf1*, *bga1*, *cip2*, *cel74a*, and *xyn3*) (Hakkinen et al. 2014). In contrast, the genomes of the nonviable SAN progeny likely contain two L segments but lack the D segment. The lack of D segment is the cause of meiosis-driven ascospore lethality, as it contains at least 113 annotated genes, including several putative essential genes such as an actin-like protein (ID 111468).

Due to unequal crossing-over during meiotic prophase, these two types of SAN ascospores are differentially distributed in three types of hexadecad asci. The type I hexadecad asci (~80 %) have eight viable euploidy ascospores, four viable SAN ascospores, and four nonviable SAN ascopores. The type II hexadecad asci (~10 %) contain eight viable SAN ascospores and eight nonviable SAN ascospores, but they lack euploidy ascospore. The type III hexadecad asci (~10 %) have 16 viable euploidy ascospores. Accordingly, these three types of hexadecad asci were generated via one, two, or no crossing-over during meiosis (Chuang et al. 2015).

Notably, these findings are consistent with the classic chromosomal speciation model (White 1978) stating that chromosome rearrangements cause or contribute to heterozygous hybrid infertility and serve as a genetic barrier between recently diverged species. Chromosomal rearrangements also contributed to reproductive isolation in two closely related species, e.g., two *Schizosaccharomyces* strains (*S. pombe* vs *S. kombucha*) (Zanders et al. 2014), two *Saccharomyces* strains (*S. cerevisiae* vs *S. mikatae or S. paradoxus*) (Delneri et al. 2003), and two occasionally hybridizing North American *Drosophila* species (*D. pseudoobscura* and *D. persimilis*) (Noor et al. 2001). In all cases, sexual crossing resulted in viable hybrid diploids that efficiently completed meiosis but frequently generated nonviable gametes.

QM6a had been subjected to multiple rounds of random mutagenesis and selection to generate cellulase hypersecreting mutants (e.g., QM9414 and RUT-C30). These mutants have been widely used for various industrial applications. Recent comparative genome sequence analyses revealed that their genomes, compared to that of QM6a, carry multiple chromosomal translocations and deletions [reviewed in Seiboth et al. (2011)]. Consistent with the classic chromosomal speciation model (White 1978), sexually crossing of CBS999.97 (1-1) with these industrial strains mostly produces nonviable acsospores (Chuang et al. 2015). Therefore, applying sexual development in improving these industrial strains can be expected to be challenging.

VII. Repeat-Induced Point Mutation

In *N. crassa*, sexual development initiates a mechanism which is aimed to remove repetitive sequences from the genome as defense against mobile genetic elements. This mechanism, called repeat-induced point mutation (RIP), detects and mutates duplicated sequences of more than 400 base pairs hence rendering the respective genomic area unfunctional (Galagan and Selker 2004; Ni et al. 2011). As also the genome of *T. reesei* contains a surprisingly low number of duplicated genes, the operation of RIP was suspected (Martinez et al. 2008).

If operative in *T. reesei*, RIP is of tremendous importance for industrial strain improvement. As industrial high-performance strains usually contain multiple copies of regulators or duplications introduced during mutation cycles, the risk of losing the benefits of these regulators upon sexual crossing has to be considered. In order to evaluate this risk, we performed crossing tests with strains containing

multiple copies of G-protein alpha subunits (Schmoll et al. 2009; Seibel et al. 2009) and tested progeny for mutations in the respective genes. However, the operation of RIP could not be confirmed (Schuster and Schmoll unpublished observations). Additionally, we used sexual crossing to prepare double mutants. Since the respective deletion strains contained a duplication of the *pki* promotor and *cbh2* terminator, which were introduced due to their presence in the deletion cassette (Mach et al. 1994), we could not exclude that RIP might interfere with the function of these genes. Again no defect could be detected (Seibel et al. 2012a, b). Nevertheless, it cannot be excluded that RIP occurs, albeit likely at a low extent. Hence we deleted the homologue of *N. crassa rid-1* (RIP-deficient 1; Freitag et al. 2002), which abolishes RIP and should consequently prevent RIP from mutating target genes. The phenotype of the resulting strain was similar to the wild type (Schuster et al. 2012b). This strategy can be applied if RIP is observed upon strain improvement by crossing, although since RIP has not been detected in *T. reesei*, we could also not test if *T. reesei rid1* can indeed abolish this mechanism.

VIII. The Pheromone System

A. Peptide Pheromone Precursors

Prior to initiation of sexual development, signals are exchanged, which enable mate recognition. Peptide pheromones are crucial in this communication (Jones and Bennett 2011), although also chemical signals play important roles (Leeder et al. 2011). Of these small, diffusible pheromones, which act via specific G-protein-coupled receptors (Bolker and Kahmann 1993; Ni et al. 2011), one encodes a polypeptide with multiple repeats of a putative pheromone sequence bordered by protease-processing sites and is related to the *Saccharomyces cerevisiae* alpha factor precursor (Singh et al. 1983). The second pheromone precursor gene encodes a CAAX domain protein, which upon maturation is modified to end with a carboxymethylated cysteine. Peptide pheromone precursors with these characteristics are not only found in *S. cerevisiae* a-strains and numerous ascomycetes but also in basidiomycetes (Ni et al. 2011) and sometimes in unexpectedly high numbers (Martinez et al. 2009).

In contrast to other fungi, in which pheromone receptors and pheromone precursor genes are regulated in a strictly mating-type-dependent manner (Bobrowicz et al. 2002; Coppin et al. 2005; Shen et al. 1999; Zhang et al. 1998), this is surprisingly not the case in *T. reesei* (Schmoll et al. 2010b). While some preference for mating-type-dependent regulation is observed, both pheromone receptors and precursors are transcribed in both mating types. Studies in other fungi showed that this is the case for other species as well and that asymmetry in pheromones is not required for sexual development (Goncalves-Sa and Murray 2011).

Pheromone precursor genes are known to be essential for male fertility in their cognate mating type in fungi and also in *T. reesei*, while their receptors are crucial for female fertility (Coppin et al. 2005; Kim and Borkovich 2006; Schmoll et al. 2010b; Seibel et al. 2012a).

Despite largely similar components as found in other ascomycetes, the *T. reesei* pheromone system also shows intriguing alterations. The alpha-type peptide pheromone precursor PPG1 shows the expected structure with repeats and KEX-domains. However, the function of the a-type peptide pheromone precursor is assumed by HPP1, the first characterized member of a novel type of peptide pheromone precursors named "h-type" (Schmoll et al. 2010b). Its initial detection in the genome of *T. reesei* happened rather by chance in a screen for cellulase regulators (Schmoll et al. 2004). As the sequence of the respective short mRNA sequence suggested it might encode a peptide pheromone precursor, analyses to investigate the potential of *T. reesei* to undergo sexual development were initiated after it had been considered asexual earlier.

HPP1 is characterized by a C-terminal CAAX domain, which is part of the consensus motif (LI)GC(TS)VM that occurs three times in the predicted protein sequence. Consequently, HPP1 comprises characteristics of a-type as well as alpha-type peptide pheromone precur-

sors, reflecting a "hybrid type" named h-type. Additionally, *T. reesei* HPP1 contains two KEX-protease sites and a cAMP-dependent phosphorylation site (Schmoll et al. 2010b). These additional sites are not essential for sexual development, and their relevance remains to be determined.

Besides the structure and domain composition, also the genomic location of *hpp1* is different compared to the largely conserved position of a-type peptide pheromone precursor genes in other fungi in the vicinity of a cyanate lyase. The latter, conserved locus shows a deletion of several kilobases in *Trichoderma* spp., and *hpp1* is found on a different scaffold in the vicinity of a *sec23* homologue (Schmoll et al. 2010b). In *Trichoderma* spp. the consensus sequences of *hpp1* are relatively conserved, but KEX-domains and the cAMP-dependent phosphorylation site are not. Additionally, the assumed *hpp1* homologue of *T. atroviride* is shorter and only contains one instead of three consensus motifs (Schmoll 2013; Schmoll et al. 2010b).

These findings would point at an evolutionary event, where a lost pheromone precursor gene in *Trichoderma* spp. has been complemented by a newly evolved gene. However, genes encoding pheromone precursors with similar characteristics, also at the conserved locus close to a cyanate lyase, were detected in *Nectria* spp., *Gibberella* spp. and other fungal species (Schmoll et al. 2010b).

In most cases the potential homologues are located in the vicinity of a cyanate lyase in the genome or even fused to the respective gene model. Moreover, next to *hpp1* gene models, also putative pheromone precursor genes resembling a-type genes are detected. With the increase in fungal genome sequences available, also some more potential HPP1 homologues in addition to those already known (Schmoll et al. 2010b) were detected. For example, in several *Gibberella/Fusarium* spp., *Acremonium strictum*, or *Ilyonectria* spp., *Hpp1*-homologues were also found within gene models spanning its genomic location, and additional analyses would be required to confirm whether these homologues are indeed specifically transcribed and translated. Nevertheless, still no homologue has been detected in orders other than Hypocreales, hence indicating a very specific function in this group of fungi.

Investigation of the role of HPP1 in sexual development so far did not reveal any differences to a-type peptide pheromone precursors (Schmoll et al. 2010b; Seibel et al. 2012a). Only its isolation in a screening for cellulase regulators points at an additional function in responding to nutrient conditions, which are important for fungal development. Although preliminary data indeed support such a function, more analyses are necessary to confirm this hypothesis.

B. Pheromone Receptors

The pheromone receptors belonging to the heterotrimeric G-protein pathway sense the presence of a compatible mating partner. In heterothallic ascomycetes, they are associated with one of the two mating types and sense alpha-type peptide pheromone precursors (Ste2p-type in MAT1-2) or a-type peptide pheromone precursors (Ste3p-type in MAT1-1). *T. reesei* has two pheromone receptors, HPR1 (related to Ste3p) and HPR2 (related to Ste2p), which are essential for female fertility in their cognate mating types (Seibel et al. 2012a). As in other fungi (Mayrhofer et al. 2006), also in *T. reesei*, a compatible pair of pheromone precursor and receptor in both mating partners (PPG1-HPR2 or HPP1-HPR1) is required for mating to occur (Seibel et al. 2012a). Besides their function in pheromone signal reception, pheromone receptors moreover have a function in ascosporogenesis in *T. reesei*.

Comparing transcription patterns of pheromone precursors and pheromone receptors between the fully fertile nature isolate CBS999.97 and QM6a revealed that the basic patterns are similar, but still severalfold (up to 20-fold) differences in transcript levels occur (Seibel et al. 2012a). While the relevance of this imbalance is not yet entirely clear, a contribution to the defect of QM6a in female fertility cannot be excluded.

IX. Regulation of Sexual Development

One of the major advantages of sexual development lies in the ability to overcome problems of a deteriorating environment by creating progeny with different characteristics due to the recombination of the genomic content of two parents. Accordingly, sensing conditions that would require adaptation of an individual or a whole population is of crucial importance (Pöggeler et al. 2006). In *T. reesei*, so far studies on the relevance of nutrient signaling via the heterotrimeric G-protein pathway, the cAMP signaling, and the light response pathway are available (Fig. 17.3) along with some preliminary findings for other regulators.

A. The Heterotrimeric G-Protein Pathway

As one of the most important signaling pathways for environmental sensing, heterotrimeric G-protein signaling also plays an important role in sexual development. The two pheromone receptors are G-protein coupled, and once they sense the pheromones of a potential mating partner, the signaling cascade involving predominantly G-protein alpha, beta, and gamma subunits is initiated (Li et al. 2007). Keeping in mind that the *T. reesei* genome contains roughly 50G-protein-coupled receptors (GPCRs), but only 3G-alpha (GNA1, GNA2, and GNA3), one G-beta (GNB1), and one G-gamma subunits (GNG1) (Gruber et al. 2013; Schmoll 2008), it can be expected that at this signal transmission step, also signal integration occurs.

As co-chaperones presumably supporting correct folding of G-beta and G-gamma subunits, phosducin-like proteins exert a regulatory function in the pathway (Willardson and Howlett 2007). A function of phosducin-like proteins in sexual development of fungi was first shown in *Aspergillus nidulans* (Seo and Yu 2006). In *T. reesei* the gene encoding the class I phosducin-like protein, *phlp1*, was shown to be regulated in response to light and involved in cellulase gene expression. Additionally, the regulatory targets of PhLP1, GNB1, and GNG1 include numerous genes associated with sexual development (Tisch et al. 2011a). Moreover, PhLP1 was found to be one of the potentially several factors connecting light response with nutrient and environmental signaling through the G-protein pathway via regulatory interaction with the photoreceptor ENV1 (Tisch et al. 2014). Investigation of sexual development in mutants of *phlp1* and *gnb1* indeed revealed a role of these genes in development of female reproductive structures (fruiting body development) and reproductive efficiency (ascospore discharge) while *gng1* had no significant effect. Lack of *phlp1* significantly decreased fruiting body development, while ascospore discharge was lower in *phlp1* mutants and virtually abolished in *gnb1* mutants (Tisch et al. 2011a).

Interestingly, transcript levels of the h-type pheromone precursor-encoding gene *hpp1* are clearly decreased in mutants of *phlp1*, *gnb1*, and *gng1* in light, which is the preferred condition for sexual development of *T. reesei*. In addition, the putative homologue of the pheromone receptor gene *ste6* is significantly regulated by all three genes (Tisch et al. 2011a).

With respect to the G-protein alpha subunits, evaluation of strains with constitutive activation of the G-alpha subunit and deletion or knockdown of *gna1*, *gna2*, and *gna3* (Schmoll et al. 2009; Schuster et al. 2012a; Seibel et al. 2009) did not abolish sexual development (Schuster et al. 2012a; Schmoll and Seibel unpublished observations). As all the mutant strains used in these experiments are female sterile due to their strain background as derivatives of QM6a, it can be concluded that the G-protein alpha subunits do not exert an essential function in sexual development in *T. reesei* and are dispensable for male fertility. Functions in female fertility as shown in other fungi for certain components of the heterotrimeric G-protein pathway (Kim et al. 2012) remain to be evaluated.

B. The cAMP Pathway

cAMP levels are influenced by the heterotrimeric G-protein pathway also in *T. reesei* (Schmoll et al. 2009), and the cAMP pathway represents one of the output pathways of

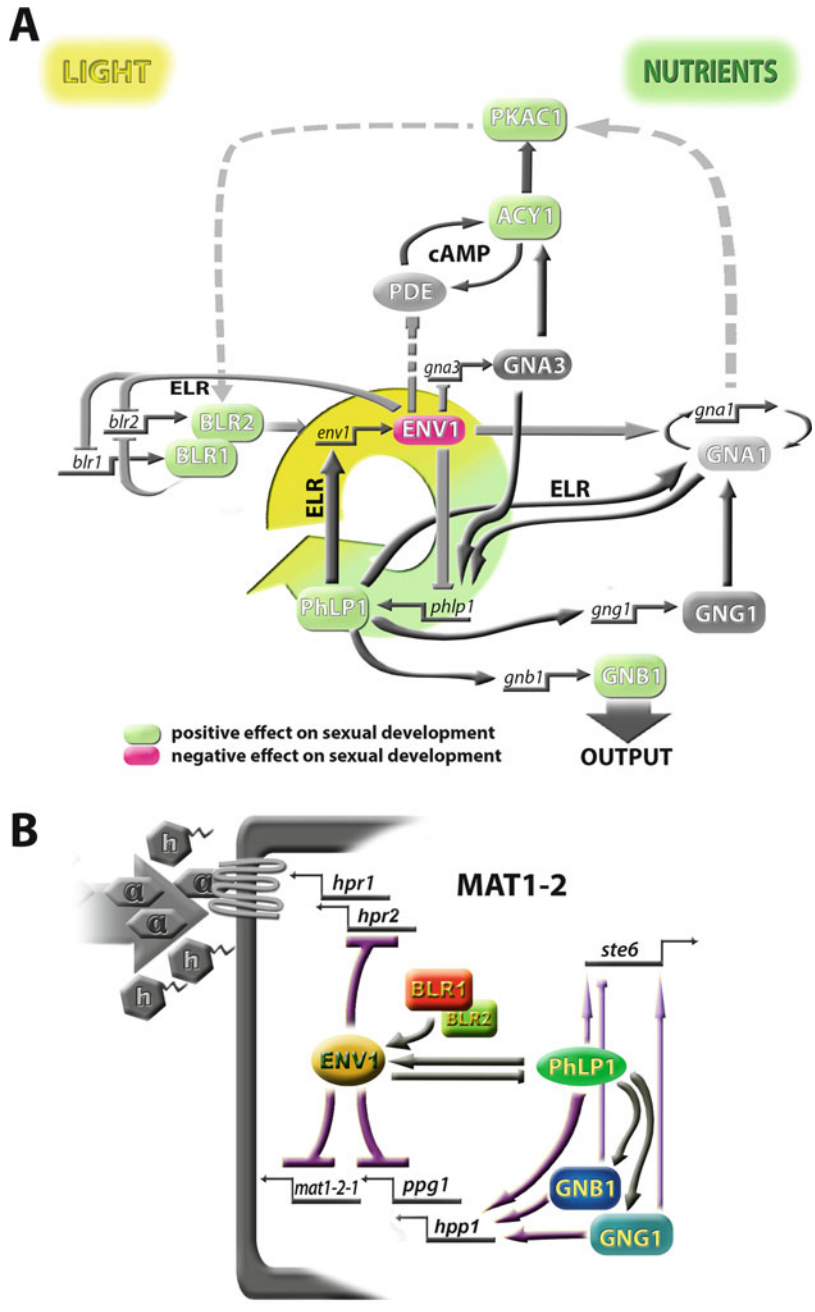


Fig. 17.3 Regulation of sexual development in *T. reesei*. (**a**) Influence of signal transduction components of the heterotrimeric G-protein pathway, the cAMP pathway, and the light response pathway on sexual development. (**b**) Regulatory interconnections between the phosducin-like protein PhLP1, the G-protein beta and gamma subunits (GNB1 and GNG1), and the light response pathway with respect to regulation of pheromone precursors and pheromone receptors. Revised and reproduced with permission from Schmoll (2013)

G-protein signaling, which indicates a function in development as well. Components of the cAMP pathway were shown to influence sexual development in terms of delayed fruiting body formation or reduced fruiting body size (Ivey et al. 2002; Kamerewerd et al. 2008). In *T. reesei* both adenylate cyclase 1 (ACY1) and protein kinase A (catalytic subunit PKAc1) play a role in sexual development. Lack of ACY1 or PKAc1 caused delayed fruiting body formation, but no significant alteration in fruiting body size (Schuster et al. 2012a).

In the latter study, crossing was also used to evaluate segregation of the severe growth phenotype of ACY1 and PKAc1 mutant strains with the selection marker (Schuster et al. 2012a). Along with an earlier study on the ubiquitin pathway in *T. reesei* (Denton and Kelly 2011), the results corroborate the applicability of crossing for confirming mutant phenotypes, a method used in other fungi already for decades.

C. The Light Response Pathway

Accumulating evidence indicates that light is a key environmental factor affecting vegetative mycelial growth, asexual conidiation, and sexual development in several model fungi, including *A. nidulans*, *Coprinopsis cinerea, N. crassa*, *Mucor circinelloides*, and *T. reesei* (Fuller et al. 2014; Idnurm et al. 2010; Rodriguez-Romero et al. 2010; Steyaert et al. 2010). In many of them, the balance between sexual development and asexual development is considerably influenced by light (Debuchy et al. 2010; Rodriguez-Romero et al. 2010). In *T. reesei*, sexual development preferentially occurs in daylight (light–dark cycles) and is clearly delayed in constant darkness (Seibel et al. 2012b; Seidl et al. 2009). In darkness, perithecia develop more slowly, are larger in volume, are located deeper inside the stromata, and have longer necks toward the upper surface. In contrast, fruiting body formation is perturbed in constant light (Chen et al. 2012).

BLR1, BLR2 (blue light receptors 1 and 2), and ENV1 (envoy 1) are the blue light photoreceptors characterized so far from *T. reesei* (Castellanos et al. 2010; Schmoll et al. 2005, 2010a).

Despite the important role of light in sexual development in *T. reesei*, the photoreceptors BLR1 and BLR2 are not essential for this process, neither individually nor in a double mutant. Additionally, no specific effect on male or female fertility was detected. Lack of BLR1 or BLR2 or both also did not result in the dark phenotype with respect to sexual development. Instead, deletion mutants showed larger but fewer fruiting bodies, with only a slight decrease in total dry mass of stromata. Hence BLR1 and BLR2 positively influence fruiting body formation (Chen et al. 2012; Seibel et al. 2012b).

The negative effect of constant light on sexual development was however found to be mediated by BLR1 and BLR2 (Chen et al. 2012).

In contrast to BLR1 and BLR2, ENV1 considerably influences sexual development. ENV1 is essential for female fertility specifically in light, but not in darkness, which is in agreement with its function as photoreceptor (Chen et al. 2012; Seibel et al. 2012b). ENV1 is known to regulate cAMP levels, which is assumed to occur via dampening phosphodiesterase activity (Tisch et al. 2011b). Moreover it was recently shown that the effect of ENV1 is in part due to its effect on cAMP levels (Tisch et al. 2014). Therefore the phosphodiesterase inhibitor caffeine was used to mimick ENV1 function in ENV1 mutants, but still the defect in female fertility in light could not be rescued. Hence, the effect of ENV1 on female fertility is not regulated via its effect on cAMP levels (Chen et al. 2012). Interestingly, the defect could be alleviated by deletion of *blr1* in a strain lacking ENV1 in light or in a 12:12 photoperiod (Chen et al. 2012).

With respect to regulation of the pheromone system, all three photoreceptors were involved. BLR1, BLR2, and ENV1 all negatively regulate transcript levels of the pheromone precursor genes *hpp1* and *ppg1* and the pheromone receptor genes *hpr1* and *hpr2* (Seibel et al. 2012b). For *hpp1* this regulation pattern is specific for MAT1-1 with BLR1 and BLR2, and the strongly negative effect of ENV1 on *hpp1* transcript abundance was even observed upon shake flask cultivation on cellulose media, where sexual development does not occur

(Schmoll et al. 2010b). The effect of ENV1 on *hpp1* was mating-type dependent. Lack of ENV1 caused up to 100-fold increased transcript levels of *hpp1* in MAT1-1, but in the cognate mating type of hpp1, MAT1-2, the increase was around 100,000-fold. Also the effect on *ppg1* is mating-type dependent but much less pronounced reaching differences of around 100-fold in its cognate mating type MAT1-1. In case of BLR1 and BLR2, some mating-type-dependent differences were observed, but the extent was only severalfolds here (Seibel et al. 2012b). Also for the pheromone receptors, the negative effect of ENV1 on transcript levels was clearly more pronounced than that of BLR1 or BLR2 with differences to wild-type strains of up to around 30-fold. Considering the severe misregulation of the pheromone system in *env1*-mutant strains, the observed female fertility upon growth in light might be due to lost sexual identity and failure in mate recognition in light (Seibel et al. 2012b). Additionally, the finding that deletion of *hpp1* rescues the ability of fruiting body formation in constant light is in agreement with this interpretation (Chen et al. 2012).

Nevertheless, mating of *env1* mutants with female fertile partners of opposite mating type, which are obviously able to compensate for the abolished control mechanism, is possible. Moreover, in photoreceptor mutants, transcript levels of the genes of the *T. reesei* pheromone system are at wild-type levels in darkness (Seibel et al. 2012b).

Besides regulation of the pheromone system, BLR1, BLR2, and ENV1 also negatively regulate the mating-type gene *mat1-2-1*, again with ENV1 having the most severe effect. Hence, the photoreceptors assume important functions in sexual development in *T. reesei* and appear to act at least for some functions upstream of the mating-type genes in the regulatory cascade (Seibel et al. 2012b).

A further factor important for regulation of development in fungi is VELVET, which is best studied in *Aspergilli* (Bayram and Braus 2012), and now its function has also been investigated in *T. reesei*. Initial analysis indicated a function of VEL1 in sexual development, albeit these analyses were performed in the background of a cellulase high-producer mutant (QM9414) (Karimi Aghcheh et al. 2014), which is female sterile already (Seidl et al. 2009). Hence female fertility could not be tested in this system.

Our recent detailed analyses using strains with a female fertile background indicated a function of VEL1 in female fertility in light, while VEL1 is essential for sexual development in darkness. Additionally, VEL1 was found to impact regulation of the pheromone system and appears to be involved in communication of (potential) mating partners via pheromone response and secretion of specific patterns of secondary metabolites (Bazafkan et al. 2015). This regulation of fungal communication by VEL1 now connects the functions of this group of proteins in light response, development, and secondary metabolism to a biologically important role in reproduction.

D. Other Factors

The ability to undergo sexual development has been tested with several mutant strains albeit without detailed investigation and in the female sterile strain background of QM6a derivatives. For example, the ubiquitin C-terminal hydrolase CREB (Denton and Kelly 2011), protein kinase C, phosphodiesterases 1 and 2 as well as several transporters and transcription factors (Schuster et al. 2012b) were shown not to be essential for sexual development.

Using female fertile *Trichoderma* strains and mutants derived from them, a transcriptome analysis comparing conditions under which sexual development occurred (termed sexually potent) with those that did not permit sexual development (sexually impotent) revealed considerable differential gene regulation. Roughly 200 genes exhibited more than twofold downregulation, and 287 genes showed higher transcript levels under sexually potent conditions in comparison with those under sexually impotent conditions. Interestingly, among the genes significantly upregulated under sexually potent conditions, significant enrichment of genes involved in cellulose and hemicellulose metabolism, electron transport, redox regulation, and protein folding was detected. Specifically, more than 30 glycoside hydrolases or cellulose-binding protein, several peptidases

and proteases, metabolic proteins, G-protein-coupled receptors, various transporters, and permeases were upregulated under sexually potent conditions versus impotent conditions (Chen et al. 2012). Consequently, these results point at a hierarchically organized regulatory network with complex regulation, which is not limited to the function of the mating-type transcription factors. Moreover, this regulation reflects an influence of both environmental and nutritional cues on sexual development.

References

Atanasova L, Druzhinina I, Jaklitsch W (2013a) Two hundred *Trichoderma* species recognized on the basis of molecular phylogeny. In: Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M (eds) *Trichoderma* – biology and applications, vol 2. CAB International, Oxfordshire, UK, pp 10–42

Atanasova L, Knox BP, Kubicek CP, Druzhinina IS, Baker SE (2013b) The polyketide synthase gene *pks4* of *Trichoderma reesei* provides pigmentation and stress resistance. Eukaryot Cell 12:1499–1508

Bayram O, Braus GH (2012) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol Rev 36:1–24

Bazafkan H, Dattenböck C, Böhmdorfer S, Tisch D, Stappler E, Schmoll M (2015) Mating type dependent partner sensing as mediated by VEL1 in *Trichoderma reesei*. Mol Microbiol 96(6):1103–18

Bissett J (1984) A revision of the genus *Trichoderma*. I. Section *T. longibrachiatum* sect. nov. Can J Botany 62:924–931

Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ (2002) The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804

Böhm J, Hoff B, O'Gorman CM, Wolfers S, Klix V, Binger D, Zadra I, Kurnsteiner H, Pöggeler S, Dyer PS, Kück U (2013) Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus *Penicillium chrysogenum*. Proc Natl Acad Sci USA 110:1476–1481

Bolker M, Kahmann R (1993) Sexual pheromones and mating responses in fungi. Plant Cell 5:1461–1469

Castellanos F, Schmoll M, Martinez P, Tisch D, Kubicek CP, Herrera-Estrella A, Esquivel-Naranjo EU (2010) Crucial factors of the light perception machinery and their impact on growth and cellulase gene transcription in *Trichoderma reesei*. Fungal Genet Biol 47:468–476

Chen CL, Kuo HC, Tung SY, Hsu PW, Wang CL, Seibel C, Schmoll M, Chen RS, Wang TF (2012) Blue light acts as a double-edged sword in regulating sexual development of *Hypocrea jecorina* (*Trichoderma reesei*). PLoS One 7, e44969

Chuang YC, Li WC, Chen CL, Hsu PW, Tung SY, Kuo HC, Schmoll M, Wang TF (2015) *Trichoderma reesei* meiosis generates segmentally aneuploid progeny with higher xylanase-producing capability. Biotechnol Biofuels 8:30

Coppin E, de Renty C, Debuchy R (2005) The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Eukaryot Cell 4:407–420

Debuchy R, Turgeon BG (2006) Mating-Type Structure, Evolution and Function in Euascomycetes. In: Kues U, Fischer R (eds) The Mycota I. Springer, Berlin, pp 293–323

Debuchy R, Berteaux-Lecellier V, Silar P (2010) Mating systems and sexual morphogenesis in ascomycetes. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi, vol 33. ASM Press, Washington, DC, pp 501–535

Delneri D, Colson I, Grammenoudi S, Roberts IN, Louis EJ, Oliver SG (2003) Engineering evolution to study speciation in yeasts. Nature 422:68–72

Deng F, Allen TD, Nuss DL (2007) Ste12 transcription factor homologue CpST12 is down-regulated by hypovirus infection and required for virulence and female fertility of the chestnut blight fungus *Cryphonectria parasitica*. Eukaryot Cell 6:235–244

Denton JA, Kelly JM (2011) Disruption of *Trichoderma reesei cre2*, encoding an ubiquitin C-terminal hydrolase, results in increased cellulase activity. BMC Biotechnol 11:103

Druzhinina IS, Komon-Zelazowska M, Atanasova L, Seidl V, Kubicek CP (2010) Evolution and ecophysiology of the industrial producer *Hypocrea jecorina* (anamorph *Trichoderma reesei*) and a new sympatric agamospecies related to it. PLoS One 5, e9191

Freitag M, Williams RL, Kothe GO, Selker EU (2002) A cytosine methyltransferase homologue is essential for repeat-induced point mutation in *Neurospora crassa*. Proc Natl Acad Sci USA 99:8802–8807

Frisvad JC, Petersen LM, Lyhne EK, Larsen TO (2014) Formation of sclerotia and production of indoloterpenes by *Aspergillus niger* and other species in section Nigri. PLoS One 9, e94857

Fuller KK, Loros JJ, Dunlap JC (2014) Fungal photobiology: visible light as a signal for stress, space and time. Curr Genet 61(3):275–288

Galagan JE, Selker EU (2004) RIP: the evolutionary cost of genome defense. Trends Genet 20:417–423

Geiser DM, Arnold ML, Timberlake WE (1996) Wild chromosomal variants in *Aspergillus nidulans*. Curr Genet 29:293–300

Glass NL, Schmoll M, Cate JH, Coradetti S (2013) Plant cell wall deconstruction by ascomycete fungi. Annu Rev Microbiol 67:477–498

Goncalves-Sa J, Murray A (2011) Asymmetry in sexual pheromones is not required for ascomycete mating. Curr Biol 21:1337–1346

Gruber S, Omann M, Zeilinger S (2013) Comparative analysis of the repertoire of G protein-coupled receptors of three species of the fungal genus *Trichoderma*. BMC Microbiol 13:108

Hakkinen M, Valkonen MJ, Westerholm-Parvinen A, Aro N, Arvas M, Vitikainen M, Penttila M, Saloheimo M, Pakula TM (2014) Screening of candidate regulators for cellulase and hemicellulase production in *Trichoderma reesei* and identification of a factor essential for cellulase production. Biotechnol Biofuels 7:14

Heitman J, Sun S, James TY (2013) Evolution of fungal sexual reproduction. Mycologia 105:1–27

Hornok L, Waalwijk C, Leslie JF (2007) Genetic factors affecting sexual reproduction in toxigenic *Fusarium* species. Int J Food Microbiol 119:54–58

Idnurm A, Verma S, Corrochano LM (2010) A glimpse into the basis of vision in the kingdom Mycota. Fungal Genet Biol 47:881–892

Ivey FD, Kays AM, Borkovich KA (2002) Shared and independent roles for a Galpha(i) protein and adenylyl cyclase in regulating development and stress responses in *Neurospora crassa*. Eukaryot Cell 1:634–642

Jaklitsch WM (2009) European species of *Hypocrea* Part I. The green-spored species. Stud Mycol 63:1–91

Jaklitsch WM (2011) European species of *Hypocrea* part II: species with hyaline ascospores. Fungal Divers 48:1–250

Jones SK Jr, Bennett RJ (2011) Fungal mating pheromones: choreographing the dating game. Fungal Genet Biol 48:668–676

Kamerewerd J, Jansson M, Nowrousian M, Pöggeler S, Kück U (2008) Three alpha-subunits of heterotrimeric G proteins and an adenylyl cyclase have distinct roles in fruiting body development in the homothallic fungus *Sordaria macrospora*. Genetics 180:191–206

Karimi Aghcheh R, Nemeth Z, Atanasova L, Fekete E, Paholcsek M, Sandor E, Aquino B, Druzhinina IS, Karaffa L, Kubicek CP (2014) The VELVET A orthologue VEL1 of *Trichoderma reesei* regulates fungal development and is essential for cellulase gene expression. PLoS One 9, e112799

Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52:1781–1798

Kim H, Borkovich KA (2006) Pheromones are essential for male fertility and sufficient to direct chemotropic polarized growth of trichogynes during mating in *Neurospora crassa*. Eukaryot Cell 5:544–554

Kim MJ, Choi JW, Park SM, Cha BJ, Yang MS, Kim DH (2002) Characterization of a fungal protein kinase from *Cryphonectria parasitica* and its transcriptional upregulation by hypovirus. Mol Microbiol 45:933–941

Kim H, Wright SJ, Park G, Ouyang S, Krystofova S, Borkovich KA (2012) Roles for receptors, pheromones, G proteins, and mating type genes during sexual reproduction in *Neurospora crassa*. Genetics 190:1389–1404

Klein D, Eveleigh DE (1998) Ecology of trichoderma. In: Kubicek CP, Harman GE (eds) Trichoderma and gliocladium, vol 1. Taylor & Francis, London, pp 57–74

Klittich C, Leslie JF (1988) Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118:417–423

Kubicek CP, Mikus M, Schuster A, Schmoll M, Seiboth B (2009) Metabolic engineering strategies for improvement of cellulase production by *Hypocrea jecorina*. Biotechnol Biofuels 2:19

Kuhls K, Lieckfeldt E, Samuels GJ, Kovacs W, Meyer W, Petrini O, Gams W, Borner T, Kubicek CP (1996) Molecular evidence that the asexual industrial fungus *Trichoderma reesei* is a clonal derivative of the ascomycete *Hypocrea jecorina*. Proc Natl Acad Sci USA 93:7755–7760

Leeder AC, Palma-Guerrero J, Glass NL (2011) The social network: deciphering fungal language. Nat Rev Microbiol 9:440–451

Leslie JF, Klein KK (1996) Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics 144:557–567

Li L, Wright SJ, Krystofova S, Park G, Borkovich KA (2007) Heterotrimeric G protein signaling in filamentous fungi. Annu Rev Microbiol 61:423–452

Lieckfeldt E, Kullnig CM, Samuels GJ, Kubicek CP (2000) Sexually competent, sucrose- and nitrate-assimilating strains of *Hypocrea jecorina* (*Trichoderma reesei*) from South American soils. Mycologia 92:374–380

Lobuglio KF, Pitt JI, Taylor JW (1993) Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of a sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*. Mycologia 85:592–604

Mach RL, Schindler M, Kubicek CP (1994) Transformation of *Trichoderma reesei* based on hygromycin B resistance using homologous expression signals. Curr Genet 25:567–570

Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EG, Grigoriev IV, Harris P, Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, de Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmoll M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barbote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman N, Ward M, Brettin TS (2008) Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nat Biotechnol 26:553–560

Martinez D, Challacombe J, Morgenstern I, Hibbett D, Schmoll M, Kubicek CP, Ferreira P, Ruiz-Duenas FJ, Martinez AT, Kersten P, Hammel KE, Vanden Wymelenberg A, Gaskell J, Lindquist E, Sabat G, Bondurant SS, Larrondo LF, Canessa P, Vicuna R, Yadav J, Doddapaneni H, Subramanian V, Pisabarro AG, Lavin JL, Oguiza JA, Master E, Henrissat B, Coutinho PM, Harris P, Magnuson JK, Baker SE, Bruno K, Kenealy W, Hoegger PJ, Kues U, Ramaiya P, Lucas S, Salamov A, Shapiro H, Tu H, Chee CL, Misra M, Xie G, Teter S, Yaver D, James T, Mokrejs M, Pospisek M, Grigoriev IV, Brettin T, Rokhsar D, Berka R, Cullen D (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. Proc Natl Acad Sci USA 106:1954–1959

Mayrhofer S, Weber JM, Poggeler S (2006) Pheromones and pheromone receptors are required for proper sexual development in the homothallic ascomycete *Sordaria macrospora*. Genetics 172:1521–1533

Metzenberg RL, Glass NL (1990) Mating type and mating strategies in *Neurospora*. Bioessays 12:53–59

Moore-Landecker E (1992) Physiology and biochemistry of ascocarp induction and development. Mycol Res 96:705–716

Mukherjee PK, Horwitz BA, Herrera-Estrella A, Schmoll M, Kenerley C (2013) *Trichoderma* research in the genome era. Annu Rev Phytopathol 51:105–129

Ni M, Feretzaki M, Sun S, Wang X, Heitman J (2011) Sex in fungi. Annu Rev Genet 45:405–430

Nieuwenhuis BP, Aanen DK (2012) Sexual selection in fungi. J Evol Biol 25:2397–2411

Noor MA, Grams KL, Bertucci LA, Reiland J (2001) Chromosomal inversions and the reproductive isolation of species. Proc Natl Acad Sci USA 98:12084–12088

Perkins DD, Turner BC, Barry EG (1976) Strains of *Neurospora* collected from nature. Evolution 30:281–313

Persoon CH (1794) Disposita methodica fungorum. Römer′s Neues Mag Bot 1:81–128

Pöggeler S, Nowrousian M, Kück U (2006) Fruiting body development in ascomycetes. In: Kües U, Fischer R (eds) The mycota I, Fungal growth, differentiation and sexuality, vol I, 2nd edn. Springer, Heidelberg, New York, pp 325–355

Reese ET (1976). History of the cellulase program at the US Army Natick Development Center. Biotechnol Bioeng Symp (6)**:**9–20

Reese ET, Levinson HS, Downing M, White WL (1950) Quartermaster culture collection. Farlowia 4:45–86

Rodriguez-Romero J, Hedtke M, Kastner C, Muller S, Fischer R (2010) Fungi, hidden in soil or up in the air: light makes a difference. Annu Rev Microbiol 64:585–610

Ropars J, Lopez-Villavicencio M, Dupont J, Snirc A, Gillot G, Coton M, Jany JL, Coton E, Giraud T (2014) Induction of sexual reproduction and genetic diversity in the cheese fungus *Penicillium roqueforti*. Evol Appl 7:433–441

Samuels GJ (2006) *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96:195–206

Schmoll M (2008) The information highways of a biotechnological workhorse – signal transduction in *Hypocrea jecorina*. BMC Genomics 9:430

Schmoll M (2013) Sexual development in *trichoderma* – scrutinizing the aspired phenomenon. In: Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M (eds) Trichoderma: biology and applications. CAB International, Oxfordshire, pp. 67–86

Schmoll M, Zeilinger S, Mach RL, Kubicek CP (2004) Cloning of genes expressed early during cellulase induction in *Hypocrea jecorina* by a rapid subtraction hybridization approach. Fungal Genet Biol 41:877–887

Schmoll M, Franchi L, Kubicek CP (2005) Envoy, a PAS/LOV domain protein of *Hypocrea jecorina* (anamorph *Trichoderma reesei*), modulates cellulase gene transcription in response to light. Eukaryot Cell 4:1998–2007

Schmoll M, Schuster A, Silva Rdo N, Kubicek CP (2009) The G-alpha protein GNA3 of *Hypocrea jecorina* (anamorph *Trichoderma reesei*) regulates cellulase gene expression in the presence of light. Eukaryot Cell 8:410–420

Schmoll M, Esquivel-Naranjo EU, Herrera-Estrella A (2010a) *Trichoderma* in the light of day – physiology and development. Fungal Genet Biol 47:909–916

Schmoll M, Seibel C, Tisch D, Dorrer M, Kubicek CP (2010b) A novel class of peptide pheromone precursors in ascomycetous fungi. Mol Microbiol 77:1483–1501

Schmoll M, Seiboth B, Druzhinina I, Kubicek CP (2014). Genomics analysis of biocontrol species and industrial enzyme producers from the genus *Trichoderma*. In: Nowrousian M (ed) Fungal genomics, vol XIII, 10. Elsevier, Amsterdam, pp. 233–264

Schuster A, Schmoll M (2010) Biology and biotechnology of *trichoderma*. Appl Microbiol Biotechnol 87:787–799

Schuster A, Tisch D, Seidl-Seiboth V, Kubicek CP, Schmoll M (2012a) Roles of protein kinase A and adenylate cyclase in light-modulated cellulase regulation in *Trichoderma reesei*. Appl Environ Microbiol 78:2168–2178

Schuster A, Bruno KS, Collett JR, Baker SE, Seiboth B, Kubicek CP, Schmoll M (2012b) A versatile toolkit for high throughput functional genomics with *Trichoderma reesei*. Biotechnol Biofuels 5:1

Seibel C, Gremel G, Silva RD, Schuster A, Kubicek CP, Schmoll M (2009) Light-dependent roles of the G-protein subunit GNA1 of *Hypocrea jecorina* (anamorph *Trichoderma reesei*). BMC Biol 7:58

Seibel C, Tisch D, Kubicek CP, Schmoll M (2012a) The role of pheromone receptors for communication and mating in *Hypocrea jecorina* (*Trichoderma reesei*). Fungal Genet Biol 49:814–824

Seibel C, Tisch D, Kubicek CP, Schmoll M (2012b) ENVOY is a major determinant in regulation of

sexual development in *Hypocrea jecorina* (*Trichoderma reesei*). Eukaryot Cell 11:885–895

Seiboth B, Ivanova C, Seidl-Seiboth V (2011) *Trichoderma reesei*: a fungal enzyme producer for cellulosic biofuels. In: dos Santos Bernardes MA (ed) Biofuel production – recent developments and prospects. Intech, Rijeka, Croatia, pp 309–340

Seidl V, Seibel C, Kubicek CP, Schmoll M (2009) Sexual development in the industrial workhorse *Trichoderma reesei*. Proc Natl Acad Sci USA 106:13909–13914

Seo JA, Yu JH (2006) The phosducin-like protein PhnA is required for Gbetagamma-mediated signaling for vegetative growth, developmental control, and toxin biosynthesis in *Aspergillus nidulans*. Eukaryot Cell 5:400–410

Shen WC, Bobrowicz P, Ebbole DJ (1999) Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet Biol 27:253–263

Simmons EG (1977) Classification of some cellulase producing *Trichoderma* species. In: Bigelow HE, Simmons E (eds) Second international mycological congress. University of South Florida, Tampa, p 618

Singh A, Chen EY, Lugovoy JM, Chang CN, Hitzeman RA, Seeburg PH (1983) *Saccharomyces cerevisiae* contains two discrete genes coding for the alpha-factor pheromone. Nucleic Acids Res 11:4049–4063

Steyaert JM, Weld RJ, Mendoza-Mendoza A, Stewart A (2010) Reproduction without sex: conidiation in the filamentous fungus *trichoderma*. Microbiology 156:2887–2900

Sun Q, Choi GH, Nuss DL (2009) Hypovirus-responsive transcription factor gene *pro1* of the chestnut blight fungus *Cryphonectria parasitica* is required for female fertility, asexual spore development, and stable maintenance of hypovirus infection. Eukaryot Cell 8:262–270

Taylor J, Jacobson D, Fisher M (1999) The evolution of asexual fungi: reproduction, speciation and classification. Annu Rev Phytopathol 37:197–246

Tisch D, Kubicek CP, Schmoll M (2011a) The phosducin-like protein PhLP1 impacts regulation of glycoside hydrolases and light response in *Trichoderma reesei*. BMC Genomics 12:613

Tisch D, Kubicek CP, Schmoll M (2011b) New insights into the mechanism of light modulated signaling by heterotrimeric G-proteins: ENVOY acts on *gna1* and *gna3* and adjusts cAMP levels in *Trichoderma reesei* (*Hypocrea jecorina*). Fungal Genet Biol 48:631–640

Tisch D, Schuster A, Schmoll M (2014) Crossroads between light response and nutrient signaling: ENV1 and PhLP1 act as mutual regulatory pair in *Trichoderma reesei*. BMC Genomics 15:425

Tulasne LR, Tulasne C (1865) Selecta fungorum carpologia. Jussu, Paris

White MJD (1978) Modes of speciation, a series of books in biology. W.H. Freeman, San Francisco, p 455

Willardson BM, Howlett AC (2007) Function of phosducin-like proteins in G protein signaling and chaperone-assisted protein folding. Cell Signal 19:2417–2427

Xu JR, Leslie JF (1996) A genetic map of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*). Genetics 143:175–189

Zanders SE, Eickbush MT, Yu JS, Kang JW, Fowler KR, Smith GR, Malik HS (2014) Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. Elife 3, e02630

Zhang L, Baasiri RA, Van Alfen NK (1998) Viral repression of fungal pheromone precursor gene expression. Mol Cell Biol 18:953–959

18 Velvet Regulation of Fungal Development

Ana M. Calvo[1], Jessica M. Lohmar[1], Beatriz Ibarra[1], Timothy Satterlee[1]

CONTENTS

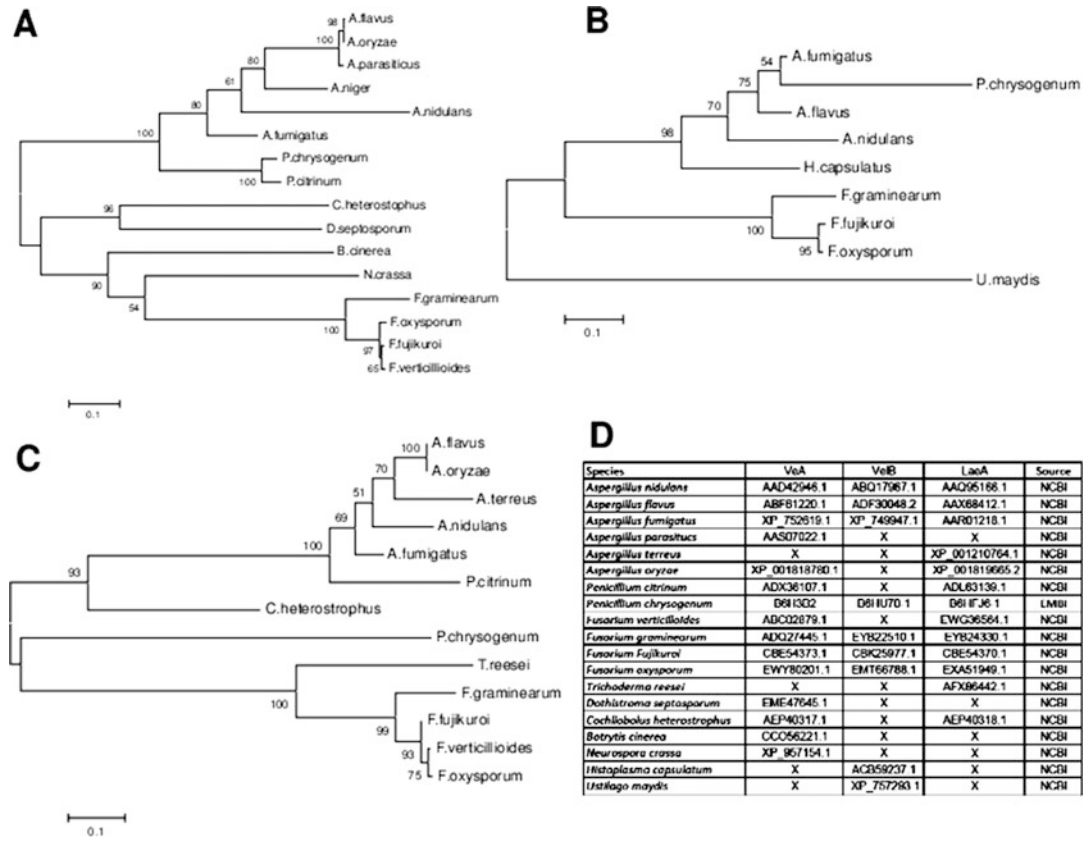
[1]Department of Biological Sciences, Northern Illinois University, 155 Castle Drive, Dekalb, IL, 60115, USA; e-mail: amcalvo@niu.edu

I. Introduction

Morphogenesis studies of the model fungus *A. nidulans* have greatly facilitated our understanding of this sophisticated developmental process. Genomics, together with classical genetics and the state-of-the-art molecular biology techniques of this model system, has accelerated discovery of the intricate regulatory genetic network governing morphology in fungi. The dissemination mechanism of *A. nidulans* is common in many other fungal species and relies on the production of asexual spores called conidia (or conidiospores) on specialized structures called conidiophores. In addition, this fungus also presents a sexual stage, forming fruiting bodies denominated cleistothecia that contain meiospores called ascospores. Other species, such as the aflatoxin (AF) producers *Aspergillus flavus* and *Aspergillus parasiticus*, produce vestiges of cleistothecia (in most cases barren of spores) that act as resistant structures, which is important for the survival of these fungi under environmental extremes (Coley-Smith and Cooke 1971; Malloch and Cain 1972; Wicklow 1987).

It is known that both the development of asexual and sexual structures is under a tight temporal and spatial genetic regulation (Calvo et al. 2002; Adams and Yu 1998; Fischer and Kues 2006). A major component of this network is the *velvet* regulatory system. The first steps in the investigation of this regulatory system, unique to fungi, began with the discovery of the "*velvet*" gene, or *veA* by Käfer, who generated a mutant allele called *veA1* (Kafer 1965). *A. nidulans* strains with a *veA* wild-type

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)



Species	VeA	VelB	LaeA	Source
Aspergillus nidulans	AAD42946.1	ABQ17967.1	AAQ95166.1	NCBI
Aspergillus flavus	ABF81220.1	ADF30048.2	AAX68412.1	NCBI
Aspergillus fumigatus	XP_752619.1	XP_749947.1	AAR01218.1	NCBI
Aspergillus parasiticus	AAS07022.1	X	X	NCBI
Aspergillus terreus	X	X	XP_001210764.1	NCBI
Aspergillus oryzae	XP_001818780.1	X	XP_001819665.2	NCBI
Penicillium citrinum	ADX36107.1	X	ADL63139.1	NCBI
Penicillium chrysogenum	D6IB02	D6IIU70.1	D6IIFJ6.1	EMBI
Fusarium verticilloides	ADC02879.1	X	EWG36564.1	NCBI
Fusarium graminearum	ADQ27445.1	EYB22510.1	EYB24330.1	NCBI
Fusarium Fujikuroi	CBE54373.1	CBK25977.1	CBE54370.1	NCBI
Fusarium oxysporum	EWY80201.1	EMT66788.1	EXA51949.1	NCBI
Trichoderma reesei	X	X	AFX86442.1	NCBI
Dothistroma septosporum	EME47645.1	X	X	NCBI
Cochliobolus heterostrophus	AEP40317.1	X	AEP40318.1	NCBI
Botrytis cinerea	CCO56221.1	X	X	NCBI
Neurospora crassa	XP_957154.1	X	X	NCBI
Histoplasma capsulatum	X	ACB59237.1	X	NCBI
Ustilago maydis	X	XP_757793.1	X	NCBI

Fig. 18.1 Phylogenetic analysis of several experimentally characterized proteins of the Velvet regulatory system. Analysis of VeA (**a**), VelB (**b**), and LaeA (**c**). (**d**) Species and accession numbers corresponding to the amino acid sequences used in this analysis and their source (NCBI or EMBI). The "X" represents that the gene has not been experimentally characterized. Images were generated using MEGA6 (http://megasoftware.net/) (Tamura et al. 2013). Sequences were aligned using MUSCLE and visualized in a maximum likelihood model using a bootstrap value of 1000

allele develop asexually when the colonies are exposed to light, while in the dark the fungus develops fruiting bodies (Champe et al. 1981; Yager 1992). However, the *veA1* strains develop mainly asexually and present a reduced and delayed sexual stage independent of illumination. The *veA* mechanism of action remained unknown for many years due to the fact that the VeA protein did not show homology with other proteins of known function. Additional genetic studies, including generation of deletion mutants, as well as protein–protein interaction studies and genomics have provided abundant and valuable information on the mode of action of the *velvet* regulatory system and its functional roles.

The sequencing of numerous fungal genomes has also facilitated the rapid identification and characterization of putative *veA* homologs and *veA*-related regulators in other fungal species (Myung et al. 2012) (Fig. 18.1). Efforts from our group and others have contributed to the functional characterization of several of these *veA* homologs and regulators (Baidya et al. 2014; Calvo et al. 2004; Dhingra et al. 2012; Dreyer et al. 2007; Duran et al. 2007; Li et al. 2006; Zhang et al. 2004). Overall these studies indicate that the function of the *velvet* regulatory system is similar in different fungi. However some of these studies also denote a certain degree of specialization leading to a different regulatory output depending on species.

II. The *Velvet* System in the Model Filamentous Fungus *A. nidulans*

In 1965, after performing a random mutagenesis, Käfer obtained the first *veA* mutant in *A. nidulans*, denominated *veA1* (Kafer 1965). The *veA1* mutant allele presents a change in the initiation codon (G to T at position +3). The next possible initiation codon corresponds to the 37th codon in this mutant allele. This results in a truncated gene product with partial function that lacks the first 36 amino acids. In strains with a *veA1* allele, conidiation is promoted, while cleistothecial formation is delayed and reduced (Yager 1992). Morphogenesis in *veA1* strains is independent of light, while illumination clearly determines the developmental stage of *A. nidulans* strains with a *veA* wild-type allele (Champe et al. 1981; Mooney and Yager 1990; Yager 1992). Due to this practical characteristic present in the *veA1* mutant, this genetic background has been and is still used by many *A. nidulans* research groups investigating subjects such as asexual development or certain aspects of fungal metabolism.

The absence of known functional domains in the VeA protein contributed to the delay in its characterization. The study of additional *veA* mutants, a deletion (Δ*veA*) and overexpression *veA* strains, further contributed to our understanding of this regulatory gene (Kim et al. 2002). The Δ*veA* strain was unable to produce cleistothecia, while conidiation was enhanced. The opposite was observed in the *veA* overexpression strain. These findings further supported *veA* as negative regulator of asexual development while positively regulating sexual development in *A. nidulans* (Champe et al. 1981; Yager 1992; Kim et al. 2002). Further studies demonstrated that *veA* affects the *brlA* α/β transcript ratio that modulates the initiation of asexual development in *A. nidulans* (Kato et al. 2003).

Another milestone in the elucidation of the function of *veA* was the discovery of its regulatory role as master regulator of secondary metabolism in *A. nidulans* (Kato et al. 2003). Additional studies further supported that its role as genetic link between development and secondary metabolism is conserved in many other fungi (i.e., Baidya et al. 2014; Lan et al. 2014). Transcriptome analysis in *A. nidulans* (Lind et al. 2015) and in other *Aspergillus*, *A. flavus* (Cary et al. 2007; Cary et al. 2015) and in *A. fumigatus* (Dhingra et al. 2013; Lind et al. 2015), revealed that *veA* regulates the expression of hundreds of genes. We also recently discovered additional roles of *veA* in hydrolytic activity and oxidative stress response (Duran et al. 2014). This review is mainly focused on *veA*-dependent developmental regulation; however, in another section, we will briefly describe other *veA*-associated cellular processes as a reflection of its broad regulatory scope in fungi.

The search for VeA-interacting proteins has provided valuable additional insight on the VeA mechanism of action (Fig. 18.2). VeA is transported to the nucleus by the alpha-importin KapA (Stinnett et al. 2007; Araujo-Bazan et al. 2009). This transport is particularly notable in the dark, while in the light this process is not efficient. Once in the nucleus, VeA is able to establish interactions with other proteins, such as FphA, a red phytochrome-like protein (Purschwitz et al. 2008). FphA acts as a bridge between VeA and two blue light-sensing proteins, LreA and LreB. Deletion of *fphA* results in a reduction of asexual development tipping the developmental balance to the formation of fruiting bodies even under light, while deletion of *lreA* or *lreB* genes results in opposite effects (Purschwitz et al. 2008). Importantly, FphA also has a negative effect on the transport of VeA to the nucleus in the light (Purschwitz et al. 2008). Other proteins, such as a putative methyltransferase called LlmF, also have a similar effect on VeA transport (Palmer et al. 2013). It is unknown whether FphA and LlmF act coordinately to antagonize VeA migration to the nucleus in the presence of light. Additionally, LlmF has been shown to negatively regulate sexual development (Palmer et al., 2013). VeA also interacts with another putative methyltransferase termed LaeA, as part of the denominated *velvet* complex (Bayram et al. 2008a). LaeA has been described to be involved in chromatin conformational changes regulating secondary metabolism (Bok et al. 2006a, b; Reyes-Dominguez et al. 2010; Keller et al. 2005). In *A. nidulans* LaeA is also

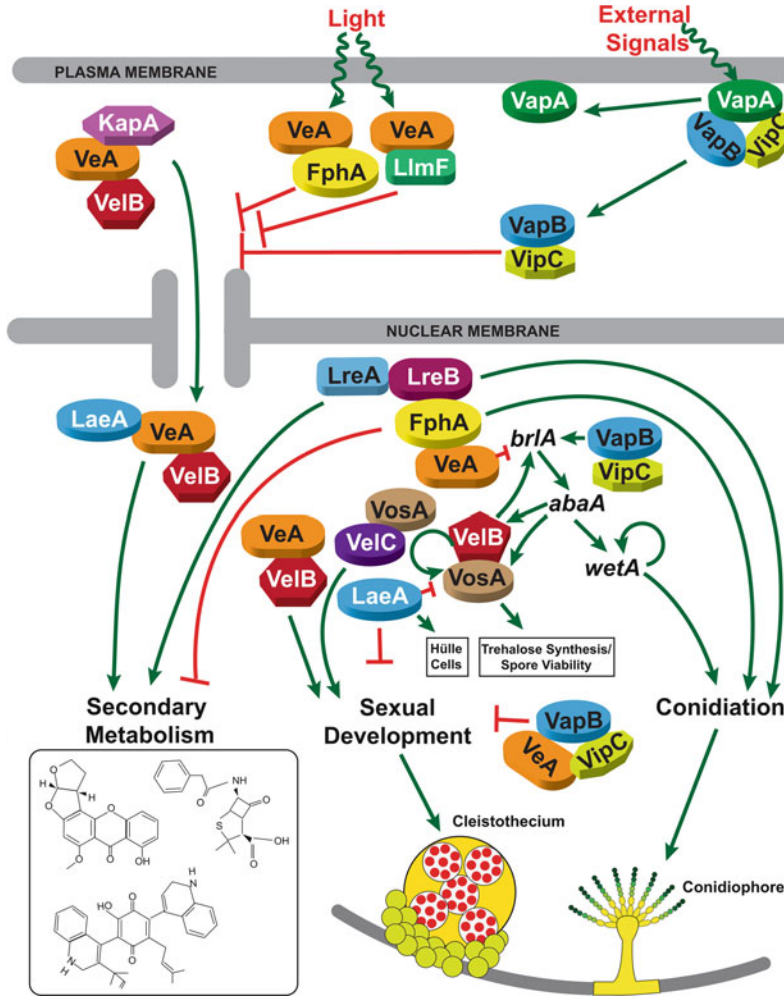


Fig. 18.2 Model representing interactions among *velvet* family proteins, LaeA and other proteins in the model fungus *Aspergillus nidulans*. The α-importin KapA transports the VeA-VelB dimer to the nucleus, particularly in the dark. This migration of KapA-VeA-VelB is negatively affected by other proteins, including FphA, LlmF, and VipC-VapB dimer under light. Once in the nucleus, VeA-VelB promotes sexual development and is able to interact with LaeA, forming the denominated *velvet* complex. In addition, VeA also interacts with FphA, which interacts with LreB-LreA forming a light-responsive protein complex. VelB affects asexual development and forms homodimers and heterodimers with VosA, which is necessary for trehalose biosynthesis and spore viability. VosA also interacts with VelC, which positively influences sexual development. VipC and VapB also positively regulate conidiation and are found associated with VeA in the nucleus-repressing sexual development. These protein complexes govern development and secondary metabolism in a coordinated manner

involved in the inhibition of sexual development in response to light (Sarikaya-Bayram et al. 2010). VelB is another VeA-interacting protein and component of the *velvet* complex (Bayram et al. 2008a; Park et al. 2012a). VelB, together with VeA, VosA, and VelC (Ni and Yu 2007; Park et al. 2014), forms the denominated *velvet* protein family, where VelB interacts with VeA in the cytoplasm and they are carried together to the nucleus (Bayram et al. 2008a). Deletion of *velB* results in a strain with a light-independent conidiation pattern and lack of sexual stage (Bayram et al. 2008a). VelB also forms dimers and heterodimers with VosA (Bayram et al. 2010). Park et al.

(2012a) demonstrated that VosA is required for normal trehalose production, affecting spore viability. Interestingly, these researchers also showed that the known developmental regulator AbaA positively affects the expression of *velB* and *vosA* directly binding to their promoters, while VelB positively affects the central regulatory pathway (Park et al. 2012a) composed of *brlA*, *abaA*, and *wetA* (Adams et al. 1998). Furthermore, the *velvet* domain in VelB and VosA has been shown to bind DNA in *A. nidulans* (Ahmed et al. 2013) as well as in *Histoplasma capsulatum* (Beyhan et al. 2013). The VelC *velvet* protein also contributes to regulate development in *A. nidulans*, positively regulating sexual development (Park et al. 2014).

Other recently characterized VeA-interacting proteins include VipC-VapB. These are methyltransferases released from the VapA-VipC-VapB membrane-bound complex in response to environmental signals (Sarikaya-Bayram et al. 2014). Released VipC-VapB reduces VeA-VelB nuclear import, resulting in decreased sexual development. At the same time, VapB also counteracts histone 3 lysine 9 trimethylation (H3K9me3), promoting conidiation.

VeA is posttranslationally modified. For example, a LaeA-dependent VeA modification has been reported in *A. nidulans* (Sarikaya-Bayram et al. 2010). It is predictable that these modifications could have an effect on the *velvet* complex function. Phosphorylation of VeA was demonstrated by Purschwitz et al. (2009). Later Bayram et al. (2012) showed that MpkB is involved in this phosphorylation. The MAP kinase gene *mpkB*, homolog of *FUS3* in *S. cerevisiae*, was first characterized in *A. nidulans* by Paoletti et al. (2007) and by Atoui et al. (2008). *mpkB* transcription increased during sexual development, and deletion of the *mpkB* gene resulted in sterility (Paoletti et al. 2007). MpkB is also a genetic link between development and biosynthesis of natural products; deletion of *mpkB* also results in decreased *laeA* transcription and in a reduction in the expression of several secondary metabolite gene clusters (Atoui et al. 2008).

As mentioned above, VeA influences the expression of many genes, among them are the oxylipin genes, or psi factor genes, that affect the developmental balance between asexual and sexual development (Tsitsigiannis et al. 2004). For example, *veA* regulates *ppoA* expression in *A. nidulans* (Tsitsigiannis et al. 2004). Interestingly, psi factor genes also modulate *veA* expression; the triple mutant *ppo*A/B/C presented an increase in the expression of this regulatory gene (Tsitsigiannis et al. 2005), suggesting a feedback loop regulation between *veA* and oxylipin genes. This regulation could be affected by other oxylipins of cross-kingdom origin, mimicking or interfering with fungal psi factors.

III. The Role of VeA in the Morphology of Other *Aspergillus* spp.

A. *Aspergillus flavus*

Aspergillus flavus is a saprophytic fungus and an important opportunistic plant pathogen which infects oilseed crops worldwide contaminating them with potent mycotoxins such as AFs. As in the case of *A. nidulans*, *A. flavus* also disseminates by producing conidiospores that lead to field infestations. Additionally, this fungus produces sclerotia that can be dormant for long periods and allow for survival under adverse conditions (Coley-Smith and Cooke 1971; Malloch and Cain 1972; Wicklow 1987). When the environment is favorable again, sclerotia produce hyphae or conidiophores directly contributing to dissemination. Genetic and genomic evidence strongly supports the origin of these resistant structures as vestigial forms of cleistothecia (Ramirez-Prado et al. 2008). In most cases these resting bodies are unable to produce meiospores. However, Horn et al. (2014) described ascospore-bearing ascocarps embedded within sclerotia of *A. flavus*. The fact that cleistothecia and sclerotia might possess a common origin suggests that the genetic regulatory mechanism controlling the formation of cleistothecia might also control the formation of sclerotia. The VeA regulatory system is one of these conserved mechanisms. The role of VeA was first characterized in *A. flavus* by Duran et al. (2007). The absence of *veA* resulted in an increase in conidiogenesis and the inability to produce sclerotia (Duran

et al. 2007). The requirement of *veA* for production of sclerotia was also demonstrated in another phylogenetically close AF-producer species, *A. parasiticus* (Calvo et al. 2004). In both species *veA* was also shown to regulate the synthesis of AF and other secondary metabolites (Calvo et al. 2004; Duran et al. 2007).

Other elements of the VeA regulatory mechanism are also conserved in *A. flavus*. The LaeA homolog is necessary for normal production of conidia and for the production of sclerotia, as well as for normal secondary metabolism (Amaike and Keller 2009; Kale et al. 2008). Further description of the role of *laeA* on conidiation was provided by Chang et al. (2012a). The *laeA* deletion mutants showed a reduction in conidial chain elongation, increased production of conidiophores, and decreased colony hydrophobicity compared to the wild type (Chang et al. 2012a). At transcriptional level, *laeA* has been shown to negatively regulate *veA* in *A. flavus*, possibly contributing to maintain the stoichiometry of the subunits in these multiprotein complexes necessary to properly perform their biological functions. Chang et al. (2013) also reported interactions among the *velvet* proteins in *A. flavus* (Chang et al. 2013). VeA interacts with both LaeA and VelB, but LaeA does not interact with VelB. Interactions between LaeA and VelB with FluG (Chang et al. 2012b, 2013), a developmental regulatory protein previously described in *A. nidulans* (Lee and Adams 1994), were also reported. In this model organism, FluG is involved in the inactivation of the FadA heterotrimeric G-protein signaling pathway, resulting in inhibition of vegetative growth and promotion of sexual and asexual development, as well as production of the mycotoxin sterigmatocystin (ST) (Hicks et al. 1997). FadA function was also found to be conserved in *A. parasiticus* (Hicks et al. 1997) and most likely in *A. flavus*. Initial evidence supporting a connection between *fluG* and *veA* was previously shown in *A. nidulans* by Yager and collaborators (Yager et al. 1998; Mooney and Yager 1990). Mooney et al. (1990) described extragenic *veA1* suppressor mutations that restored the light-dependent conidiation that corresponded to *fluG* alleles, suggesting a link between *veA* and *fluG* functions. In *A. flavus*, FluG has antagonistic role with respect to VeA, LaeA, and VelB influencing sclerotial formation (Chang et al. 2012b). While deletion of *velB* or *veA* prevents sclerotial formation, deletion of *fluG* results in overproduction of these structures. In addition, a notable decrease in condiation and inability to produce AF was observed in deletion *velB* strains. Chang et al. (2013) postulated that a balance among the interacting proteins VeA, VelB, FluG, and LaeA is needed to maintain conidiation and sclerotial formation as well as secondary metabolism (Chang et al. 2013).

The nutritional environment influences the *veA*-dependent regulation of *A. flavus* conidiation (Duran et al. 2007, 2009). While the deletion *veA* strain presented greater levels of conidiation than the wild type when grown on laboratory media, a decrease was observed when the mutant was grown on oilseeds, accompanied by a reduction in colonization on the host plant substrate. This reduction in conidiation was also observed in deletion *veA* strains of *A. parasiticus* (Calvo et al. 2004). In both fungi, *veA* is required for the production of sclerotia and AF when colonizing seeds.

B. *Aspergillus fumigatus*

Aspergillus fumigatus is an opportunistic human fungal pathogen and the leading cause of invasive aspergillosis, with a mortality rate ranging between 40 and 90 % among affected immunodepressed patients (Dhingra et al. 2012). The role of *veA* and other *velvet* genes in *A. fumigatus* is of interest due to their potential effect on production of inoculum and virulence factors that affect fungal infection. Several groups investigating the role of *veA* in conidiation reported apparently contradictory results. Krappmann et al. (2005) described a reduction of conidiation on nitrate-containing medium in the *veA* deletion mutant (Krappmann et al. 2005). Studies by Dhingra et al. (2012) showed a reduction in conidiation accompanied by a decrease in the expression of *brlA* in this pathogen (Dhingra et al. 2012). However, Park et al. (2012a) reported a notable increase in conidiophore development in the absence of *veA*, even when grown in liquid submerged cultures, suggesting that *veA* acts as a negative regulator of conidiation in *A. fumigatus*.

Differences in media and culture conditions utilized in these studies could account for the observed differences.

Transcriptome analysis also further contributed to the elucidation of the broad role of the *veA* regulator in *A. fumigatus* (Dhingra et al. 2013; Lind et al. 2015), establishing that the expression of hundreds of genes is influenced by *veA* in this organism. In addition, a similar VeA protein interaction pattern to that described in *A. nidulans* was also described in *A. fumigatus*. Using tandem affinity purification, Park et al. (2012b) showed interaction of VelB and LaeA with the VeA protein. Other *velvet* genes were characterized in *A. fumigatus*. *velB* was shown to be a negative regulator of conidiation, together with *vosA* (Park et al. 2012b). The *A. fumigatus abaA* also activates *vosA* and *velB* expression during the mid to late phase of conidiation. Furthermore, *vosA* and *velB* are also required for proper spore viability and tolerance to environmental stresses. In contrast with the clear effect of other *velvet* genes on development in *A. fumigatus*, no noticeable phenotype associated with the deletion of *velC* was observed in this fungus (Park et al. 2012b).

laeA was also characterized in *A. fumigatus* (Bok and Keller 2004), where it was reported to regulate secondary metabolism. Other roles have also been described for this regulator, among them are its effects on spore surface and spore germination, relevant features contributing to the *Aspergillus* infection process (Bok et al. 2005; Dagenais et al. 2010). Deletion of *laeA* results in decreased virulence (Bok et al. 2005). Further studies indicate a reduction in RodAp levels in Δ*laeA* spores (Dagenais et al. 2010). Bok et al. (2005) also reported that *laeA* regulates *alb1*, a known virulence factor in *A. fumigatus* responsible for the formation of conidial pigment. According to these authors, deletion of *laeA* causes a delay in *alb1* expression. However, Sugui et al. (2007) did not find a *laeA*-dependent regulation of *alb1* expression in relation to conidiogenesis, but a decrease in *alb1* transcription levels in mycelium. They also did not find changes in conidial morphology, speculating that the differences observed in these two studies could be medium dependent (Sugui et al. 2007). Perrin et al. (2007) analyzed the transcriptome of *laeA* in *A. fumigatus*. This microarray analysis indicated that numerous genes are under the control of *laeA*; many of them are genes involved in secondary metabolism.

C. *Aspergillus carbonarius*

Aspergillus carbonarius is a plant pathogen of grapes, raisins, coffee, cocoa, peanuts, and maize (i.e., Battilani et al. 2006; Joosten et al. 2001). *A. carbonarius* is a main producer of the mycotoxin ochratoxin A (Creppy 1999; Kuiper-Goodman and Scott 1989). Deletion of *veA* resulted in marked radial grooves, as well as a decrease in radial growth rate when compared to wild type. Deletion of *laeA* results in similar radial grooves; however, under light conditions, *laeA* mutants exhibited a slightly faster growth rate than wild-type strains. Additionally, both deletion mutants presented a decrease in conidiation independently of light (Crespo-Sempere et al. 2013). Besides their role in development, *laeA* and *veA* are also involved in the activation of ochratoxin A genes (Crespo-Sempere et al. 2013).

IV. *Velvet* in *Fusarium* spp.

A. *Fusarium verticillioides*

The study of the *veA* homolog *FvVE1* in *F. verticillioides* was the first characterization of a *velvet* gene in a fungal species outside *Aspergillus* (Li et al. 2006) (Fig. 18.3). This mycotoxigenic fungus is the most common cause of corn ear rot worldwide. The absence of *FvVE1* results in a suppression of aerial hyphal growth and reduced colony surface hydrophobicity, alterations in hyphal polarity and branching, hyperconidiation, and budding yeast-like growth in submerged cultures. Deletion of *FvVE1* also increases the ratio of macroconidia to microconidia, reduces sexual development, and results in changes in cell wall integrity, particularly related to mannoprotein content (Li et al. 2006). Furthermore, Myung et al. showed that *FvVE1* controls secondary metab-

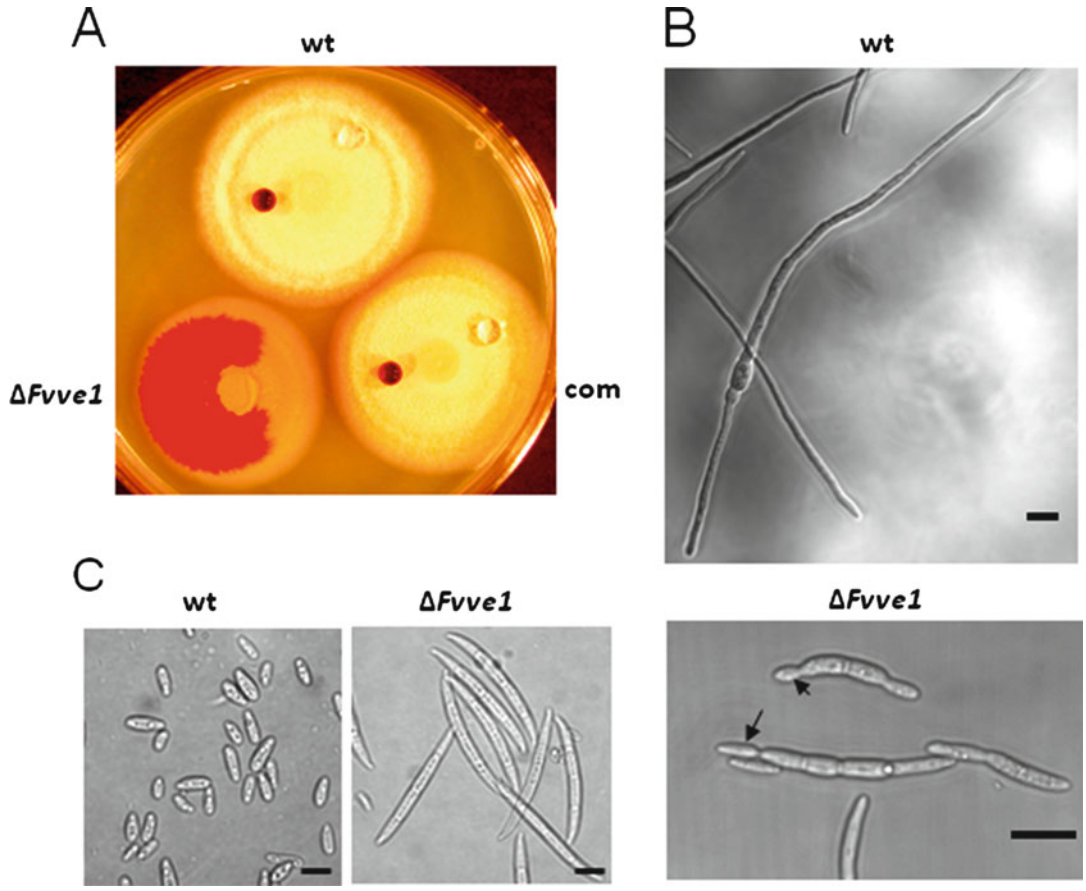


Fig. 18.3 Phenotype of the Δ*Fvve1* mutant in *Fusarium verticillioides*. (**a**) Colonies of wild type (wt), Δ*Fvve1* and complementation strain (com). Water or 1% acid fuchsin droplets (*red spots*) were loaded on colony surfaces. (**b**) Micrographs of conidia produced by wt and Δ*Fvve1* mutant strain. Bar, 10 μm. (**c**) Images obtained from submerged cultures of wt and Δ*Fvve1*. Conidia produced under these conditions are indicated with *arrows*. Bar, 30 μm (Li et al. 2006)

olism (Myung et al. 2009) and is necessary for pathogenicity (Myung et al. 2012) in *F. verticillioides*. Additionally, pull-down studies showed FvVE1 interactions with other *velvet* proteins, FvVelB and FvVelC (Lan et al. 2014). Deletion of *FvVelB* presents a similar phenotype to that described by Li et al. (2006) for *FvVE1* mutants, with changes in hydrophobicity and increased the ratio of macroconidia to microconidia. Deletion of *FvVelC*, however, did not show a phenotype. The *laeA* homolog, *lae1*, has also been found in *F. verticillioides* (Butchko et al. 2012). Studies of the *lae1* transcriptome confirmed its role regulating secondary metabolism. However, a developmental role has not been associated with *lae1* in this fungus (Butchko et al. 2012).

B. *Fusarium oxysporum*

Through the use of yeast two-hybrid systems, a preliminary concept of the *velvet* complex structure in *F. oxysporum* was proposed (Lopez-Berges et al. 2013). According to these studies, in this fungus VeA interacts with VelB, VelC, and LaeA. Additionally VelB forms homodimers or heterodimers with VelC. As in the case of FvVE1 in *F. verticillioides* (Li et al. 2006), *velvet* modulates development in *F. oxysporum*. The absence of *F. oxysporum veA* results in a reduction of aerial mycelium, a decrease in hydrophobicity, an increase in hyphal branching, and an elongation of conidia (Lopez-Berges et al. 2013). Also, as in the case of FvVE1, the presence of osmotic stabilizers

(sorbitol) reversed the effects of the *veA* mutation. The same study also revealed that both *velB* and *laeA* have some common functions with *veA*, particularly *velB*, but less pronounced (Lopez-Berges et al. 2013), with the exception of conidial elongation, which was more notable in the *velB* mutant than in the *veA* mutant. Deletion of *laeA* also showed a decrease of hydrophobicity; however, differently from the *veA* and *velB* deletion mutants, it presented a reduction in microconidia. In the case of Δ*velC*, conidia were of smaller size and of slightly increased number compared with the wild type. This study of *F. oxysporum velvet* and *laeA* genes revealed that *veA*, *velB*, *laeA*, and, to a lesser extent, *velC* have some overlapping and also some distinct roles in the development of this fungus. Additionally, this study also shows the role of both *veA* and *laeA* in chromatin remodeling, regulating not only development but also secondary metabolism and virulence in plants and animals (Lopez-Berges et al. 2013; Lopez-Berges et al. 2014).

In *A. nidulans* a connection between light-sensing proteins, such as those responsive to blue light (white collar homologs) or red light (red phytochrome-like protein), has been observed (Purschwitz et al. 2008). However, although the white collar genes have been studied in several *Fusarium* spp., a possible link between these regulators and *velvet* has not been investigated in *Fusarium*. In *F. oxysporum wc1* mutants showed developmental defects, such as a decrease in aerial mycelium, a reduction in surface hydrophobicity, a reduction in light-dependent synthesis of carotenoids and in UV-dependent photoreactivation, as well as a decrease in virulence (Ruiz-Roldán et al. 2008). Some of these phenotypes overlap with those observed in *F. oxysporum veA* mutants, suggesting a possible functional connection with *veA*.

C. *Fusarium fujikuroi*

Fusarium fujikuroi is a fungus often found on rice where it causes the *bakanae* disease, resulting in abnormal elongation of the plants, yellowish leaves, decrease in tillering, and sterility (Desjardins et al. 2000; Sun and Snyder 1981). Homologs of VeA (FfVel1), VelB (FfVel2), and LaeA (FfLae1) have been identified and studied in this fungus. An interaction between FfVel1 and FfLae1 in the nucleus was demonstrated by bimolecular fluorescence complementation (Wiemann et al. 2010). The *F. oxysporum* homologs of VeA and VelB also have similar roles in development to those originally described for FvVE1 in *F. verticillioides*, including effects on aerial mycelium and pigmentation (Wiemann et al. 2010). *Ffvel1*, *Ffvel2*, and *Fflae1* mutants presented a decrease in the production of microconidia on solid media. All three genes *Ffvel1*, *Ffvel2*, and *Fflae1* were relevant for the activation of secondary metabolism and virulence during rice infection. Interestingly, transcriptome analysis indicated that developmental genes such as *FffluG* or *FfflbC*, homologs of *fluG* and *flbC* first described in *A. nidulans* (Kwon et al. 2010; Lee and Adams 1994), are under the control of *Ffvel1*. Surprisingly, different from *A. nidulans*, fruiting body formation increased in the Δ*Ffvel1*, suggesting that *Ffvel1 is* a positive regulator of asexual development and a negative regulator of sexual development in *F. fujikuroi*.

The gene encoding the *F. fujikuroi* white collar 1 protein, WcoA, has also been characterized. The *wcoA* deletion strains showed a decline in conidial production in both light and dark conditions. Interestingly, a reduction in surface hydrophobicity was also observed in these mutants (Estrada and Avalos 2008). Furthermore WcoA has been shown to regulate the synthesis of several secondary metabolites. Whether WcoA function is associated with VeA is still unknown.

D. *Fusarium graminearum*

The homologs of *A. nidulans veA* and *velB* were identified in the wheat head blight pathogen *Fusarium graminearum* and were denominated *FgVEA* and *FgVELB*, respectively (Jiang et al. 2011; Jiang et al. 2012). Studies have shown that *FgVEA* and *FgVELB* are responsible for several developmental functions in *F. graminearum*. Changes in normal pigmentation, as well as a reduction in hydrophobicity and amount of aerial mycelium produced with respect to the

wild type, were also observed in the case of the *FgVEA* and *FgVELB* mutant strains (Jiang et al. 2011; Jiang et al. 2012). These two mutants also presented an increase in conidial production. Additionally, a delay in conidial germination was detected in the Δ*FgVEA* strain. Large lipid droplets accumulated in the conidia of these mutants. This, accompanied by gene expression analysis, indicated that *F. graminearum velvet* genes have a role of in lipid metabolism. Secondary metabolism was also affected in the absence of *FgVEA or FgVELB*, specifically deoxynivalenol biosynthesis, as well as pathogenicity on flowering wheat head. Jiang et al. (2012) also identified the homolog of LaeA in *F. graminearum* (*FgLaeA1*). In these studies, differently from the case of *A. nidulans*, an interaction between FgVeA and FgLaeA1 or between FgVeA and FgVelB proteins was not found. Furthermore, six different putative methyltransferases were found to interact with FgVeA, suggesting a different organization of the *velvet* regulatory system in *F. graminearum*.

A second study also contributed to the characterization of the *veA* homolog in *F. graminearum*, denominated by this research group as *FgVe1* (Merhej et al. 2012). This study also showed similar results to those described by Jiang et al. (2012). However the two studies show a discrepancy regarding the effect of this gene on conidia production. While Jiang et al. (2012) reported an increase in conidiation in the absence of the *veA* homolog, Merhej et al. (2012) reported a severe reduction in conidial production in comparison to the wild type.

A different independent study also investigated the role of *FgVelB* in asexual development in *F. graminearum* (Lee et al. 2012). The Δ*FgVelB* strains lack the ability to produce fruiting bodies, but retained male fertility. Conidiation was delayed but increased compared to the wild type. The *FgVelB* transcript greatly accumulates during sexual development. Transcriptome analysis indicated that *velB* is involved in diverse biological functions in addition to development. Furthermore, infection of wheat spikes demonstrated that *FgVelB* is required for pathogenicity in *F. graminearum*.

As in the case of *FgVeA* and *FgVelB*, *FgLaeA* has been shown to regulate morphogenesis, sexual development, and virulence in *F. graminearum* (Kim et al. 2013a). *FgLaeA* was shown to affect pigmentation and production of aerial mycelium. In the Δ*FgLaeA* strain, only the central part of the colony presents alterations, while the colony edge retains wild-type phenotype. The oldest part of the colony shows albinism and a reduction in aerial mycelia (Kim et al. 2013a). In this albino region, a decrease in hydrophobicity was observed (Kim et al. 2013a). Loss of *FgLaeA* also results in a decrease of conidiation, particularly in the dark (Kim et al. 2013a), and precocious production of fruiting bodies, accompanied by an abnormal earlier expression of MAT genes. Virulence tests also demonstrated that *FgLaeA* is necessary for pathogenicity in *F. graminearum* on wheat spikes (Kim et al. 2013a). Differently from Jiang et al. (2012), using a split luciferase complementation assay, Kim et al. (2013a) found interaction between FgVeA and FgLaeA proteins.

Fgwc-1 and *Fgwc-2* have also been characterized in *F. graminearum*. Both deletion strains show defects in the light-dependent biosynthesis of carotenoids and hyperconidia under conditions nonconducive for wild-type conidiation, indicating that *Fg*WC-1 and *Fg*WC-2 regulate asexual development. Additionally, these genes are necessary for normal maturity of perithecia (Kim et al. 2013b).

V. *Velvet* in Other Ascomycetes

A. *Neurospora crassa*

Developmental differentiation in *N. crassa* is dependent on multiple environmental cues, including light sensed by WC-1 and WC-2- (Linden et al. 1997; Talora et al. 1999), VIVID (Crosson et al. 2003; Schwedtfeger and Linden 2003) or *veA*. In 2008 the *N. crassa veA* ortholog, *ve-1*, was identified and characterized (Bayram et al. 2008b). Similarly to the *A. nidulans veA* deletion strain, the *N. crassa* Δ*veA* mutant showed an increase in asexual sporulation, indicating that *ve-1* acts as a repressor of conidiation. In addition, this mutant also presented defects in the formation of aerial hypha and disturbances in hyphal branching pattern. Furthermore, *ve-1* rescues wild-type pheno-

types in the *A. nidulans* Δ*veA* mutants, suggesting a similar mechanism of action for this regulator in both fungal species.

B. *Penicillium chrysogenum*

Penicillium chrysogenum is the major producer of the β-lactam antibiotic penicillin in industry (Hoff et al. 2010). Hoff et al. (2010) studied the role of *PcvelA* and *PclaeA* in development. PcVelA acts as a negative regulator in light-dependent conidiation; although conidiophore morphogenesis is not affected. However, PcLaeA is involved in modulating conidiophore differentiation as well as the rate of conidial production in *P. chrysogenum* (Hoff et al. 2010). In addition, both PcVelA and PcLaeA are positive regulators of penicillin biosynthesis, affecting the expression of penicillin genes (Hoff et al. 2010). Further studies (Kopke et al. 2013) also investigated the *velB*, *vosA*, and *velC* homologs in *P. chrysogenum*, *PcvelB*, *PcvosA*, and *PcvelC*, respectively. *PcvelB* and *PcvosA* promote conidiation, while *PcvelC* is a negative regulator in this process. Interestingly, antagonistic roles have also been described for members of the *velvet* protein family with respect to their regulatory role in penicillin biosynthesis in *P. chrysogenum*. PcVelC, as well as PcVelA and PcLaeA, activates penicillin biosynthesis, while PcVelB has opposite effect (Kopke et al. 2013).

The core of the *velvet* complex in *P. chrysogenum* was similar to that described *in A. nidulans*; PcVelA interacts with PcVelB and PcLaeA (Hoff et al. 2010). However, other interacting subunits have also been described. These studies also provided information on the dynamics of *velvet* interaction in this fungus. Yeast two-hybrid system and biomolecular fluorescence complementation experiments showed that PcVelA, PcVelB, and PcVosA directly interact with each other in the nucleus. In addition, PcVelC interacts with PcVosA and PcVelA (Kopke et al. 2013), suggesting that in this fungus both PcVelA and PcVosA function as keystones to maintain the integrity of these protein complexes.

C. *Acremonium chrysogenum*

Acremonium chrysogenum is a major industrial antibiotic producer of the β-lactam antibiotic cephalosporin B. Thus far, the *veA* homolog, *AcveA*, is the only member of the *velvet* genes to be characterized in this fungus. *AcveA* was first characterized by Dreyer et al. (2007). Deleting *AcveA* results in delayed hyphal fragmentation, as well as defective hyphal branching when grown in liquid cultures. Additionally, *AcveA* controls the production of the antibiotic cephalosporin C in this fungus by regulating the expression of six cephalosporin C biosynthetic genes (Dreyer et al. 2007).

D. *Mycosphaerella graminicola*

Mycosphaerella graminicola is a dothideomycete that causes *Septoria tritici* blotch disease in wheat. A *velvet* protein homolog of *veA* has been identified and characterized in this fungus, denoted *MVE1* (Choi and Goodwin 2010). Δ*MVE1* are albino colonies with abnormal hyphal swelling. Deletion of *MVE1* also negatively affects aerial mycelial formation regardless of light conditions and reduces colony hydrophobicity. Despite the pleiotropic effects observed in this mutant, no effect on the pathogenicity was observed when the fungus was spray inoculated on wheat (Choi and Goodwin 2010).

E. *Dothistroma septosporum*

Dothistroma septosporum is another dothideomycete. This fungus is a plant pathogen that causes disease in conifers, affecting mainly pine needles. Chettri et al. (2012) identified and characterized the *veA* homolog. The *Ds-veA* deletion mutants showed a drastic decrease in conidiation under light or dark conditions. This was associated with a reduction in the expression of a putative *stuA* developmental regulatory gene. Although a teleomorph of *D. septosporum* has been identified, *Mycosphaerella pini*, sexual development in the lab setting has yet to be observed, and thus there is no

information on the effect of *Ds-veA* on the formation of fruiting bodies. *Ds-veA* is also a positive regulator of secondary metabolism in this fungus, including the synthesis of the mycotoxin dothistromin. Similarly to the case of *M. graminicola*, the absence of *Ds-veA* did not affect the capacity of this pathogen to infect pine seedlings and complete its life cycle.

F. *Trichoderma reesei*

Trichoderma reesei is an industrially relevant producer of several monomers utilized to generate biofuel (Seiboth et al. 2012). This group studied the *laeA* ortholog, *lae1*, and showed that this gene acts as a positive regulator of conidiation. Absence of *lae1* not only results in a reduction in conidial production but also in the lack of the pigment associated with wild-type *T. reesei* strains. Overexpression of *lae1* results in an increase in conidiation levels. Conidiation is not affected by light exposure in wild-type or Δ*lae1* strains; however, the increased conidial phenotype observed in overexpression *lae1* strain was only observed in the dark.

Transcriptome analysis also demonstrated the *lae1* is a genetic link between development and secondary metabolism in this fungus (Seiboth et al. 2012). Furthermore, chromatin immunoprecipitation sequencing with antibodies against histone modifications showed numerous genes exhibiting a correlation between either H3K4me2 or H3K4me3 and regulation by LAE1 (Seiboth et al. 2012). Another study has also revealed that *lae1* is negatively regulated by PKA1 (Karimi-Aghcheh et al. 2013), similar to the case in *A. nidulans* (Bok and Keller 2004). In addition, the *T. reesei* LAE1 is required for *vel1* expression (Karimi-Aghcheh et al. 2013). With a yeast two-hybrid assay, these authors demonstrated that the *T. reesei* VeA ortholog, VEL1, interacts with LAE1 (Karimi-Aghcheh et al. 2013). However, *T. reesei* LAE1 does not interact with the *A. nidulans* VeA protein. Furthermore, expression of *lae1* in an *A. nidulans* deletion *laeA* strain does not rescue wild-type phenotype, suggesting a different structure for LaeA and LAE1 in these fungal species.

G. *Botrytis cinerea*

Botrytis cinerea is an important necrotic plant pathogen able to infect over 200 crop hosts worldwide. Two *velvet* homologs have been identified in *B. cinerea* (Yang et al. 2013), *BcVEA* and *BcVELB*. Yang's group demonstrated that BcVeA and BcVelB interact in this fungus, and both function as negative regulators of conidiation. Similarly to *A. flavus* and *A. parasiticus*, *B. cinerea* produces sclerotia, and the VeA and VelB homologs also regulate the production of these resistant structures. These two regulators are also necessary for full virulence in *B. cinerea*. Some strains produce bikaverin. Schumacher et al. (2013) postulates that BcVEL1 acts as a positive regulator in bikaverin biosynthesis.

H. *Histoplasma capsulatum*

Histoplasma capsulatum is a dimorphic fungal pathogen of animals that presents filamentous growth in the soil and yeast-like budding in the mammalian host causing histoplasmosis, a life-threatening respiratory and potentially systemic disease (Webster and Sil 2008). This dimorphism is regulated by temperature changes. Webster and Sil identified genes encoding orthologs of the *velvet* family, specifically *vosA*, *velB*, denominated *RYP2*, and *RYP3*, respectively, and demonstrated that these two regulatory genes are necessary for the characteristic dimorphism of *H. capsulatum*. *RYP2* and *RYP3* are differentially expressed in response to temperature, showing greater expression at 37°C during yeast-like growth compared to room temperature, during filamentous growth. Additional studies by this group revealed that both Ryp2 and Ryp3 are DNA-binding proteins (Beyhan et al. 2013).

Ryp1, a transcription factor of the WOPR family, is another Ryp protein involved in thermally driven dimorphism in *H. capsulatum* (Nguyen and Sil 2008). Chromatin immunoprecipitation studies indicated that Ryp1, Ryp2, and Ryp3, in addition to their role regulating the expression of genes involved in the switch to yeast phase, also regulate a large set of

common genomic loci that include virulence genes directly involved in pathogenicity (Beyhan et al. 2013). A fourth recently discovered Ryp, the DNA-associated Ryp4, is also required for yeast-phase growth and displays interdependent regulation with Ryp1, Ryp2, and Ryp3 (Beyhan et al. 2013). Beyhan et al. (2013) also revealed an interaction between Ryp2 and Ryp3 proteins, and that the presence of both proteins is necessary for their binding to DNA motifs, also defined in this study (Beyhan et al. 2013).

In addition, Laskowski-Peak et al. (2012) analyzed the role of the *veA* homolog in *H. capsulatum*, *VEA1*, and showed that *VEA1* negatively regulates the expression of *RYP3*. Additionally, *VEA1* is necessary for the production of cleistothecia in this species (Laskowski-Peak et al. 2012). Silenced *VEA1* strains also switch to mycelial phase faster, preventing the switch to the yeast phase once in mycelial phase. Complementation of the *A. nidulans veA* deletion mutant with *H. capsulatum VEA1* partially rescues wild-type morphological and metabolic phenotype, indicating conserved functionality. Virulence in mice and macrophages is attenuated in *VEA1*-silenced strains. These silenced strains demonstrate increased sensitivity during growth under acidic conditions (Laskowski-Peak et al. 2012).

I. *Cochliobolus heterostrophus*

Cochliobolus heterostrophus is a plant pathogen that causes southern corn leaf blight in maize. Wu et al. (2012) identified and characterized the orthologs of VeA and LaeA, ChVel1, and ChLae1, respectively. Under conditions conducive to sexual development, deletion of *Chvel1* or either deletion or overexpression of *Chlae1* resulted in strains with female sterility. These results indicate that both *Chvel1* and *Chlae1* are positive regulators of sexual differentiation in *C. heterostrophus*. The fact that excessive expression of *Chlae1* resulted in sterility suggests that a stoichiometric balance between ChLae1 and other components in this regulatory network would be important to achieve fertility. Additionally, Chlae1 and Chvel1 mutants also show decreased aerial hyphal formation as well as an increase in asexual sporulation, while overexpression results in fluffy colonies, revealing their negative regulatory role in the activation of conidiation while decreasing vegetative growth (Wu et al. 2012). In addition, Wu et al. (2012) demonstrated that both *Chvel1* and *Chlae1* are important for the production of secondary metabolites in this fungus, including T-toxin, and full virulence during maize infection (Wu et al. 2012).

VI. An Example of *Velvet* in Basidiomycetes

A. *Ustilago maydis*

The basidiomycete *Ustilago maydis* is a plant pathogen that is known to colonize maize crops causing corn smut-forming galls in the infected tissue (Karakkat et al. 2013). *U. maydis* grows in a yeast-like form, producing budding cells; however, upon infection in maize, there is a morphological change to filamentous growth. After mating of two compatible types, hyphal branching results in the production of teliospores, which spread from the rupture of galls on the plant surface. Germination of teliospores results in haploid basidiospores, exhibiting only one mating type (Karakkat et al. 2013). These authors identified three *velvet* homologs, *umv1*, *umv2*, and *umv3*. Phylogenetic analysis indicated Umv1 to be the homolog of VosA, while Umv2 and Umv3 are homologs of VelB. Both deletion *umv1* and *umv2* mutants showed a reduction in virulence when inoculated on maize seedlings. No significant differences on virulence were observed in Δ*umv3*. Δ*umv1* strains showed shortened hyphal branches and failed to produce galls or teliospores when inoculated on maize leaves. Δ*umv2* mutants presented slightly shortened hyphal branches with respect to wild type, as well as a delay in the production of teliospores. These results were not duplicated in adult maize tissue, suggesting that the roles of Umv1 and Umv2 in pathogenicity depend on the developmental stage of the host.

VII. Other Biological Roles Associated with *Velvet*

The *velvet* global regulatory mechanism not only governs fungal development but also controls other important biological processes. In this section we will briefly review the influence of *velvet* on some of the processes that are connected to development by this master regulatory system unique to fungi.

A. Secondary Metabolism

Fungi have the capacity to produce a wide range of bioactive compounds called secondary metabolites or natural products. Some of these compounds have detrimental effects, for instance, mycotoxins (i.e., AFs, T-toxin, fumonisins), often found contaminating food and feed crops, whereas others are beneficial, such as those used as therapeutic agents (e.g., statins, antibiotics, and antitumoral drugs). This attractive side leads to a continuous effort to increase production as well as to mine new fungal natural products for pharmaceutical development. Elucidation of cellular mechanisms controlling secondary metabolism could contribute to reducing the negative effects of fungi as well as to promoting their positive impact on human health and the economy. *Velvet* is one of the most influential regulatory mechanisms controlling fungal secondary metabolism. This work was pioneered by Kato et al. (2003) when they discovered that *A. nidulans* VeA, eponymous for the *velvet* protein family, is necessary for the production of several secondary metabolites, including the mycotoxin ST and the beta-lactam antibiotic penicillin, establishing VeA as global regulator and genetic link between development and secondary metabolism. This discovery, together with the fact that VeA is conserved in numerous fungal species, catalyzed the initiation of many research studies by other groups that also contributed to the current knowledge of the *velvet* regulatory mechanism in *A. nidulans* and in other fungi. As mentioned above, not only VeA but also other proteins of the *velvet* family and LaeA have been shown to be unique to fungi and conserved across fungal genera. VeA, VelB, VelC, VosA, and LaeA along with other interacting proteins such as FphA, LreA, LreB, and LlmF have been extensively characterized in fungi, including their role regulating the biosynthesis of natural products.

1. *Mycotoxins*

Mycotoxins are fungal compounds known to adversely affect health by causing disease or death in humans and other vertebrates. The biosynthesis of many of these compounds has been shown to be regulated by *velvet* and *velvet*-interacting proteins. In the model fungus *A. nidulans*, the synthesis of the mycotoxin ST, which is the penultimate precursor in the biosynthetic pathway leading to the formation of the carcinogenic mycotoxin AF (Bhatnagar et al. 2002; Cary et al. 2000; Payne and Brown 1998; Sweeney and Dobson 1999; Trail et al. 1995), is positively regulated by *veA*, *laeA*, *lreA*, and *lreB* and negatively regulated by *llmF* and *fphA*. In *A. flavus* aflatrem, cyclopiazonic acid and AF are controlled by *veA* (Duran et al. 2007, 2009). AF production is also *veA*-dependent in the closely related *A. parasiticus* (Calvo et al. 2004), where it was shown that *veA* is necessary for normal production of aflatoxisomes (Chanda et al. 2009). Other studies reported *laeA* as a master regulator of secondary metabolism in *A. nidulans* and in *A. flavus* (Bok et al. 2005; Kale et al. 2008). The Δ*laeA* strain did not produce ST or AF in these two fungi, respectively. In addition, a metabolomic approach revealed that deletion of *A. flavus laeA* negatively affects the synthesis of other secondary metabolites including kojic acid, oryzachlorin, and asperfuran, while overexpression *laeA* produced metabolites that were not observed in the wild type such as paspaline/paspalicine and aflavinines (Kale et al. 2008).

Velvet also regulates the synthesis of natural products in the most common causative agent of invasive aspergillosis, *A. fumigatus*. This medically important opportunistic pathogen has been shown to have the capability to produce around 226 bioactive secondary metabolites (Frisvad et al. 2009). Among them are

gliotoxin, identified as a genotoxic and cytotoxic metabolite (Nieminen et al. 2002), and a known virulence factor during *A. fumigatus* infection. Dhingra et al. (2013) showed that gliotoxin genes and concomitant gliotoxin production in *A. fumigatus* is positively regulated by *veA*. Furthermore, Dhingra et al. (2013) also revealed by transcriptome and metabolomic analyses the broad spectrum of *veA* regulatory output in *A. fumigatus*. The expression of many other secondary metabolite gene clusters and their products are also *veA* dependent, including fumagillin, fumitremorgin G, fumigaclavine C, and glionitrin A (Dhingra et al. 2013). The production of gliotoxin is also regulated by *laeA* in *A. fumigatus* (Bok et al. 2005). Sugui et al. (2007) further confirmed this finding. Perrin et al. (2007) conducted a microarray analysis that revealed many secondary metabolite gene clusters under the regulation of *laeA*.

In the plant pathogen *Aspergillus carbonarius*, *veA* and *laeA* homologs positively regulate the synthesis of ochratoxin A (Crespo-Sempere et al. 2013), a nephrotoxic, teratogenic, and immunotoxic mycotoxin (Creppy 1999; Kuiper-Goodman and Scott 1989).

In *F. verticillioides* (Butchko et al. 2012; Li et al. 2006; Myung et al. 2009), well known for the production of fumonisins and fusarins when colonizing maize, Myung et al. (2009) demonstrated that FvVE1 is necessary for production of these two toxins as well as for the development of symptoms during maize colonization (Myung et al. 2012). This study also includes a broad phylogenetic analysis revealing the extent of *veA* conservation in fungi. Butchko et al. (2012) reported *lae1*-dependent positive and negative regulation of the expression of multiple secondary metabolite gene clusters. While production of fumonisins was not altered in the absence of *lae1*, bikaverin and fusarin production were both reduced compared to wild type (Butchko et al. 2012). In *Fusarium oxysporum*, the synthesis of beauvericin, a virulence factor in *F. oxysporum*, as well as the synthesis of siderophores is positively regulated by *veA*, *velB*, and *laeA* (Lopez-Berges et al. 2013). Additionally, in *Fusarium fujikuroi*, while *Ffvel1* and *Fflae1* positively regulate gibberellins, fumonisins, and fusarin C production, they negatively regulate bikaverin biosynthesis (Wiemann et al. 2010). *veA*, *velB*, and *laeA* homologs also play a role in mycotoxin production in *F. graminearum* (Jiang et al. 2011; Jiang et al. 2012; Kim et al. 2013a; Kim et al. 2013b; Lee et al. 2012). *FgVEA* positively regulates the biosynthesis of deoxynivalenol (DON), a virulence factor in *F. graminearum* plant infections (Desjardins et al. 1996; Jiang et al. 2011; Proctor et al. 1995; Seong et al. 2009). Similarly, *FgVELB* has been shown to positively affect the biosynthesis of DON and also zearalenone and trichothecenes (Jiang et al. 2012; Lee et al. 2012). *FgLaeA1* is also necessary for both DON and zearalenone biosynthesis (Kim et al. 2013b). Furthermore, overexpression of *FgLaeA1* produced higher levels of additional mycotoxins not present in the Δ*FgLaeA1* strain such as butenolide and culmorin indicating that *FgLaeA1* is also necessary for the production of these compounds (Kim et al. 2013b).

Production of the T-toxin by *C. heterostrophus* is dependent of ChVeA and ChLaeA (Wu et al. 2012). These proteins promote T-toxin production, while Llm1 (Bi et al. 2013), the homolog of LlmF in *A. nidulans* (Palmer et al. 2013), acts as a negative regulator of T-toxin production, impacting virulence. ChVel1 and ChLae1 (Wu et al. 2012) also have a negative role regulating the formation of melanins, as in the case of *B. cinerea* BcVeA and BcVelB mutants (Yang et al. 2013), and opposite to Mve1 in *M. graminicola* where this regulator promotes melanin (Choi and Goodwin 2010). Production of the mycotoxin dothistromin in the pine needle pathogen *Dothistroma septosporum*, similar in structure to the AF-precursor versicolorin B (Bradshaw and Zhang 2006), is also positively regulated by the *veA* homolog, *Ds-veA* (Chettri et al. 2012).

2. Antibiotics and Other Medically Important Fungal Secondary Metabolites

Fungi produce a broad variety of valuable bioactive compounds, including antibiotics, cholesterol-lowering drugs, and secondary metabolites with antitumoral properties among others. For example, *Aspergillus nidulans*

produces the β-lactam antibiotic penicillin and terrequinone A, a compound with antitumoral properties. Both of these compounds are positively regulated by *veA* and *laeA* (Bok et al. 2006a, b; Bok and Keller 2004; Kato et al. 2003). Penicillin is industrially produced mainly by *P. chrysogenum*, formally known as *Penicillium notatum* (Samson et al. 1977). *PcvelA*, *PclaeA*, and *PcvelC* are positive regulators of penicillin biosynthesis (Hoff et al. 2010; Kopke et al. 2013), while *PcvelB* acts as a negative regulator in this process (Kopke et al. 2013). The species *Penicillium citrinum* is another member of the *Penicillium* genus that produces a secondary metabolite called ML-236B, which is a substrate in the cholesterol-lowering drug pravastatin. Production of these compounds is regulated by homologs of *veA* and *laeA* in this organism (Baba et al. 2012). In addition, in *A. chrysogenum*, another industrially important fungus, the biosynthesis of the antibiotic cephalosporin C is regulated by *AcveA* (Dreyer et al. 2007).

3. Other Industrially Produced Secondary Metabolites

The industrially important *Aspergillus oryzae* is another member of the *Aspergillus* genus that is genetically closely related to the opportunistic plant pathogen *A. flavus*. *A. oryzae*, unlike *A. flavus*, does not transcribe the AF gene cluster (van den Broek et al. 2001; Zhang et al. 2004). *A. oryzae* is industrially important due to its ability to produce the secondary metabolite kojic acid. Kojic acid has also been shown to be positively regulated by *laeA* in this fungus (Oda et al. 2011).

B. Production of Extracellular Enzymes

Many fungi produce a wide array of extracellular hydrolytic enzymes; some of them could contribute to host colonization in pathogenic fungi (Dolezal et al. 2013; Mellon et al. 2007). For example, in *A. flavus* extracellular enzymes allow successful colonization of plant tissue and acquisition of nutrients from the plant host. Interestingly, some of these hydrolytic activities are regulated by *veA* and *laeA* (Amaike and Keller 2009; Duran et al. 2014; Kale et al. 2008). It has been demonstrated that *veA*, necessary for full virulence during host colonization (Duran et al. 2009), is also required for normal amylase and protease activity (Duran et al. 2014). *laeA*, as with *veA*, is also required for normal plant colonization (Amaike and Keller 2009; Kale et al. 2008) and is involved in the regulation of lipase activity in *A. flavus*. The role of *veA* in regulating hydrolytic activity has also been described in other *Aspergillus* species. *A. fumigatus* produces many proteases, some of which have a role in virulence during animal infection (Bergmann et al. 2009; Jaton-Ogay et al. 1994; Kolattukudy et al. 1993; Richie et al. 2009). Dhingra et al. (Dhingra et al. 2012) showed that *veA* is necessary for normal protease activity in *A. fumigatus*. In industry, the fungus *Trichoderma reesei* produces enzymes such as cellulases that are used to catabolize polysaccharides into products that are used in biofuel production (Seiboth et al. 2012). Importantly, *lae1* is necessary for normal expression of cellulase as well as xylanase genes and concomitant enzymatic activity in *T. reesei* (Seiboth et al. 2012).

C. Response to Environmental Stresses

Fungi, like many other living organisms, sense different forms of environmental stresses, such as nutrient starvation, changes in pH and temperature, light, and oxidative and osmotic stresses. Biochemical pathways responsive to environmental stress signals, such as reactive oxygen species (ROS), have been studied in depth in the model organisms *Saccharomyces cerevisiae* and *A. nidulans* (Duran et al. 2010; Furukawa et al. 2005; Hagiwara et al. 2007; Han and Prade 2002; Hohmann et al. 2007; Kawasaki et al. 2002; Miskei et al. 2009). Interestingly, in *A. nidulans*, VelB and VosA and VeA contribute to normal responses to UV light and oxidative stress. However, VelB and VosA play a more relevant role than VeA in response to these stresses (Sarikaya-Bayram et al. 2010). This contrasts with *A. flavus* where VeA plays an important role in the survival of this fungus when exposed to an oxidative environment.

VeA regulates the expression of genes known to be involved in the response to this stress, affecting the formation of DNA-protein complexes at the promoter regions (Baidya et al. 2014). In other fungal species, such as in *C. heterostrophus*, *Chvel1* and *Chlae1* are also positive regulators of oxidative stress response (Wu et al. 2012). In *F. verticillioides*, FvVelB, and particularly FvVE1, has been shown to play a positive role in the regulation oxidative stress response (Lan et al. 2014). Response to other environmental stresses, such acidic environment, has also been associated with *veA* in *H. capsulatum* (Laskowski-Peak et al. 2012).

D. Virulence

Members of the *velvet* family and interacting proteins are associated with virulence in some important fungal pathogens, for example, in the opportunistic plant pathogen *A. flavus* (Duran et al. 2009), *A. parasiticus* (Calvo et al. 2004), *F. verticillioides* (*FvVE1*) (Myung et al. 2012), *F. graminearium* (*FgVEA*) (Jiang et al. 2011), *F. fujikuroi* (*Ffvel1*) (Wiemann et al. 2010), *B. cinerea* (*BcVEA*) (Yang et al. 2013), *C. heterostrophus* (*Chvel1*) (Wu et al. 2012), and *F. oxysporum* (*veA*) (Lopez-Berges et al. 2013). Some of these fungi, for example, *F. oxysporum*, are also opportunistic pathogens of animals, where *veA* plays a role in virulence. Similarly, *VEA1* is important in the pathogenicity of *H. capsulatum*, the causative agent of histoplasmosis (*VEA1*) (Laskowski-Peak et al. 2012). Other members of the *velvet* family, particularly *velB* and *vosA*, also influence virulence, for example, in *F. graminearium* (*FgVelB*) (Jiang et al. 2012), *F. fujikuroi* (*Ffvel2*) (Wiemann et al. 2010), *B. cinerea* (*BcVELB*) (Yang et al. 2013), *U. maydis* (*uvm1* and *uvm2*) (Karakkat et al. 2013), and *H. capsulatum* (*RYP2* and *RYP3*) (Beyhan et al. 2013). *laeA* has also been linked to virulence, for instance, in *A. flavus* (Amaike and Keller 2009; Kale et al. 2008), *A. fumigatus* (Bok et al. 2005), *F. graminearium* (*FgLaeA*) (Kim et al. 2013b), *F. fujikuroi* (*Fflae1*) (Wiemann et al. 2010), *F. oxysporum* (Lopez-Berges et al. 2013), and *C. heterostrophus* (*Chlae1*) (Wu et al. 2012). Other *velvet*-interacting proteins such as Llm1 have been reported as important for virulence in *C. heterostrophus* (Bi et al. 2013). Based on accumulating evidence, it is likely that the *velvet* regulatory system plays a role in virulence in many additional pathogenic fungi.

VIII. Conclusion

Numerous studies have revealed the importance of the *velvet* regulatory system in governing a diversity of cellular processes in fungi. Investigation carried out with the model filamentous fungus *A. nidulans* has provided valuable insight into this regulatory mechanism that is conserved in other fungi, particularly in ascomycetes. These findings led to many additional studies involving the experimental characterization of putative homologs found in fungal genomic databases, uncovering the importance of this regulatory mechanism in the fungal kingdom. Regulation of development and secondary metabolism are the two aspects of *velvet* regulatory output that have been investigated in the most depth. The information provided in this review extensively reflects details of the *velvet* mechanism of action and its effects on morphological differentiation. Comparative studies among *Aspergillus* species indicated functional conservation of the *velvet* regulatory system regarding conidiation and formation of fruiting bodies or vestiges of such structures with remodeled function as resistant bodies. Interestingly, the *velvet* regulatory output affecting morphogenesis, particularly conidiation, appears strongly influenced by environmental conditions, such as light or the nutritional environment.

Although the comparison between the function of *velvet* among different fungal species shows a clear role in the regulation of development, it also indicates certain evolutionary plasticity in which this broadly conserved regulatory mechanism has been partially rearranged, serving the specific biological requirements of different fungi adapted to their respective habitats. These differences are noted in variations in their regu-

latory function as well as in mechanistic differences, for example, differences in protein interactions among members of the *velvet* protein family and with other *velvet*-associated proteins. The diversity of roles of *velvet* in fungi is reflected in the different phenotypes observed in *Aspergillus velvet* mutants in comparison with those exhibited in mutants generated in species from other fungal genera. For example, yeast-like growth or colony hydrophobicity, observed in *Fusarium* or *Penicillium* mutants, have not been described in *Aspergillus* species. In other species, the involvement of *velvet* in developmental processes such as regulation of dimorphism has been described. However, the positive effect of *velvet* on sexual development is a commonality observed in numerous fungi. Interestingly, *velvet*-interacting proteins often present antagonistic roles with respect to their *velvet* partners, contributing to maintain a developmental balance between asexual and sexual development and other cellular functions.

It has been well established that the *velvet* regulatory system is responsive to environmental stimuli resulting in an adaptive genetic response. However, although some studies have provided insight into how environmental cues affect the function of *velvet*, currently this process is still not well understood and needs further investigation. Also, recent studies have indicated direct binding to DNA by some of the *velvet* proteins, specifically VelB and VosA. Future studies could contribute to the detailed characterization of the dynamics involving *velvet*–DNA interactions.

In recent years, several genomic studies have provided valuable information toward the understanding of this remarkable regulatory mechanism in fungi. Functional genomic analysis of *velvet* and *velvet*-related dependent genes has revealed a very broad regulatory spectrum, including the control of developmental genes as well as genes involved in other processes, such as secondary metabolism, response to oxidative stress, hydrolytic activity, and virulence among others. The diversification and rewiring of the *velvet* regulatory system is also reflected in its effect on secondary metabolism. Different fungal species often produce a distinct signature of natural products; however, the process is governed by the same conserved regulatory mechanism. This conserved regulatory role, combined with our increasing knowledge of fungal genomes, makes the *velvet* system a possible target for industrial applications to efficiently produce bioactive compounds of commercial value or to reduce the synthesis of those secondary metabolites that are detrimental. In addition, since *velvet* has also been linked to hydrolytic activity in several fungi, it is possible that manipulation of this system could lead to greater industrial production of valuable enzymes. Furthermore, the role of the global *velvet* regulatory system on morphogenesis affects the dissemination and survivability of many fungi, including pathogenic species. Lastly, in many cases *velvet* is important for virulence. This fact, and the fact that the *velvet* system is unique to fungi, could allow for the design of methodology to decrease the impact of pathogenic fungi.

References

Adams TH, Yu JH (1998) Coordinate control of secondary metabolite production and asexual sporulation in *Aspergillus nidulans*. Curr Opin Microbiol 1:674–677

Adams TH, Wieser JK, Yu JH (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Ahmed YL, Gerke J, Park HS, Bayram O, Neumann P, Ni M, Dickmanns A, Kim SC, Yu JH, Braus GH, Ficner R (2013) The velvet family of fungal regulators contains a DNA-binding domain structurally similar to NF-kappaB. PLoS Biol 11(12), e1001750

Amaike S, Keller NP (2009) Distinct roles for VeA and LaeA in development and pathogenesis of *Aspergillus flavus*. Eukaryot Cell 8:1051–1060

Araujo-Bazan L, Dhingra S, Chu J, Fernandez-Martinez J, Calvo AM, Espeso EA (2009) Importin alpha is an essential nuclear import carrier adaptor required for proper sexual and asexual development and secondary metabolism in *Aspergillus nidulans*. Fungal Genet Biol 46:506–515

Atoui A, Bao D, Kaur N, Grayburn WS, Calvo AM (2008) *Aspergillus nidulans* natural product biosynthesis is regulated by mpkB, a putative pheromone response mitogen-activated protein kinase. Appl Environ Microbiol 74:3596–3600

Baba S, Kinoshita H, Nihira T (2012) Identification and characterization of *Penicillium citrinum* VeA and

LaeA as global regulators for ML-236B production. Curr Genet 58:1–11

Baidya S, Duran RM, Lohmar JM, Harris-Coward PY, Cary JW, Hong SY, Roze LV, Linz JE, Calvo AM (2014) VeA is associated with the response to oxidative stress in the aflatoxin producer *Aspergillus flavus*. Eukaryot Cell 13:1095–1103

Battilani P, Magan N, Logrieco A (2006) European research on ochratoxin A in grapes and wine. Int J Food Microbiol 111(Suppl 1):S2–4

Bayram O, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon NJ, Keller NP, Yu JH, Braus GH (2008a) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320:1504–1506

Bayram O, Krappmann S, Seiler S, Vogt N, Braus GH (2008b) *Neurospora crassa* ve-1 affects asexual conidiation. Fungal Genet Biol 45:127–138

Bayram O, Braus GH, Fischer R, Rodriguez-Romero J (2010) Spotlight on *Aspergillus nidulans* photosensory systems. Fungal Genet Biol 47:900–908

Bayram O, Bayram OS, Ahmed YL, Maruyama J, Valerius O, Rizzoli SO, Ficner R, Irniger S, Braus GH (2012) The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. PLoS Genet 8(7), e1002816

Bergmann A, Hartmann T, Cairns T, Bignell EM, Krappmann S (2009) A regulator of *Aspergillus fumigatus* extracellular proteolytic activity is dispensable for virulence. Infect Immun 77:4041–4050

Beyhan S, Gutierrez M, Voorhies M, Sil A (2013) A temperature-responsive network links cell shape and virulence traits in a primary fungal pathogen. PLoS Biol 11(7), e1001614

Bhatnagar D, Brown DW, Ehrlich KC, Cleveland TE (2002) Mycotoxins Contaminating Cereal Grain Crops: Their Occurrence and Toxicity. In: Khachatourians GG, Arora DK (eds) Applied mycology and biotechnology, vol 2. Elsevier B.V, New York, pp 171–196

Bi Q, Wu D, Zhu X, Gillian Turgeon B (2013) *Cochliobolus heterostrophus* Llm1—a Lae1-like methyltransferase regulates T-toxin production, virulence, and development. Fungal Genet Biol 51:21–33

Bok JW, Keller NP (2004) LaeA, a regulator of secondary metabolism in *Aspergillus* spp. Eukaryot Cell 3:527–535

Bok J, Balajee SA, Marr KA, Andes D, Nielsen KF, Frisvad JC, Keller NP (2005) LaeA, a regulator of morphogenetic fungal virulence factors. Eukaryot Cell 4:1574–1582

Bok JW, Hoffmeister D, Maggio-Hall LA, Murillo R, Glasner JD, Keller NP (2006a) Genomic mining for *Aspergillus* natural products. Chem Biol 13:31–37

Bok JW, Noordermeer D, Kale SP, Keller NP (2006b) Secondary metabolic gene cluster silencing in *Aspergillus nidulans*. Mol Microbiol 61:1636–1645

Bradshaw RE, Zhang S (2006) Biosynthesis of dothistromin. Mycopathologia 162:201–213

Butchko RA, Brown DW, Busman M, Tudzynski B, Wiemann P (2012) Lae1 regulates expression of multiple secondary metabolite gene clusters in *Fusarium verticillioides*. Fungal Genet Biol 49:602–612

Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459

Calvo AM, Bok J, Brooks W, Keller NP (2004) veA is required for toxin and sclerotial production in *Aspergillus parasiticus*. Appl Environ Microbiol 70:4733–4739

Cary JW, Linz JE, Bhatnagar D (2000) Aflatoxins: biological significance and regulation of biosynthesis. In: Cary JW, Linz JE, Bhatnagar D (eds) Microbial foodborne diseases: mechanisms of pathogenesis and toxin synthesis. Technomic Publishing Co., Lancaster, PA, pp 317–361

Cary JW, Obrian GR, Nielsen DM, Nierman W, Harris-Coward P, Yu J, Bhatnagar D, Cleveland TE, Payne GA, Calvo AM (2007) Elucidation of veA-dependent genes associated with aflatoxin and sclerotial production in *Aspergillus flavus* by functional genomics. Appl Microbiol Biotechnol 76:1107–1118

Cary JW, Han Z, Yin Y, Lohmar JM, Shantappa S, Harris-Coward PY, Mack B, Ehrlich KC, Wei Q, Arroyo-Manzanares N, Uka V, Vanhaecke L, Bhatnagar D, Yu J, Nierman WC, Johns MA, Sorensen D, Shen H, De Saeger S, Diana Di Mavungu J, Calvo AM (2015) Transcriptome analysis of Aspergillus flavus reveals veA-dependent regulation of secondary metabolite gene clusters, including the novel Aflavarin cluster. Eukaryot Cell 14(10):983–997. doi:10.1128/EC.00092-15

Champe S, Kurtz M, Yager L, Butnick N, Axelrod D (1981) Spore formation in *Aspergillus nidulans*: competence and other developmental processes. In: Hohl HRTG (ed) The fungal spores: morphogenic controls. Academic, New York, pp 255–276

Chanda A, Roze LV, Kang S, Artymovich KA, Hicks GR, Raikhel NV, Calvo AM, Linz JE (2009) A key role for vesicles in fungal secondary metabolism. Proc Natl Acad Sci U S A 106:19533–19538

Chang PK, Scharfenstein LL, Ehrlich KC, Wei Q, Bhatnagar D, Ingber BF (2012a) Effects of laeA deletion on *Aspergillus flavus* conidial development and hydrophobicity may contribute to loss of aflatoxin production. Fungal Biol 116:298–307

Chang PK, Scharfenstein LL, Mack B, Ehrlich KC (2012b) Deletion of the *Aspergillus flavus* orthologue of *Aspergillus nidulans fluG* reduces conidiation and promotes production of sclerotia but

does not abolish aflatoxin biosynthesis. Appl Environ Microbiol 78:7557–7563

Chang PK, Scharfenstein LL, Li P, Ehrlich KC (2013) *Aspergillus flavus* VelB acts distinctly from VeA in conidiation and may coordinate with FluG to modulate sclerotial production. Fungal Genet Biol 58–59:71–79

Chettri P, Calvo AM, Cary JW, Dhingra S, Guo Y, McDougal RL, Bradshaw RE (2012) The veA gene of the pine needle pathogen *Dothistroma septosporum* regulates sporulation and secondary metabolism. Fungal Genet Biol 49:141–151

Choi YE, Goodwin SB (2010) MVE1, encoding the velvet gene product homolog in *Mycosphaerella graminicola*, is associated with aerial mycelium formation, melanin biosynthesis, hyphal swelling, and light signaling. Appl Environ Microbiol 77:942–953

Coley-Smith J, Cooke R (1971) Survival and germination of fungal sclerotia. Annu Rev Phytopathol 9:65–92

Creppy EE (1999) Human ochratoxicosis. J Toxicol Toxin Rev 18:273–293

Crespo-Sempere A, Marin S, Sanchis V, Ramos AJ (2013) VeA and LaeA transcriptional factors regulate ochratoxin A biosynthesis in *Aspergillus carbonarius*. Int J Food Microbiol 166:479–486

Crosson S, Rajagopal S, Moffat K (2003) The LOV domain family: photoresponsive signaling modules coupled to diverse output domains. Biochemistry 42:2–10

Dagenais T, Giles S, Aimanianda V, Latge J, Hull C, Keller NP (2010) *Aspergillus fumigatus* LaeA-mediated phagocytosis is associated with a decreased hydrophobin layer. Infect Immun 78:823–829

Desjardins AE, Proctor RH, Bai GH, McCormick SP, Shaner G (1996) Reduced virulence of trichothecene toxin mutants of *Gibberella zeae* in wheat field tests. Mol Plant-Microbe Interact 9:775–781

Desjardins AE, Manandhar HK, Plattner RD, Manandhar GG, Poling SM, Maragos CM (2000) *Fusarium* species from nepalese rice and production of mycotoxins and gibberellic acid by selected species. Appl Environ Microbiol 66:1020–1025

Dhingra S, Andes D, Calvo AM (2012) VeA regulates conidiation, gliotoxin production, and protease activity in the opportunistic human pathogen *Aspergillus fumigatus*. Eukaryot Cell 11:1531–1543

Dhingra S, Lind AL, Lin HC, Tang Y, Rokas A, Calvo AM (2013) The fumagillin gene cluster, an example of hundreds of genes under veA control in *Aspergillus fumigatus*. PLoS One 8(10), e77147

Dolezal AL, Obrian GR, Nielsen DM, Woloshuk CP, Boston RS, Payne GA (2013) Localization, morphology and transcriptional profile of *Aspergillus flavus* during seed colonization. Mol Plant Pathol 14:898–909

Dreyer J, Eichhorn H, Friedlin E, Kurnsteiner H, Kuck U (2007) A homologue of the *Aspergillus* velvet gene regulates both cephalosporin C biosynthesis and hyphal fragmentation in *Acremonium chrysogenum*. Appl Environ Microbiol 73:3412–3422

Duran RM, Cary JW, Calvo AM (2007) Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* is regulated by veA, a gene necessary for sclerotial formation. Appl Microbiol Biotechnol 73:1158–1168

Duran RM, Cary JW, Calvo AM (2009) The Role of *veA* in *Aspergillus flavus* Infection of Peanut, Corn and Cotton. Open Mycol J 3:27–36

Duran R, Cary JW, Calvo AM (2010) Role of the osmotic stress regulatory pathway in morphogenesis and secondary metabolism in filamentous fungi. Toxins (Basel) 2:367–381

Duran RM, Gregersen S, Smith TD, Bhetariya PJ, Cary JW, Harris-Coward PY, Mattison CP, Grimm C, Calvo AM (2014) The Role of *Aspergillus flavus* veA in the production of extracellular proteins during the growth on starch substrates. Appl Microbiol Biotechnol 5081–5094

Estrada A, Avalos J (2008) The White Collar protein WcoA of *Fusarium fujikuroi* is not essential for photocarotenogenesis, but is involved in the regulation of secondary metabolism and conidiation. Fungal Genet Biol 45:705–718

Fischer R, Kues U (2006) Asexual sporulation in mycelial fungi. In: Kues U, Fischer R (eds) The mycota: growth, differentiation and sexuality, vol I, 2nd edn. Springer, Berlin, Heidelberg, pp 263–292

Frisvad JC, Rank C, Nielsen KF, Larsen TO (2009) Metabolomics of *Aspergillus fumigatus*. Med Mycol 47(Suppl 1):S53–71

Furukawa K, Hoshi Y, Maeda T, Nakajima T, Abe K (2005) *Aspergillus nidulans* HOG pathway is activated only by two-component signalling pathway in response to osmotic stress. Mol Microbiol 56:1246–1261

Hagiwara D, Asano Y, Marui J, Furukawa K, Kanamaru K, Kato M, Abe K, Kobayashi T, Yamashino T, Mizuno T (2007) The SskA and SrrA response regulators are implicated in oxidative stress responses of hyphae and asexual spores in the phosphorelay signaling network of *Aspergillus nidulans*. Biosci Biotechnol Biochem 71:1003–1014

Han KH, Prade RA (2002) Osmotic stress-coupled maintenance of polar growth in *Aspergillus nidulans*. Mol Microbiol 43:1065–1078

Hicks JK, Yu JH, Keller NP, Adams TH (1997) *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA G alpha protein-dependent signaling pathway. EMBO J 16:4916–4923

Hoff B, Kamerewerd J, Sigl C, Mitterbauer R, Zadra I, Kurnsteiner H, Kuck U (2010) Two components of a velvet-like complex control hyphal morphogenesis, conidiophore development, and penicillin

biosynthesis in *Penicillium chrysogenum*. Eukaryot Cell 9:1236–1250
Hohmann S, Krantz M, Nordlander B (2007) Yeast osmoregulation. Methods Enzymol 428:29–45
Horn BW, Sorensen RB, Lamb MC, Sobolev VS, Olarte RA, Worthington CJ, Carbone I (2014) Sexual reproduction in *Aspergillus flavus* sclerotia naturally produced in corn. Phytopathology 104:75–85
Jaton-Ogay K, Paris S, Huerre M, Quadroni M, Falchetto R, Togni G, Latge JP, Monod M (1994) Cloning and disruption of the gene encoding an extracellular metalloprotease of *Aspergillus fumigatus*. Mol Microbiol 14:917–928
Jiang J, Liu X, Yin Y, Ma Z (2011) Involvement of a velvet protein FgVeA in the regulation of asexual development, lipid and secondary metabolisms and virulence in *Fusarium graminearum*. PLoS One 6(11), e28291
Jiang J, Yun Y, Liu Y, Ma Z (2012) FgVELB is associated with vegetative differentiation, secondary metabolism and virulence in *Fusarium graminearum*. Fungal Genet Biol 49:653–662
Joosten HM, Goetz J, Pittet A, Schellenberg M, Bucheli P (2001) Production of ochratoxin A by *Aspergillus carbonarius* on coffee cherries. Int J Food Microbiol 65:39–44
Kafer E (1965) Origins of translocations in *Aspergillus nidulans*. Genetics 52:217–232
Kale SP, Milde L, Trapp MK, Frisvad JC, Keller NP, Bok JW (2008) Requirement of LaeA for secondary metabolism and sclerotial production in *Aspergillus flavus*. Fungal Genet Biol 45:1422–1429
Karakkat BB, Gold SE, Covert SF (2013) Two members of the *Ustilago maydis* velvet family influence teliospore development and virulence on maize seedlings. Fungal Genet Biol 61:111–119
Karimi-Aghcheh R, Bok JW, Phatale PA, Smith KM, Baker SE, Lichius A, Omann M, Zeilinger S, Seiboth B, Rhee C, Keller NP, Freitag M, Kubicek CP (2013) Functional analyses of *Trichoderma reesei* LAE1 reveal conserved and contrasting roles of this regulator. G3 (Bethesda) 3:369–378
Kato N, Brooks W, Calvo AM (2003) The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by *veA*, a gene required for sexual development. Eukaryot Cell 2:1178–1186
Kawasaki L, Sanchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45:1153–1163
Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3:937–947
Kim H, Han K, Kim K, Han D, Jahng K, Chae K (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80
Kim HK, Lee S, Jo SM, McCormick SP, Butchko RA, Proctor RH, Yun SH (2013a) Functional roles of FgLaeA in controlling secondary metabolism, sexual development, and virulence in *Fusarium graminearum*. PLoS One 8(7), e68441
Kim H, Son H, Lee YW (2013b) Effects of light on secondary metabolism and fungal development of *Fusarium graminearum*. J Appl Microbiol 116 (2):380–389
Kolattukudy PE, Lee JD, Rogers LM, Zimmerman P, Ceselski S, Fox B, Stein B, Copelan EA (1993) Evidence for possible involvement of an elastolytic serine protease in aspergillosis. Infect Immun 61:2357–2368
Kopke K, Hoff B, Bloemendal S, Katschorowski A, Kamerewerd J, Kuck U (2013) Members of the *Penicillium chrysogenum* velvet complex play functionally opposing roles in the regulation of penicillin biosynthesis and conidiation. Eukaryot Cell 12:299–310
Krappmann S, Bayram O, Braus GH (2005) Deletion and allelic exchange of the *Aspergillus fumigatus* veA locus via a novel recyclable marker module. Eukaryot Cell 4:1298–1307
Kuiper-Goodman T, Scott PM (1989) Risk assessment of the mycotoxin ochratoxin A. Biomed Environ Sci 2:179–248
Kwon NJ, Garzia A, Espeso EA, Ugalde U, Yu JH (2010) FlbC is a putative nuclear C2H2 transcription factor regulating development in *Aspergillus nidulans*. Mol Microbiol 77:1203–1219
Lan N, Zhang H, Hu C, Wang W, Calvo AM, Harris SD, Chen S, Li S (2014) Coordinated and distinct functions of velvet proteins in *Fusarium verticillioides*. Eukaryot Cell 13:909–918
Laskowski-Peak MC, Calvo AM, Rohrssen J, Smulian AG (2012) VEA1 is required for cleistothecial formation and virulence in Histoplasma *capsulatum*. Fungal Genet Biol 49:838–846
Lee BN, Adams TH (1994) The *Aspergillus nidulans* fluG gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev 8 (6):641–651
Lee J, Myong K, Kim JE, Kim HK, Yun SH, Lee YW (2012) FgVelB globally regulates sexual reproduction, mycotoxin production and pathogenicity in the cereal pathogen *Fusarium graminearum*. Microbiology 158(Pt 7):1723–1733
Li S, Myung K, Guse D, Donkin B, Proctor RH, Grayburn WS, Calvo AM (2006) FvVE1 regulates filamentous growth, the ratio of microconidia to macroconidia and cell wall formation in *Fusarium verticillioides*. Mol Microbiol 62:1418–1432
Lind AL, Wisecaver JH, Smith TD, Feng X, Calvo AM, Rokas A (2015) Examining the evolution of the regulatory circuit controlling secondary metabolism and development in the fungal genus Aspergillus. PLoS Genet 11(3), e1005096. doi:10.1371/journal.pgen.1005096
Linden H, Ballario P, Macino G (1997) Blue light regulation in *Neurospora Crassa*. Fungal Genet Biol 22:141–150
Lopez-Berges MS, Hera C, Sulyok M, Schafer K, Capilla J, Guarro J, Di Pietro A (2013) The velvet complex governs mycotoxin production and virulence of

Fusarium oxysporum on plant and mammalian hosts. Mol Microbiol 87:49–65

Lopez-Berges MS, Schafer K, Hera C, Di Pietro A (2014) Combinatorial function of velvet and AreA in transcriptional regulation of nitrate utilization and secondary metabolism. Fungal Genet Biol 62:78–84

Malloch D, Cain R (1972) The Trichocomataceae: ascomycetes with *Aspergillus*, *Paecilomyces* and *Penicillium* imperfect states. Can J Bot 50:2613–2628

Mellon JE, Cotty PJ, Dowd MK (2007) *Aspergillus flavus* hydrolases: their roles in pathogenesis and substrate utilization. Appl Microbiol Biotechnol 77:497–504

Merhej J, Urban M, Dufresne M, Hammond-Kosack KE, Richard-Forget F, Barreau C (2012) The velvet gene, FgVe1, affects fungal development and positively regulates trichothecene biosynthesis and pathogenicity in *Fusarium graminearum*. Mol Plant Pathol 13:363–374

Miskei M, Karanyi Z, Pocsi I (2009) Annotation of stress-response proteins in the Aspergilli. Fungal Genet Biol 46(Suppl 1):S105–120

Mooney J, Yager L (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Mooney JL, Hassett DE, Yager LN (1990) Genetic analysis of suppressors of the *veA1* mutation in *Aspergillus nidulans*. Genetics 126:869–874

Myung K, Li S, Butchko RA, Busman M, Proctor RH, Abbas HK, Calvo AM (2009) FvVE1 regulates biosynthesis of the mycotoxins fumonisins and fusarins in *Fusarium verticillioides*. J Agric Food Chem 57:5089–5094

Myung K, Zitomer NC, Duvall M, Glenn AE, Riley RT, Calvo AM (2012) The conserved global regulator VeA is necessary for symptom production and mycotoxin synthesis in maize seedlings by *Fusarium verticillioides*. Plant Pathol 61:152–160

Nguyen VQ, Sil A (2008) Temperature-induced switch to the pathogenic yeast form of *Histoplasma capsulatum* requires Ryp1, a conserved transcriptional regulator. Proc Natl Acad Sci U S A 105:4880–4885

Ni M, Yu JH (2007) A novel regulator couples sporogenesis and trehalose biogenesis in *Aspergillus nidulans*. PLoS One 2(10), e970

Nieminen SM, Karki R, Auriola S, Toivola M, Laatsch H, Laatikainen R, Hyvärinen A, von Wright A (2002) Isolation and identification of *Aspergillus fumigatus* mycotoxins on growth medium and some building materials. Appl Environ Microbiol 68:4871–4875

Oda K, Kobayashi A, Ohashi S, Sano M (2011) *Aspergillus oryzae* laeA regulates kojic acid synthesis genes. Biosci Biotechnol Biochem 75:1832–1834

Palmer JM, Theisen JM, Duran RM, Grayburn WS, Calvo AM, Keller NP (2013) Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. PLoS Genet 9(1), e1003193

Paoletti M, Seymour FA, Alcocer MJ, Kaur N, Calvo AM, Archer DB, Dyer PS (2007) Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. Curr Biol 17:1384–1389

Park HS, Ni M, Jeong KC, Kim YH, Yu JH (2012a) The role, interaction and regulation of the velvet regulator VelB in *Aspergillus nidulans*. PLoS One 7(9), e45935

Park HS, Bayram O, Braus GH, Kim SC, Yu JH (2012b) Characterization of the velvet regulators in *Aspergillus fumigatus*. Mol Microbiol 86:937–953

Park HS, Nam TY, Han KH, Kim SC, Yu JH (2014) VelC positively controls sexual development in *Aspergillus nidulans*. PLoS One 9(2), e89883

Payne GA, Brown MP (1998) Genetics and physiology of aflatoxin biosynthesis. Annu Rev Phytopathol 36:329–362

Perrin R, Fedorova N, Bok JW, Cramer RA, Wortman JR, Kim HS, Nierman WC, Keller NP (2007) Transcriptional regulation of chemical diversity in *Aspergillus fumigatus* by LaeA. PLoS Pathog 3(4), e50

Proctor RH, Hohn TM, McCormick SP (1995) Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol Plant Microbe Interact 8:593–601

Purschwitz J, Muller S, Kastner C, Schoser M, Haas H, Espeso EA, Atoui A, Calvo AM, Fischer R (2008) Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. Curr Biol 18:255–259

Purschwitz J, Muller S, Fischer R (2009) Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the White Collar protein LreB. Mol Genet Genomics 281:35–42

Ramirez-Prado J, Moore G, Horn B, Carbone I (2008) Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet Biol 45:1292–1299

Reyes-Dominguez Y, Bok JW, Berger H, Shwab EK, Basheer A, Gallmetzer A, Scazzocchio C, Keller N, Strauss J (2010) Heterochromatic marks are associated with the repression of secondary metabolism clusters in *Aspergillus nidulans*. Mol Microbiol 76:1376–1386

Richie DL, Hartl L, Aimanianda V, Winters MS, Fuller KK, Miley MD, White S, McCarthy JW, Latge JP, Feldmesser M, Rhodes JC, Askew DS (2009) A role for the unfolded protein response (UPR) in virulence and antifungal susceptibility in *Aspergillus fumigatus*. PLoS Pathog 5(1), e1000258

Ruiz-Roldán M, Garre V, Guarro J, Mariné M, Roncero M (2008) Role of the white collar 1 photoreceptor in carotenogenesis, UV resistance, hydrophobicity, and virulence of *Fusarium oxysporum*. Eukaryot Cell: 7(7):1227–1230

Samson RA, Hadlok R, Stolk AC (1977) A taxonomic study of the *Penicillium chrysogenum* series. Antonie Van Leeuwenhoek 43:169–175

Sarikaya-Bayram O, Bayram O, Valerius O, Park HS, Irniger S, Gerke J, Ni M, Han K, Yu J, Braus GH (2010) LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. PLoS Genet 6(12), e1001226

Sarikaya-Bayram O, Bayram O, Feussner K, Kim JH, Kim HS, Kaever A, Feussner I, Chae KS, Han DM, Han KH, Braus GH (2014) Membrane-bound methyltransferase complex VapA-VipC-VapB guides epigenetic control of fungal development. Dev Cell 29:406–420

Schumacher J, Gautier A, Morgant G, Studt L, Ducrot PH, Le Pecheur P, Azeddine S, Fillinger S, Leroux P, Tudzynski B, Viaud M (2013) A functional bikaverin biosynthesis gene cluster in rare strains of *Botrytis cinerea* is positively controlled by VELVET. PLoS One 8(1), e53729

Schwedtfeger C, Linden H (2003) VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J 22:4846–4855

Seiboth B, Karimi RA, Phatale PA, Linke R, Hartl L, Sauer DG, Smith KM, Baker SE, Freitag M, Kubicek CP (2012) The putative protein methyltransferase LAE1 controls cellulase gene expression in *Trichoderma reesei*. Mol Microbiol 84:1150–1164

Seong KY, Pasquali M, Zhou X, Song J, Hilburn K, McCormick S, Dong Y, Xu JR, Kistler HC (2009) Global gene regulation by *Fusarium* transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis. Mol Microbiol 72:354–367

Stinnett SM, Espeso EA, Cobeno L, Araujo-Bazan L, Calvo AM (2007) *Aspergillus nidulans* VeA subcellular localization is dependent on the importin alpha carrier and on light. Mol Microbiol 63:242–255

Sugui JA, Pardo J, Chang YC, Mullbacher A, Zarember KA, Galvez EM, Brinster L, Zerfas P, Gallin JI, Simon MM, Kwon-Chung KJ (2007) Role of laeA in the Regulation of alb1, gliP, Conidial Morphology, and Virulence in *Aspergillus fumigatus*. Eukaryot Cell 6:1552–1561

Sun S-K, Snyder WC (1981) The bakanae disease of the rice plant. In: Nelson PE, Toussoun TA, Cook RJ (eds) *Fusarium*: diseases, biology and taxonomy. Pennsylvania State University Press, University Park, PA

Sweeney MJ, Dobson AD (1999) Molecular biology of mycotoxin biosynthesis. FEMS Microbiol Lett 175:149–163

Talora C, Franchi L, Linden H, Ballario P, Macino G (1999) Role of a white collar-1-white collar-2- complex in blue-light signal transduction. EMBO J 18:4961–4968

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Trail F, Mahanti N, Linz J (1995) Molecular biology of aflatoxin biosynthesis. Microbiology 141:755–765

Tsitsigiannis D, Zarnowski R, Keller NP (2004) The lipid body protein, PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Tsitsigiannis DI, Bok JW, Andes D, Nielsen KF, Frisvad JC, Keller NP (2005) *Aspergillus* cyclooxygenase-like enzymes are associated with prostaglandin production and virulence. Infect Immun 73:4548–4559

Van den Broek P, Pittet A, Hajjaj H (2001) Aflatoxin genes and the aflatoxigenic potential of Koji moulds. Appl Microbiol Biotechnol 57:192–199

Webster RH, Sil A (2008) Conserved factors Ryp2 and Ryp3 control cell morphology and infectious spore formation in the fungal pathogen *Histoplasma capsulatum*. Proc Natl Acad Sci USA 105:14573–14578

Wicklow D (1987) Survival of Aspergillus flavus sclerotia in soil. Trans Br Mycol Soc 89:131–134

Wiemann P, Brown DW, Kleigrewe K, Bok JW, Keller NP, Humpf HU, Tudzynski B (2010) FfVel1 and FfLae1, components of a velvet-like complex in *Fusarium fujikuroi*, affect differentiation, secondary metabolism and virulence. Mol Microbiol 972–994

Wu D, Oide S, Zhang N, Choi MY, Turgeon BG (2012) ChLae1 and ChVel1 regulate T-toxin production, virulence, oxidative stress response, and development of the maize pathogen *Cochliobolus heterostrophus*. PLoS Pathog 8(2), e1002542

Yager L (1992) Early developmental events during asexual and sexual sporulation in *Aspergillus nidulans*. In: Bennet J, Klich M (eds) *Aspergillus*: biology and industrial applications. Butterworth-Heinemann, Boston, pp 19–41

Yager LN, Lee HO, Nagle DL, Zimmerman JE (1998) Analysis of *fluG* mutations that affect light-dependent conidiation in *Aspergillus nidulans*. Genetics 149:1777–1786

Yang Q, Chen Y, Ma Z (2013) Involvement of BcVeA and BcVelB in regulating conidiation, pigmentation and virulence in *Botrytis cinerea*. Fungal Genet Biol 50:63–71

Zhang YQ, Wilkinson H, Keller NP, Tsitsigiannis DI (2004) Secondary metabolite gene clusters. In: An Z (ed) Handbook of industrial microbiology. Dekker, New York

Biosystematic Index

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)

D

R

Subject Index

Growth, Differentiation and Sexuality, 3rd Edition
The Mycota I
J. Wendland (Ed.)