Fruiting-Body Development in Ascomycetes

S. Pöggeler[1], M. Nowrousian[2], I. Teichert[2], A. Beier[2], U. Kück[2]

CONTENTS

[1] Institut für Mikrobiologie und Genetik, Genetik eukaryotischer Mikroorganismen, Universität Göttingen, Göttingen, Germany; e-mail: spoegge@gwdg.de
[2] Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, Bochum, Bochum, Germany; e-mail: ulrich.kueck@rub.de

I. Introduction

Fruiting bodies are multicellular structures, which protect the products of meiosis, the sexual spores. They occur during the sexual life cycle of the *Dikarya*, a group that encompasses the ascomycetes and basidiomycetes (Hibbett et al. 2007; Peraza-Reyes and Malagnac 2016) (Fig. 1). However, only filamentous species show the development of fruiting bodies, while yeasts never exhibit comparable structures.

In this chapter, which is an extension and update of a previous review in this series (Pöggeler et al. 2006b), we will give an overview of the development of fruiting bodies in ascomycetes, including an outline of some model ascomycetes, which have been used to study fruiting-body development at the molecular level. Further, we will summarize factors that can either be environmental or endogenous, which control this process. Finally, regulatory networks will be mentioned that govern fruiting-body development. This includes signal transduction pathways, protein degradation mechanisms, and transcriptional regulatory networks. Ultimately, we observe that novel experimental approaches such as quantitative mass spectrometry, functional genomics, or super resolution microscopy have begun to improve our knowledge about the mechanistic

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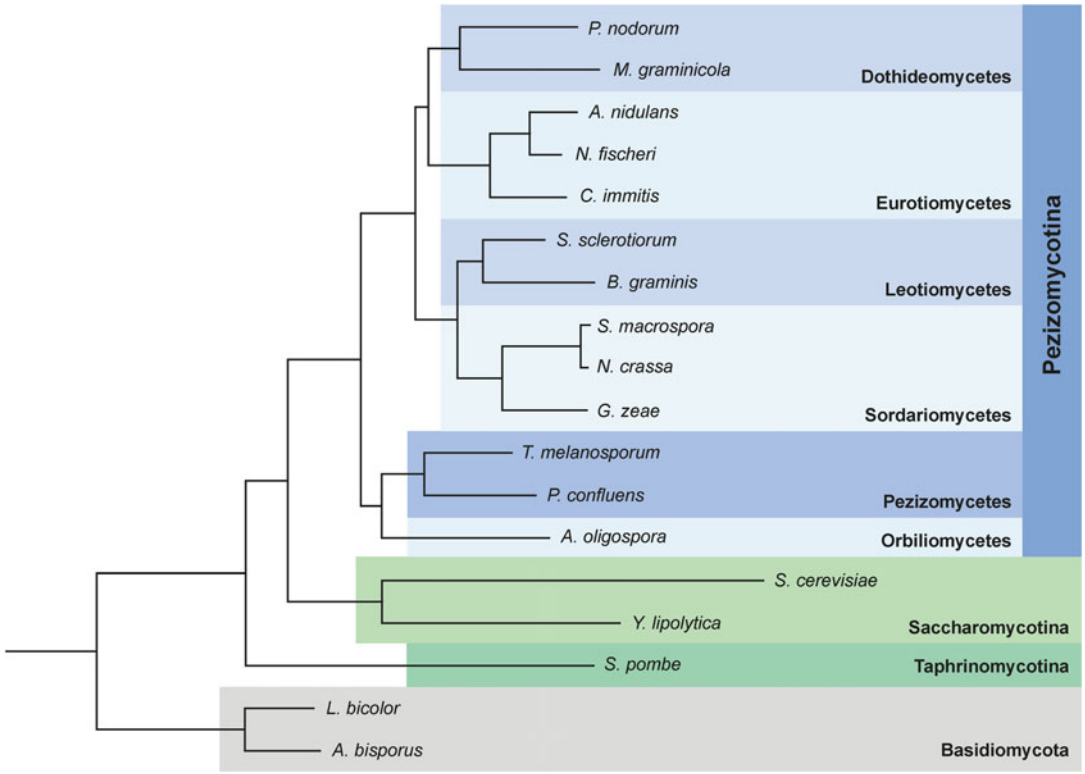

Fig. 1 Phylogenetic tree of *Ascomycota*. Characteristic species are given as examples. Branch lengths are proportional to genetic distances [adapted from Traeger et al. (2013)]. Species used to construct the phylogenetic tree: *Agaricus bisporus*, *Arthrobotrys oligospora*, *Blumeria graminis*, *Coccidioides immitis*, *Aspergillus nidulans*, *Gibberella zeae*, *Laccaria bicolor*, *Mycosphaerella graminicola*, *Neosartorya fischeri*, *Neurospora crassa*, *Phaeosphaeria nodorum*, *Pyronema confluens*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Sclerotinia sclerotiorum*, *Sordaria macrospora*, *Tuber melanosporum*, *Yarrowia lipolytica*. *S. pombe*, *L. bicolor*, and *A. bisporus* served as outgroups

processes that lead to the formation of multicellular structures.

A. Fungal Sexual Development

Fungi propagate either asexually or sexually. Asexual propagation is characterized by mitotic divisions, and as a result, endospores within sporangia or exospores like conidia are generated. In contrast, sexual propagation is characterized by karyogamy and meiotic divisions, and fungi share this feature with most other eukaryotes. Generally, sexual reproduction is thought to be the source of genetic diversity. During meiotic divisions, recombination occurs between chromosomes of two heterogenetic mating partners (Peraza-Reyes and Malagnac 2016). As a result of meiotic divisions, fungi produce four haploid spores, which may be doubled or multiplied by one or several postmeiotic mitoses.

The ascus is the meiosporangium of the Ascomycota. These sac-like sporangia carry the ascospores, the products of meiosis. In mycelial ascomycetes, asci are usually formed inside developmentally complex fruiting bodies that are called the ascomata or ascocarps. In contrast to filamentous ascomycetes, ascospores of unicellular ascomycetes (yeasts) are never found in fruiting bodies. The development of fruiting bodies is a rather complex cellular process that requires special environmental and genetic conditions, which control

the expression of developmentally regulated genes. Fruiting bodies are highly complex structures, which contain several different tissues protecting the asci. For example, 15 different cell types were recognized in fruiting bodies of the Sordariomycete *Neurospora crassa* (Bistis et al. 2003). For a coordinated fruiting-body development, enzymes involved in cell wall biogenesis and metabolism are required, as well as genes responsible for the cytoskeleton structure and organization. Here we will mention some representative examples, and a more detailed description on this subject can be found in our previous review (Pöggeler et al. 2006a). The *ami1* gene from *Podospora anserina*, for example, is necessary for nuclear positioning, most likely by regulating components of the dynein pathway. This gene was shown to be responsible for male fertility, and deletion results in a delayed formation of fruiting bodies in the corresponding mutants (Bouhouche et al. 2004). The outer shell of the fruiting body, the peridium, is an essential structure to protect the meiosporangia with the ascospores. The peridium consists of bundles of filamentous cells, and their cell walls have three main constituents, namely, chitin, mannan, and β-glucan. Though the related biosynthetic pathways have intensively been investigated, it has not been demonstrated with certainty that the corresponding genes are preferentially expressed in fruiting-body tissues. For *Sordaria macrospora* functional analysis of the class VII (division III) chitin synthase gene (*chs7*) has shown that it is dispensable for fruiting-body formation, but the corresponding mutant displayed sensitivity toward cell wall stress (Traeger and Nowrousian 2015). Another result comes from *Tuber borchii*, where three genes for chitin synthesis were investigated. Albeit they are constitutively expressed in vegetative mycelium, they show a differential expression in sporogenic or vegetative tissue of the fruiting bodies (Balestrini et al. 2000). In contrast, several chs mutants from *N. crassa* and *Aspergillus nidulans* show severe defects in perithecial development (Fajardo-Somera et al. 2015).

Important pigments of the cell walls are melanins. They are synthesized either through the DHN (1,8-dihydroxynaphthalene) or the DOPA (L-3,4-dihydroxyphenylalanine) pathways. Some can also be generated by the L-tyrosine degradation pathway (Langfelder et al. 2003). One of the best-characterized melanin biosynthetic pathways is the DHN melanin pathway, which has been verified for many members of the Pezizomycotina. Melanins stabilize the cell wall and provide protection against UV light-induced DNA damage. An investigation with *S. macrospora* showed that expression of melanin biosynthesis genes is correlated with fruiting-body development. For example, melanin gene expression is highly repressed in submerged cultures, where no sexual development occurs. Similarly, sterile mutants of *S. macrospora* showed only reduced transcript levels of melanin biosynthesis genes (Engh et al. 2007). Finally, mutants with a defect in melanin biosynthesis from *Ophiostoma piliferum* and *Podospora anserina* showed defects in the formation of fruiting bodies. These observations are consistent with early reports for *N. crassa*, *P. anserina*, and *Tuber* species, where correlation between melanin biosynthesis and the reproductive cycle was suggested (Hirsch 1954; Esser 1966; Prade et al. 1984; Ragnelli et al. 1992; Teichert and Nowrousian 2011).

Very important proteins of the cell wall are the hydrophobins and lectins. Although they have mainly been characterized in higher basidiomycetes, where they are implied in mushroom formation, they have also a function in the Pezizomycotina. Cryparin, a class II hydrophobin, was found mainly in the cell walls of fruiting bodies from the chestnut blight pathogen, *Cryphonectria parasitica*. Deletion mutants lacking the cryparin gene were unable to generate wild-type-like fruiting bodies. Thus, this pathogen needs hydrophobins for its fitness under natural conditions (Kazmierczak et al. 2005).

On the genetic level, there are many genes regulating the sexual cycle of ascomycetes. Important master genes involved in the general control of sexual development are part of the mating-type loci. They have been found so far in all ascomycetes, irrespective of whether they produce fruiting bodies or not. Their regulatory role during the sexual cycle has been thoroughly

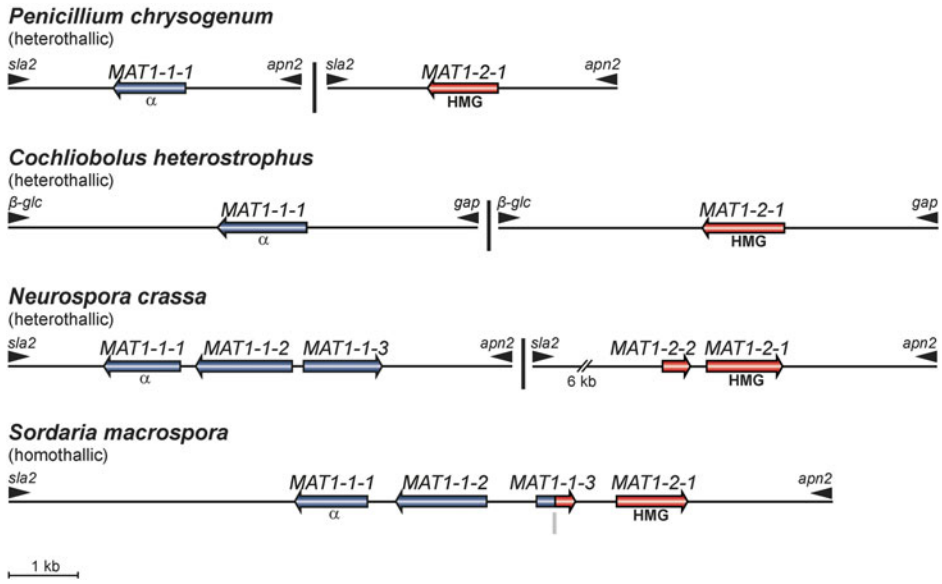

Fig. 2 Examples of mating-type loci of heterothallic members of the Dothideomycetes (*Cochliobolus heterostrophus*) (Wirsel et al. 1998), Eurotiomycetes (*Penicillium chrysogenum*) (Böhm et al. 2013, 2015), and Sordariomycetes (*N. crassa*). For comparison the mating-type locus encoding four open reading frames from the homothallic fungus *S. macrospora* (Sordariomycetes) is shown. The flanking regions often carry conserved genes, such as *sla2* (cytoskeleton assembly control factor) and *apn2* (DNA lyase). An exception is the MAT locus from *C. heterostrophus* with the following flanking genes: GAP, GTPase-activating protein; ß-Glc, ß-glucosidase. Abbreviations: "α" and "HMG" indicate genes encoding transcription factors with conserved DNA-binding domains

studied in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which produce asci, but no fruiting bodies. The role of mating-type genes during fruiting-body development is by far less well understood, although some studies have shown that mating-type genes are directly involved in fruiting-body development (Nelson and Metzenberg 1992; Pöggeler et al. 1997) (see also Sect. IV.C.1). In general, two types of fungal breeding systems are distinguished. Heterothallism involves two individuals with opposing mating types, while homothallism refers to sexual reproduction by selfing. In the latter case, individual strains do not need a mating partner to propagate sexually. Pseudohomothallism finally can be considered to be an exceptional type of heterothallism. The term was used for species that contain asci with four ascospores, each carrying two nuclei with opposite mating-type genes. Thus, after germination, these resulting heterokaryotic mycelia can undergo selfing. This type of breeding system is found, e.g., in *P. anserina* or *Neurospora tetrasperma*.

Usually the mating-type loci of heterothallic species contain dissimilar sequences, albeit they are located at identical chromosomal positions. Thus, mating-type loci do not represent alleles of a given gene but rather dissimilar DNA sequences which are called idiomorphs. MAT loci from the Pezizomycotina carry one or more open reading frames of which at least one codes for a mating-type transcription factor (TF). In general, the MAT1-1 locus of heterothallic species contains one to three open reading frames, while only a single gene is found in MAT1-2 loci. In contrast to baker's yeast, species of the Pezizomycotina carry no silent mating-type loci. Thus, mating-type switching as observed in yeast does usually not occur in heterothallic filamentous ascomycetes.

Mating-type loci encode TFs that are directly involved in the sexual life cycle. Figure 2 displays the general structure of mating-type

loci from members of the Eurotiomycetes, the *Dothideomycetes*, and the *Sordariomycetes*. The *MAT1-1-1* gene encodes a TF that is characterized by an α DNA-binding domain, while the *MAT1-2-1* gene codes for TFs with a high-mobility group (HMG) DNA-binding domain. A detailed description of mating-type locus-encoded TFs is given in Sect. IV.C.1.

B. Fruiting-Body Morphology

During their sexual life cycle, filamentous fungi of subdivision Pezizomycotina generate fruiting bodies that were historically used for their taxonomic classification. Current classification systems that rely on molecular data show that these conventional classifications contain non-monophyletic groups (Schoch et al. 2009; Ebersberger et al. 2012). However, different fruiting-body morphologies are important traits in fungal ecology, and the foremost common types of fruiting bodies (Esser 1982) are described below (Figs. 3 and 4).

1. **Cleistothecia** are closed, spherical fruiting bodies that distribute the ascospores after disintegration of the peridium of the fruiting bodies. Typically, members of the Eurotiomycetes such as *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Penicillium chrysogenum* generate cleistothecia.
2. **Pseudothecia** are spherical fruiting bodies that contain cavities (loculi) that contain the gametangia. Spores are actively discharged through openings which arise from local lysis of the peridium. Pseudothecia are, for example, found in the Dothideomycetes, e.g., *Venturia inaequalis*.
3. **Perithecia** are closed flask-like fruiting bodies that look similar to the pseudothecia. Within perithecia, sterile hyphae are found that enclose the generative tissue (hymenium). The hymenium generates asci with usually eight ascospores, which are actively discharged from the perithecium through a preformed opening, the ostiole. Perithecia are typical fruiting bodies of members of the Sordariomycetes, such as *N. crassa*, *P. anserina*, and *S. macrospora*.
4. **Apothecia** are open to cup-shaped fruiting bodies that have a hymenium layer on their surface carrying the asci. The spores are actively discharged, and examples of species that have apothecia are *Ascobolus immersus*, *Pyronema confluens*, and *Morchella* sp. within the Pezizomycetes and *Botrytis cinerea* within the Leotiomycetes.

II. Systems to Study Fruiting-Body Development

Fruiting-body development has been studied in a wide range of different ascomycetous species. Here we describe four model systems, which were used intensively for investigations on fruiting-body development. Further, we will mention some emerging model ascomycetes that were used recently for studying specific aspects of the sexual life cycle, including fruiting-body formation.

A. *Neurospora crassa*

The model fungus *Neurospora crassa* is a heterothallic species of the *Sordariaceae* and has a rather complex sexual life cycle. In general, two mating types can be distinguished, which are called “A” (MAT1-1) and “a” (MAT1-2). Both strains generate macro- and microconidia, which can be considered as male gametangia. In addition, both strains form female gametangia that are called ascogonia. Female gametangia are surrounded by supporting hyphae, which after 2–3 days generate a protoperithecium (young fruiting body). During fertilization, the female gametangium generates a distinct uptake hypha called trichogyne. This trichogyne will fuse with male gametes, which can be macro- or microconidia as described above. Alternatively, a fusion with vegetative hyphae has also been described. However, self-fertilization of each strain is prevented by an incompatibility mechanism. Thus, trichogynes can only fuse with male gametes from an opposite mating-type partner. In summary, the protoperithecium with a trichogyne from

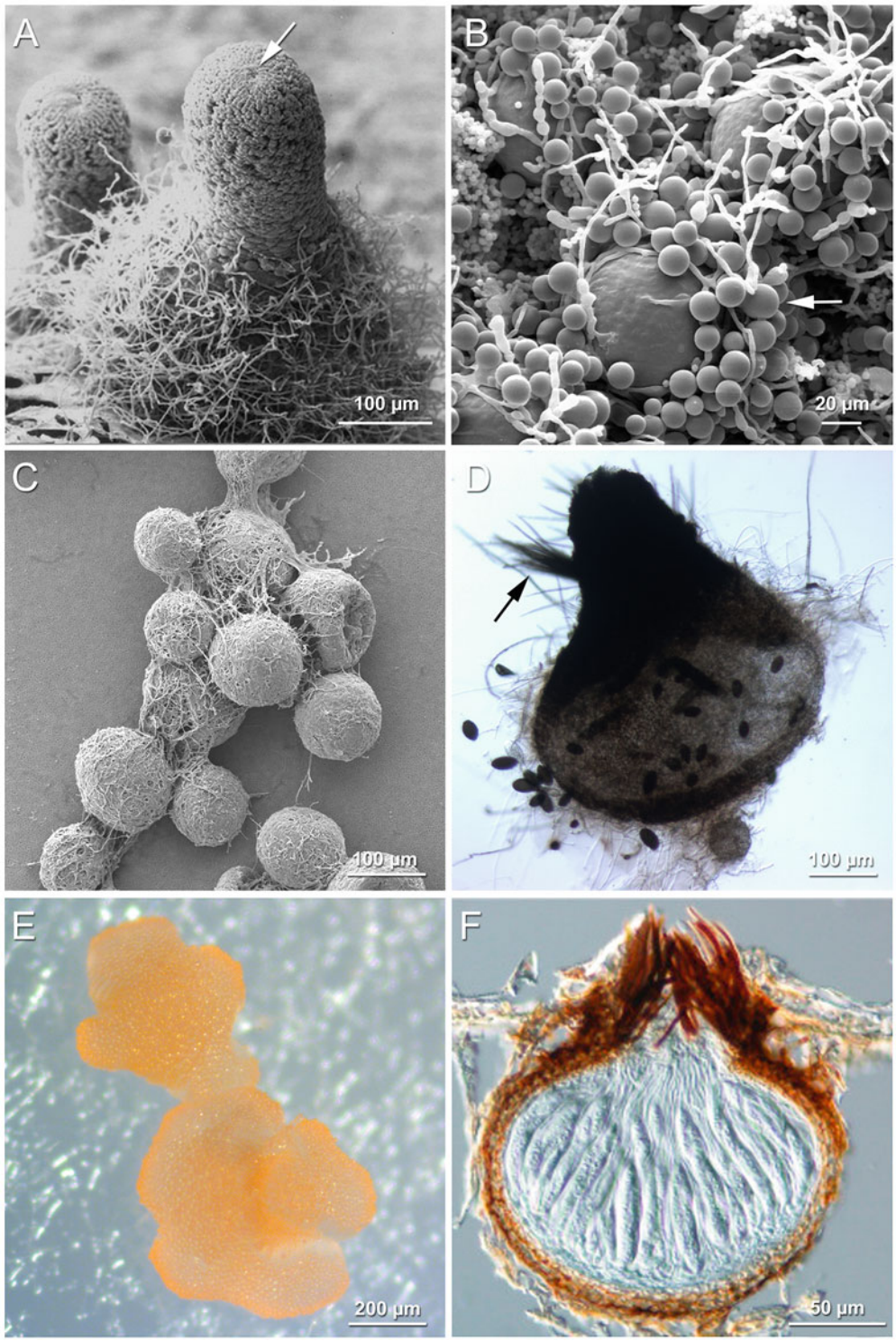

Fig. 3 Typical fruiting bodies of ascomycetes. (**a**) Perithecia of the homothallic Sordariomycete *S. macrospora*; the arrow points to the ostiolum that is used to discharge the eight-spored asci. (**b**) Cleistothecia from

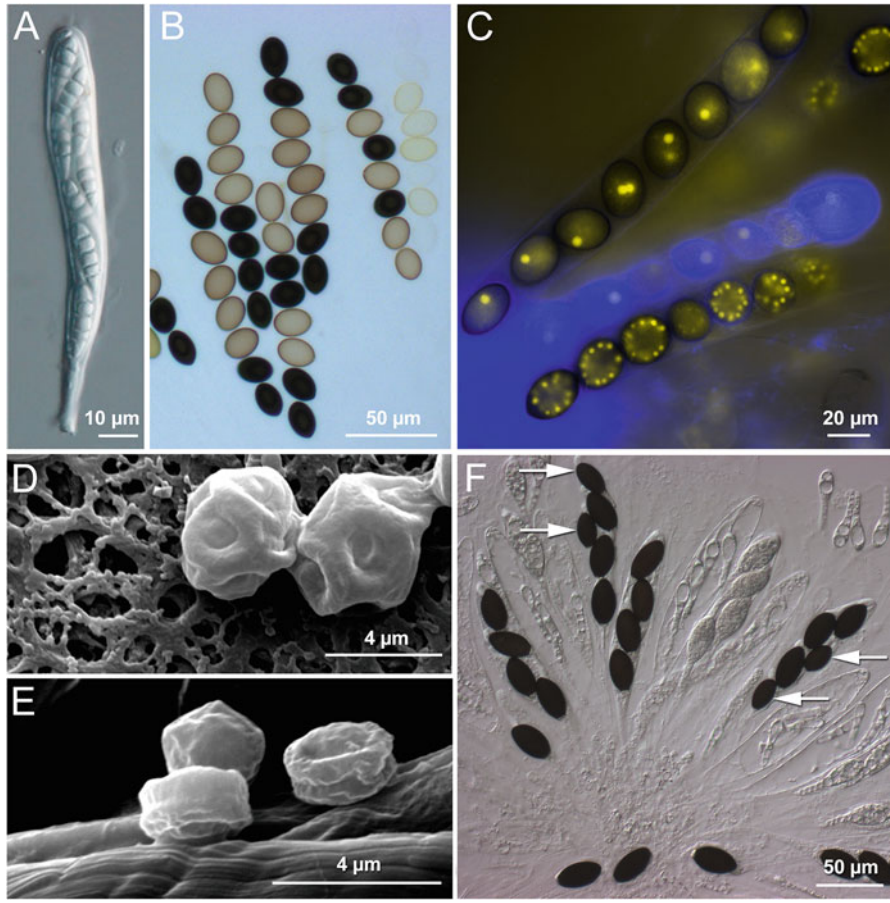

Fig. 4 Asci from different fungi of the Pezizomycotina. (**a**) Ascus from the Dothideomycete *Keissleriella quadriseptata* [from Tanaka et al. (2015)]. (**b**) Asci from *Sordaria macrospora*, obtained from a cross between a wild type (black spores) and a spore color mutant (lu with yellow spores). (**c**) Fluorescence microscopy of *S. macrospora* asci and ascospores. YFP-tagged histones label nuclei. In the upper ascus, clearly one to two nuclei are visible in each ascospore, while the lower one shows ascospores with several nuclei, which appear after several mitotic divisions. (**d**) Asci from the fungus *E. crustaceum*. (**e**) Ascospores from *E. crustaceum*. (**f**) Asci from *Podospora anserina* contain four spores or five spores. Usually, each ascospore carries two nuclei; however, in rare cases, asci contain smaller spores with only a single nucleus. Arrows indicate small spores with only a single nucleus compared to the regular ascospores with two nuclei

an "A" strain can only be fertilized by a nucleus of an "a" strain and vice versa. The fusion of the male gamete with the trichogyne will lead to the induction of the dikaryotic phase. During this phase, two genetically different nuclei exist within one cell. After several conjugated divisions, ascus development will start with the formation of ascogenous hyphae, which generate the so-called crozier cell, which undergoes conjugated divisions resulting in three cells,

Fig. 3 (continued) A. nidulans with small surrounding Hülle cells. (**c**) Cleistothecia from the homothallic fungus *Eupenicillium crustaceum*. (**d**) Perithecia from *Podospora anserina* show typical hairs (arrow) at the neck of the perithecia. (**e**) Apothecia from *P. confluens*. (**f**) Pseudothecium from the Dothideomycete *Keissleriella quadriseptata* [from Tanaka et al. (2015)]. (**a**), (**b**), and (**c**) are scanning electron micrographs and (**d**)–(**f**) light microscopy; (**b**) courtesy of G. Braus (Göttingen, Germany); (**e**) from Traeger et al. (2013); (**f**) copyright from Elsevier Press

two basal and one upper cell. The two nuclei in the upper dikaryotic cell undergo karyogamy followed by meiosis (Peraza-Reyes and Malagnac 2016). In *N. crassa*, a postmeiotic mitosis follows before spore formation starts. Thus, each ascus contains eight linearly ordered ascospores. After maturation, perithecia have a size of about 300 μm, while ascospores have a size between 15 and 30 μm. Importantly, ascospore germination occurs only after a heat shock. Fruiting-body formation in *N. crassa* was investigated in diverse genetic, biochemical, and molecular studies (Davis 1995).

B. *Podospora anserina*

P. anserina is a coprophilic fungus with a pseudohomothallic mating system, which shows similarities to the life cycle of *N. crassa*. The mating-type strains are designated "+" (MAT1-2) and "−" (MAT1-1). However, there are some distinct differences compared to *N. crassa*. As male gametes, microconidia, but no macroconidia, are generated that germinate under specific physiological conditions. Secondly, the asci usually contain only four spores, which are generated as a result of specific nuclear distribution mechanisms. After meiosis and postmeiotic mitosis, spore-wall formation covers two genetically distinct nuclei. Usually one nucleus carries the "+" and the other the "−" mating type. With a frequency of about 3%, five- or six-spored asci are generated. They carry either two or four smaller spores that carry only a single nucleus. These spores can be used to generate haploid mycelial isolates (Scheckhuber and Osiewacz 2008; Peraza-Reyes and Malagnac 2016).

C. *Sordaria macrospora*

S. macrospora is a coprophilic fungus that is taxonomically closely related to the above-described species *N. crassa* and *P. anserina*. The life cycles of all these ascomycetes are very similar, although *S. macrospora* has a homothallic mating system. In contrast to *N. crassa* however, *S. macrospora* does not generate macro- or microconidia, and thus, only the sexual cycle contributes to the propagation of this fungus. The sexual cycle can be completed in the laboratory within 1 week, since ascospores require no heat shock or resting period for germination (Esser and Straub 1958). The sexual cycle starts with the formation of ascogonia. However, so far the molecular mechanisms leading to the formation of the dikaryotic hyphae are not understood. After karyogamy of two nuclei in the abovementioned crozier cells, meiosis will follow to generate the meiotic products as a source for ascospore formation. Similar to ascus formation in *N. crassa*, meiosis is followed by a postmeiotic mitosis. As a result, eight ascospores within a single ascus are derived from a single dikaryotic mother cell.

As mentioned above, sexual reproduction is a source of genetic diversity. Usually strains of opposite mating types from heterothallic species (e.g., *N. crassa* or *P. anserina*) are used for conventional genetic recombination studies. However, it has been shown for many species that recombination can also occur between two strains of a homothallic species. In these cases, the strains are distinguished by at least a single mutation. Homothallic species such as *S. macrospora* and other *Sordaria* species are used for conventional genetic analysis (Teichert et al. 2014a).

D. *Aspergillus nidulans*

A. nidulans, which is like *S. macrospora* a homothallic species, was used extensively to study genetic recombination and fruiting-body formation. The sexual cycle starts with the formation of ascogonia and later dikaryotic hyphae, a process, which is probably very similar to the life cycle of *S. macrospora*. Within cleistothecia, spherical asci are generated containing eight ascospores. These octades are unordered and thus distinguished from the ordered asci of the abovementioned species. In recent years, several factors controlling cleistothecia formation were studied extensively, such as the velvet complex (Bayram and Braus 2012) (see Sect. IV.C).

E. Emerging Model Systems

Here we mention fungal genera or species, which were used recently to investigate fruiting-body development.

1. *Aspergillus fumigatus*, *A. flavus*, and *A. parasiticus*

The genus *Aspergillus* comprises about 340 species and was traditionally believed to contain species that generally propagate only asexually. Species of this genus having a sexual life cycle were grouped in the teleomorphic genera *Eurotium*, *Emericella*, *Neosartorya*, or *Petromyces*. Recently evidence emerged that heterothallic *Aspergillus* species, which were for long believed to propagate exclusively asexually, have also the potential to undergo sexual reproduction. These are, for example, the human pathogen *A. fumigatus* and the mycotoxin-producing fungi *A. flavus* and *A. parasiticus* (Dyer and O'Gorman 2011, 2012; Kück and Pöggeler 2009; Dyer and Kück 2017).

2. *Botrytis cinerea*

The gray mold *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana)* is a notorious plant pathogenic fungus with a wide host range and has become an important model in molecular plant pathology. This fungus has two dissimilar mating types (MAT1-1 and MAT1-2) and is therefore heterothallic (Amselem et al. 2011). Asexual propagation occurs through microconidia in the light or sclerotia in the dark. The latter represent survival structures, which also serve the fertilization process during the sexual life cycle. When microconidia of the opposite mating type are available, the sclerotia are fertilized and generate apothecia. This process is induced by light, and fruiting bodies grow in the early stages toward the light source (Schumacher 2017). However, in the laboratory, induction of the sexual life cycle is a rather time-consuming process that takes 4 to 6 months until fully developed apothecia are obtained (Faretra and Antonacci 1987).

3. *Trichoderma reesei*

Trichoderma reesei, the anamorph of *Hypocrea jecorina*, is a major industrial enzyme producer, particularly of cellulases and hemicellulases, which are used for applications in food, feed, and biorefinery businesses. Until recently, it was believed that industrial strains propagate exclusively asexually. The industrial strains are derived from a single isolate that carries the MAT1-2 mating-type locus. Crossing experiments of *T. reesei* with *H. jecorina* MAT1-1 wild-type isolates led to fertilized fruiting bodies and the production of mature ascospores. However, the industrial *T. reesei* strain, which was used for mating experiments, can be used only as male partner in crossings but is unable to produce fruiting bodies and thus has to be considered to be female sterile (Seidl et al. 2009; Linke et al. 2015; Schmoll and Wang 2016).

4. *Penicillium* species

Like *Aspergillus* species, *Penicillium* species belong to the order of *Eurotiales*. Most species were for long believed to represent the asexual teleomorphs of the genera *Eupenicillium* or *Talaromyces*. However, the recent discovery of a sexual cycle with cleistothecia in the industrial penicillin producer *P. chrysogenum* was further evidence that supposedly asexual fungi can undergo a heterothallic life cycle. Genetic analysis has provided evidence that even industrial strains have conserved their potential to undergo a recombinant genetic cycle (Böhm et al. 2013, 2015). Recently, also the cheese fungus *Penicillium roqueforti* was shown to have a sexual cycle with ascogonia, cleistothecia, and ascospores (Ropars et al. 2014). These investigations suggest that the life cycle of sexually propagating *Penicillium* species is very similar to the one of heterothallic *Aspergillus* species.

5. *Fusarium graminearum* and *F. verticillioides*

Fusarium species belong to the *Sordariomycetes*, which are characterized by perithecia formation. *F. graminearum* (syn. *Gibberella zeae*)

and *F. verticillioides* (syn. *Gibberella moniliformis*) are plant pathogenic fungi, which are responsible for high losses in the harvest of cereals. *F. graminearum* is homothallic, and its sexual spores are responsible for disease initiation. In contrast, *F. verticillioides* is heterothallic and produces only modest numbers of fruiting bodies. Both fungi have been used for comparative expression studies, using six developmental stages of perithecia (Sikhakolli et al. 2012; Geng et al. 2014). Recently, *F. graminearum* served as source in several genome-wide large-scale functional analyses to decipher developmental genes and events related to sexual development. These attempts identified targets of the mating-type locus-encoded TFs, RNA interference (RNAi) mechanisms, and perithecium-specific RNA-editing events (Kim et al. 2015; Liu et al. 2016; Son et al. 2017)

6. *Pyronema confluens*

P. confluens is a homothallic soil-living saprophytic Pezizomycete, which forms apothecia in a light-dependent manner. The eight-spored asci are generated under laboratory conditions within 6 days. In the early twentieth century, this fungus was one of the first examples to elucidate the dikaryotic phase during sexual development in filamentous ascomycetes. Extensive transcriptome analysis was recently performed to investigate the light-dependent fruiting-body formation (Traeger et al. 2013).

III. Factors Influencing Fruiting-Body Development

A. Environmental Factors

Ascomycetes generate fruiting bodies in certain environmental conditions that are species-specific. Among others, light, nutrients, temperature, physical properties of growth substrates, and atmospheric conditions are relevant for fruiting-body formation. In this review, we will focus on the influence of light and nutrients, highlighting recent advances in our understanding of how these factors integrate into developmental signaling.

1. Light

Fungi respond to light in various ways, including phototropic growth of reproductive structures, modification of (circadian) rhythms, and changes in gene expression as well as primary and secondary metabolism, among others (Casas-Flores and Herrera-Estrella 2016). Here, we will focus on the influence of light on sexual reproduction of ascomycetes.

Light-dependent fruiting-body formation, phototropism of perithecial necks, and light-dependent ascospore discharge have been described early on for a number of ascomycetes (reviewed in Moore-Landecker 1992). For example, light is required for apothecia formation in *Ascobolus magnificus*, *Pyronema confluens*, and *P. domesticum* and for perithecia formation in *Pleurage setosa* (syn. *Podospora setosa*) (Yu 1954; Carlile and Friend 1956; Callaghan 1962). In *T. reesei*, formation of stromata, harboring the perithecia, occurs only in the presence of light (Seidl et al. 2009), while light inhibits formation of *B. cinerea* sclerotia, which serve as survival structures and female mating partners (Schumacher 2017). Light-dependent positioning of the perithecial neck has been described, e.g., for *Neurospora crassa* and *P. setosa* (Callaghan 1962; Harding and Melles 1983). In *Aspergillus glaucus* and *A. nidulans*, light favors asexual reproduction, while darkness favors sexual reproduction (Chona 1932; Mooney and Yager 1990; Blumenstein et al. 2005).

Ascomycetes possess several **photoreceptors**, sensing near-UV/blue, green, and red light (Idnurm and Heitman 2005; Casas-Flores and Herrera-Estrella 2016). All of these photoreceptors sense light by physical interaction with a chromophore: flavin for near-UV/blue and blue-light receptors, retinal for green-light receptors, and linear tetrapyrroles for red-light receptors. Upon photon absorption, structural changes in the chromophore induce conformational changes in the photoreceptors, leading to changes in protein-protein interactions or

signaling via certain output domains (see below). The repertoire of photoreceptors sensing diverse light qualities differs between species, and, e.g., *A. nidulans* and *B. cinerea* encode 3 and 11 photoreceptors, respectively (Schumacher 2017).

Blue-light receptors include cryptochromes and LOV (light oxygen voltage) domain proteins. Cryptochromes sense near-UV/blue light by binding flavin dinucleotide (FAD) and pterins/folates. Most cryptochromes show strongly reduced or no photolyase activity, one exception being *A. nidulans* (Bayram et al. 2008a). The LOV domain is a specialized PAS (found in Per, Arnt, Sim) domain, which binds the flavin chromophore. LOV domain proteins may contain additional output domains, e.g., zinc finger or RGS (regulator of G-protein signaling) domains. The white collar 1 homologs are light-activated transcription factors with three PAS domains, one of which is a FAD-binding LOV domain, and a GATA-type zinc finger DNA-binding domain. WC-1 of *N. crassa* interacts with a second white collar protein, WC-2, via its PAS domains, and both control expression of light-regulated genes by binding to promoter sequences as a heterodimer (Cheng et al. 2002; Froehlich et al. 2002; He et al. 2002; Smith et al. 2010). An example for a LOV domain protein without further output domains is *N. crassa* Vivid (VVD-1) involved in photoadaptation (Chen et al. 2010).

Green-light receptors have not been extensively characterized in filamentous ascomycetes so far. These receptors are related to rhodopsins, composed of a seven-transmembrane domain opsin bound to retinal via a conserved lysine. Retinal binding in opsins leads to the formation of green-light responsive ion pumps as demonstrated for the CarO protein of *F. fujikuroi* (Garcia-Martinez et al. 2015). However, other opsins like *N. crassa* NOP-1 lack proton pump activity, indicating a putative regulatory role (Bieszke et al. 1999; Brown et al. 2001).

Fungal **phytochromes** are red-light sensors and contain multiple domains, with an N-terminal photosensory domain binding to a bilin-type linear tetrapyrrole, a histidine kinase domain, and a response regulator domain. The photosensory domain combines PAS, GAF (for vertebrate cGMP-specific phosphodiesterases, cyanobacterial adenylate cyclases, transcription activator FhlA), and PHY (phytochrome-specific PAS-related) domains. Absorption of red light leads to reversible conformational changes of the chromophore, shifting the absorption maximum to far-red light. Thus, phytochromes are able to sense red/far-red ratios (Rockwell and Lagarias 2010).

Blue- and red-light photoreceptors have roles in sexual development in various fungi (for a review, see Casas-Flores and Herrera-Estrella 2016; Dasgupta et al. 2016; Fischer et al. 2016). Besides photoreceptors, chromatin modifiers (see Sect. IV.C.3), MAPK pathways (see Sect. IV.A.1), and transcription factors (see Sect. IV.C.1) are required for light regulation of sexual development. Light perception and light signal transduction have been analyzed in great detail in few model systems, including *A. nidulans*, *N. crassa*, and *T. reesei* (Seidl et al. 2009; Bayram et al. 2010; Schmoll et al. 2010a; Dasgupta et al. 2016). An overview of photoreceptors of these three model fungi and their role in sexual development is shown in Fig. 5.

As mentioned above, *A. nidulans* reproduces mainly sexually in the dark and mainly asexually in light, and blue together with red light is the responsible light quality. Molecular genetic analysis has shown that cryptochrome CryA, the white collar proteins LreA (light response A) and LreB, as well as the phytochrome FphA are required for this light-dependent behavior (Blumenstein et al. 2005; Bayram et al. 2008a; Purschwitz et al. 2008). The cryptochrome CryA still has photolyase activity and functions in DNA repair. Notably, *A. nidulans* lacks a vivid and a functional, i.e., retinal-binding opsin homolog and has only one phytochrome, FphA, which binds biliverdin and absorbs red as well as far-red light (Bayram et al. 2008a; Idnurm et al. 2010; Fischer et al. 2016). CryA and FphA repress sexual development in light conditions, while LreA and LreB stimulate sexual development, and *lreA* as well as *lreB* deletion mutants generate no perithecia in the light (Blumenstein et al. 2005; Bayram et al. 2008a; Purschwitz et al. 2008).

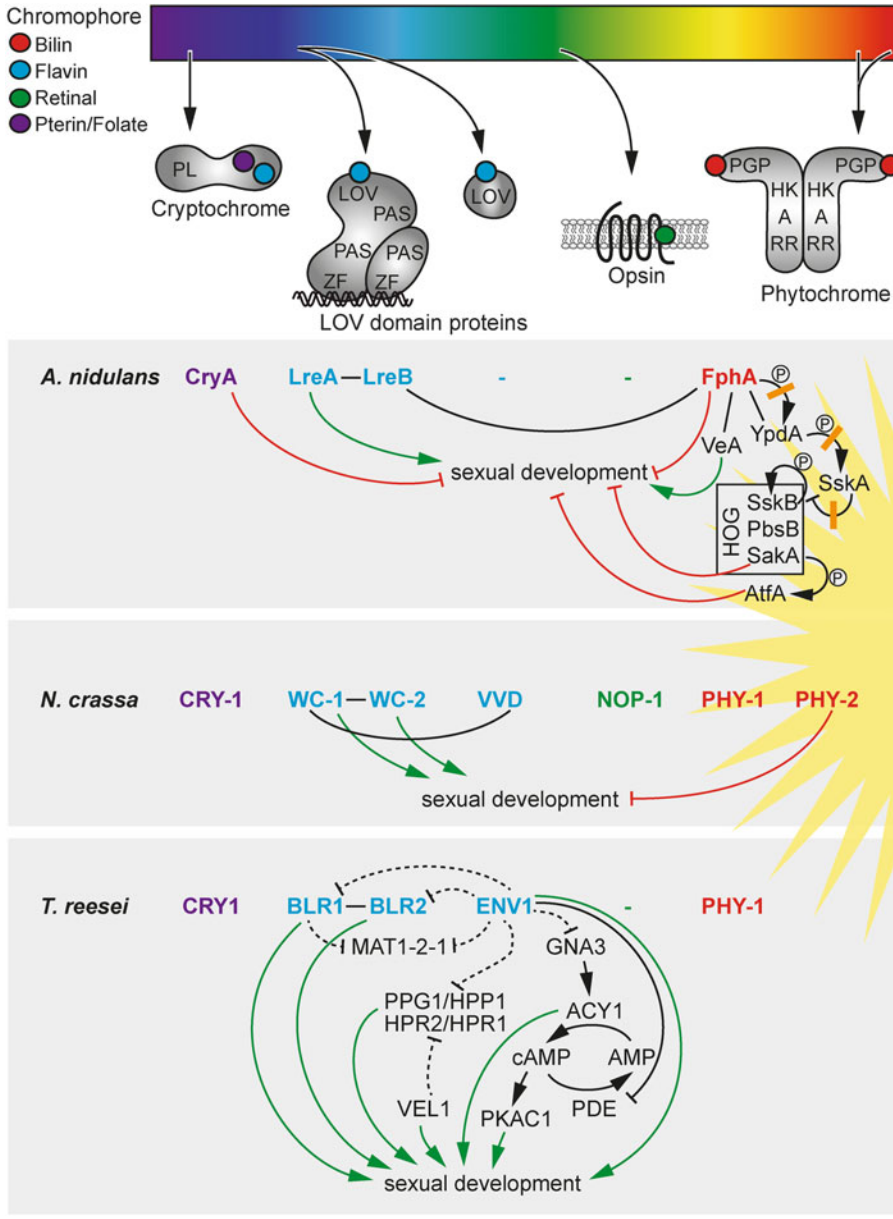

Fig. 5 Regulatory role of photoreceptors in sexual development of model ascomycetes. Data are summarized from recent reviews and research articles (Casas-Flores and Herrera-Estrella 2016; Dasgupta et al. 2016; Fischer et al. 2016; Schmoll and Wang 2016; Bazafkan et al. 2017). The upper part of the figure summarizes photoreceptor types found in fungi and their respective chromophors. The lower part of the figure summarizes the occurrence of photoreceptors and current knowledge on signal transduction in light conditions in the three model fungi *A. nidulans*, *N. crassa*, and *T. reesei*. Dashed lines indicate transcriptional regulation, while continuous lines indicate physical interaction. An encircled P symbolizes phosphorylation. Bold orange lines indicate light-induced interruption of signal transduction. For details, see main text. A, ATPase domain; HK, histidine kinase; LOV, light oxygen voltage domain; PAS, found in Per, Arnt, Sim; PGP, photosensory domain of phytochromes combining PAS, GAF (for vertebrate cGMP-specific phosphodiesterases, cyanobacterial adenylate cyclases, transcription activator FhlA), and PHY (phytochrome-specific PAS-related) domains; PL, photolyase; RR, response regulator domain; ZF, GATA-type zinc finger DNA-binding domain

Light signal transduction in *A. nidulans* involves several key regulators of development, namely, the velvet complex and the high-osmolarity glycerol (HOG) MAPK pathway (Purschwitz et al. 2008; Yu et al. 2016). FphA interacts with both, LreB and the velvet protein VeA, in the nucleus, and the latter interaction is dependent on light. Furthermore, VeA nuclear accumulation is partially dependent on FphA, and VeA is required for binding of LreA to promoter sequences (Purschwitz et al. 2008; Hedtke et al. 2015). In concert, LreA and FphA modulate gene expression by inducing histone acetylation, linking light signaling and chromatin remodeling (see Sect. IV.C.3). Recently, a regulatory function of FphA in the cytoplasm was elucidated (Yu et al. 2016). FphA is the upstream component of a phosphorelay system consisting of phosphotransfer protein YpdA and response regulator SskA (Fig. 5). In the dark, phosphorylation inhibits interaction of SskA with the downstream HOG pathway, consisting of MAPKKK SskB, MAPKK PbsB, and MAK SakA (see Sect. IV.A.1). In the light, phosphorylation of FphA, YpdA, and SskA decreases, leading to activation of the HOG pathway, light-dependent translocation of the MAPK SakA to the nucleus, and activation of the transcription factor AtfA (Yu et al. 2016). SakA and AtfA are activated during asexual development and repress cleistothecia formation (Kawasaki et al. 2002), explaining why red light represses sexual development in *A. nidulans*.

In *N. crassa*, the blue-light response is best characterized. In this ascomycete, blue light regulates perithecial neck positioning and neck orientation, and protoperithecia production is induced by blue light (Harding and Melles 1983; Innocenti et al. 1983; Oda and Hasunuma 1997). All of these light responses are mediated by the white collar proteins WC-1 and WC-2, but not cryptochrome CRY-1 (Harding and Melles 1983; Degli-Innocenti and Russo 1984; Oda and Hasunuma 1997; Froehlich et al. 2010) (Fig. 5). The role of the WC complex in circadian rhythm has been studied in great detail (reviewed in Hurley et al. 2015; Montenegro-Montero et al. 2015), but its regulatory function in sexual development remains largely elusive. As for *A. nidulans*, an interplay of the WC complex (WCC) and histone acetylation in the activation of light-induced genes has been described (Grimaldi et al. 2006). Further, it has been suggested that WCC activity is modulated by other photoreceptors, namely, CRY-1, opsin NOP-1, and one of the two phytochromes, PHY-2, and that some light responses require the velvet homolog VE-1 (Olmedo et al. 2010). Recently, phytochrome PHY-2 was described to regulate proper timing of sexual development, since deletion of *phy-2* led to early induction of protoperithecia formation in red light (Wang et al. 2016b) (Fig. 5). Taken together, regulation of sexual development by light may use the same protein machinery in *N. crassa* as in *A. nidulans*, but the wiring of light signaling needs to be analyzed further.

Unlike *A. nidulans*, *N. crassa*, and most other ascomycetes, *T. reesei* requires light for timely sexual reproduction (Seidl et al. 2009; Chen et al. 2012). *T. reesei* exhibits all photoreceptor types but an opsin homolog, and an influence on sexual development has been described for the blue-light receptors BLR1 and BLR2 and the Vivid homolog ENVOY (ENV1) (reviewed in Schmoll et al. 2010a, 2016) (Fig. 5). While ENV1 is required for female fertility in light, BLR1 and BLR2 are not, but mutants lacking these receptors produce fewer and larger fruiting bodies (Chen et al. 2012; Seibel et al. 2012a). ENV1 effects transcription of several light-regulated genes, including pheromone receptor and peptide pheromone precursor genes as well as genes for G-protein α subunits *gna1* and *gna3* (Chen et al. 2012; Seibel et al. 2012a; Tisch and Schmoll 2013). GNA3 acts in the cAMP pathway by activating adenylyl cyclase ACY1, which generates cAMP, which in turn activates protein kinase PKAC1. Both ACY1 and PKAC1 are required for sexual development in *T. reesei* (Schuster et al. 2012) (Fig. 5). ENV1 further intervenes with the cAMP pathway by inhibiting phosphodiesterase PDE, which is required for cAMP degradation (Tisch et al. 2011) (Fig. 5). As in *A. nidulans*, velvet plays a role in light signaling. Like ENV1, the *T. reesei* VEL1 protein is required for female fertility in light,

and ENV1 and VEL1 act in concert to regulate pheromone response genes in a mating-type-dependent manner (Bazafkan et al. 2015, 2017).

Since this section focused on the influence of light on fruiting-body formation, we would like to refer the interested reader to several recent reviews that focused on other aspects of fungal life affected by light (Casas-Flores and Herrera-Estrella 2016; Dasgupta et al. 2016; Fischer et al. 2016; Schumacher 2017).

2. Nutrients

Nutrients play an important role in the induction and completion of fruiting-body formation in filamentous ascomycetes, as has been reviewed before (Moore-Landecker 1992; Pöggeler et al. 2006a; Debuchy et al. 2010). In short, most fungi generate fruiting bodies at low-nutrient conditions, although there are exceptions, e.g., *A. nidulans* and *S. macrospora* (Molowitz et al. 1976; Han et al. 2003). A certain C/N ratio seems to be required for induction of fruiting-body formation, and this ratio is in the range of 1:3 to 1:10 (Moore-Landecker 1992). Several fungi need additional nutrients for fruiting-body formation, e.g., vitamins or certain amino acids. *S. fimicola* and *S. macrospora* require biotin for perithecia formation, and arginine is required by *S. macrospora* for timely completion of the sexual cycle (Barnett and Lilly 1947; Molowitz et al. 1976).

In recent years, research has focused on signaling pathways sensing nutrient status and on transport routes. In general, fungi sense nutrients using plasma membrane proteins, among them **G-protein-coupled receptors** (GPCRs) (Van Dijck et al. 2017). The *A. nidulans* GPCRs GprD and GprH are required for repression of sexual development in low-nutrient conditions. GprD probably represses sexual development via regulation of glucose and amino acid metabolism, while GprH senses glucose and tryptophan and acts upstream of the cAMP-PKA pathway (de Souza et al. 2013b; Brown et al. 2015). Proton-coupled dipeptide transporters (PTR2s) support sexual development in *F. graminearum*, since mutants lacking FgDPTR2A, FgDPTR2C, or FgDPTR2D formed fewer perithecia than wild type (Droce et al. 2017). In *T. reesei*, the gene encoding a homolog of yeast ABC transporter Ste6p is strongly upregulated on lactose. Since Ste6p exports the **a** pheromone in yeast in an ATP-dependent manner, this upregulation indicates an effect of carbon sources on fruiting-body formation in *T. reesei* (Ivanova et al. 2013; Schmoll et al. 2016).

Signaling proteins have also been shown to simultaneously influence nutrient utilization and fruiting-body formation. The *F. graminearum* sucrose nonfermenting 1 (GzSNF1) protein kinase is involved in utilization of alternative carbon sources and sexual development. A GzSNF1 mutant generated 30% less perithecia than wild type, and asci were abnormal in containing one to eight ascospores that were abnormally shaped (Lee et al. 2009b). In *N. crassa*, the kinase IME-2 (inducer of meiosis 2) supposedly downregulates protoperithecia formation by inhibiting the transcription factor VIB-1 (see Table 2) in the presence of nitrogen (reviewed in Irniger 2011). Ime2 kinases are conserved S/T kinases that function in meiotic control in *S. cerevisiae*. Ime2 homologs from various fungi are involved in repression of fruiting-body formation in response to environmental signals. As mentioned above (Sect. III.A.1), the small LOV domain protein ENV1 is a central regulator of sexual development in *T. reesei*. ENV1 and the phosducin-like protein PhLP1 involved in G-protein signaling were found in a mutual interrelationship to downregulate nutrient signaling as an early response to light, supposedly to enable protective measures prior to metabolic adaptation (Tisch et al. 2014). How exactly ENV1 and other signaling proteins integrate diverse environmental signals to control fruiting-body formation remains to be determined. Transcription factors might be the targets of these pathways (see Sect. IV.C.1).

B. Endogenous Factors

In addition to environmental factors described above, fungi need endogenous substances to reproduce sexually. Nutrients are processed

via metabolic processes, and primary as well as secondary metabolites are required for or modulate fruiting-body formation. Moreover, pheromones are necessary at distinct stages of fruiting-body formation.

1. Primary and Secondary Metabolites

The influence of metabolites on fruiting-body formation has been documented by the sterility of mutants defective in diverse metabolic pathways, as reviewed before (Pöggeler et al. 2006a). The occurrence of these mutants indicates a requirement for certain metabolites during sexual reproduction. An example is sterile mutant pro4 from *S. macrospora* with a defect in *leu1*, encoding β-isopropylmalate dehydrogenase involved in leucine biosynthesis (Kück 2005). Similarly, mutants defective in mitochondrial respiration have been reported as (female) sterile in different ascomycetes, supposedly because of the massive energy demand during sexual reproduction, as also discussed for protein degradation processes and autophagy (see Sect. IV.B) (Videira and Duarte 2002). Here, we will review recent progress concerning the role of **primary and secondary metabolites** in fruiting-body formation.

As mentioned above, many ascomycetes produce fruiting bodies upon nutrient starvation and thus rely on reserve compounds such as carbohydrate or lipid reserves. Mobilization of storage lipids requires peroxisomal functions, namely, β-oxidation and the glyoxylate pathway. Several peroxisomal import and peroxisomal metabolism mutants have been described as defective in fruiting-body formation (reviewed in Peraza-Reyes and Berteaux-Lecellier 2013). For example, in *A. nidulans*, induction of cleistothecium formation by oleic acid is abolished in mutants lacking PexF, a recycling factor for a peroxisomal import receptor (Hynes et al. 2008). In *P. anserina*, mutants of RING finger complex components, required for peroxisomal matrix protein import and subsequent export of the import receptor, are blocked in sexual development. This block occurs prior to karyogamy, leading to fruiting bodies containing no ascospores (Bonnet et al. 2006; Peraza-Reyes et al. 2008). Mutants lacking the glyoxylate cycle enzyme isocitrate lyase (ICL1) have sexual developmental defects in *F. graminearum*. Transcription of *icl1* ceases in the late sexual stage, indicating a shutdown of the glyoxylate cycle or other peroxisomal metabolic processes for fruiting-body formation in this fungus (Lee et al. 2009a). Interestingly, linoleic acid has been shown to accumulate during *Nectria haematococca* perithecium formation, and exogenous linoleic, linolenic, oleic, and palmitoleic acid stimulated perithecia formation (Dyer et al. 1993).

Nitric oxide (NO) has recently been shown to affect fruiting-body formation (Canovas et al. 2016). *A. nidulans* shows elevated levels of NO immediately after switching from vegetative growth to sexual or asexual development. Thus, NO, together with reactive oxygen species (see Sect. IV.A.3), may be an early signal triggered by or triggering development. The balance of CO_2 and HCO_3^- is another factor affecting sexual development. In *A. nidulans*, increasing CO_2 levels by sealing agar plates promotes sexual reproduction (Dyer and O'Gorman 2012). Carbonic anhydrases (CAS) catalyze the reversible interconversion of CO_2 and bicarbonate (HCO_3^-). Deletion of all four *cas* genes in *S. macrospora* led to immature perithecia that were embedded in the agar and devoid of ascospores (Lehneck et al. 2014).

A metabolic enzyme affecting fruiting-body formation is ATP citrate lyase (ACL). This enzyme generates cytoplasmic acetyl-CoA from mitochondria-derived citrate and has been shown to function in sexual development in *A. nidulans*, *F. graminearum*, and *S. macrospora*, as have *F. graminearum* acetyl-CoA synthase ACS and components of carnitine-dependent acetyl-CoA transport (Nowrousian et al. 1999; Hynes and Murray 2010; Lee et al. 2011; Son et al. 2011a, 2012). Citric acid itself may regulate sexual development. Cleistothecia formation in *A. glaucus* is strongly enhanced by citric acid, and deletion of the citrate synthase gene *citA* in *A. nidulans* abolished meiosis, leading to cleistothecia without any ascospores (Cai et al. 2010; Murray and Hynes 2010). ACL-generated acetyl-CoA is commonly used as a precursor for fatty acid and sterol biosynthesis.

Interestingly, ACL function is required for increasing histone acetylation during mammalian differentiation and has also been linked to histone acetylation in fungi (Wellen et al. 2009; Son et al. 2011a). If the sexual defects in all fungal ACL mutants are caused by defects in chromatin modification (see Sect. IV.C.2) remains to be determined.

Secondary metabolites have recently emerged as central regulators of sexual development, although a connection of secondary metabolite production and developmental processes has been known for a long time (reviewed in Calvo et al. 2002). In many ascomycetes, fruiting bodies and/or ascospores are pigmented, and often this pigment is the polyketide melanin (see Introduction). Supposedly, melanin protects ascospores from environmental damage and may also structurally reinforce three-dimensional structures by strengthening the cell wall (Langfelder et al. 2003). However, melanin per se is not required for fruiting-body formation, since mutants unable to synthesize melanin still are fertile (e.g., Engh et al. 2007; Nowrousian et al. 2012). Other secondary metabolites may function as regulators of sexual development. Loss of the polyketide synthase *pks4* gene in *S. macrospora* leads to a block of perithecia formation, while overexpression of *pks4* results in large aberrant perithecia (Schindler and Nowrousian 2014). The above-described PTR2 transporters of *F. graminearum* are required for fusarielin H production, and the gene cluster responsible for its production is upregulated during perithecia formation (Sorensen et al. 2013; Droce et al. 2017).

Oxylipins are secondary metabolites derived from peroxidation of fatty acids, and they occur in mammals, plants, and fungi (Brodhun and Feussner 2011). The function of oxylipins has been mainly analyzed in *A. nidulans*. Here, the so-called psi (precocious sexual inducer) factors regulate the balance between sexual and asexual development. Psi factor is a mixture of PsiA, PsiB, and PsiC, consisting mainly of hydroxylated oleic and linoleic acid, and generated by psi-factor producing oxygenases PpoA, PpoB, and PpoC (Tsitsigiannis et al. 2005). The role of oxylipins in sexual development of other ascomycetes remains obscure, although lipids have been known to induce sexual development for a long time (see above).

Velvet has been shown to be a common regulator of (sexual) development and secondary metabolism (reviewed in Bayram and Braus 2012). It was shown recently that *T. reesei* VEL1 promotes the production of specific secondary metabolites during encounter of a mating partner. The Δvel1 mutant showed a secondary metabolite profile different from wild type in these conditions, and the wild type exhibited different secondary metabolite profiles when confronted with another wild type or a Δvel1 mating partner (Bazafkan et al. 2015). Velvet also controls fruiting-body development in response to light (Sect. III.A.1) and via regulation of gene expression (Sect. IV.C.1).

2. Pheromones

Sexual reproduction of filamentous ascomycetes involves the establishment of a dikaryotic stage with two compatible nuclei synchronously dividing in the same hyphal compartment (see Introduction). Often, heterothallic filamentous ascomycetes send out a specialized hypha (trichogyne) from the female prefruiting body, which senses a male cell of the opposite mating type, grows toward it, and fuses with the male cell. Uninucleate spermatia and microconidia, multinucleate macroconidia, or even vegetative hyphae can act as male cells. Similar to *S. cerevisiae*, diffusible peptide pheromones activate cognate G-protein-coupled receptors (GPCRs) at the surface, which activate signaling to control chemoattraction and fusion of male and female cells (Alvaro and Thorner 2016; Bennett and Turgeon 2016).

Already in the 1980s, diffusible **pheromones** have been suggested to be involved in the mating process of *N. crassa* and to be the cause for the directional growth of trichogynes toward the male fertilizing cells of the opposite mating type (Bistis 1981, 1983). In *N. crassa*, this directional growth of the trichogynes did not occur when the recipient male cells harbored mutations at the mating-type locus,

suggesting that the mating-type locus regulates the pheromone production (Bistis 1981).

Meanwhile, two different types of pheromone precursor genes have been isolated from various heterothallic and homothallic filamentous ascomycetes. These include *A. fumigatus*, *Cryphonectria parasitica*, *F. graminearum*, *H. jecorina*, *Magnaporthe grisea*, *N. crassa*, *P. chrysogenum*, *P. anserina*, and *S. macrospora* (Zhang et al. 1998; Shen et al. 1999; Pöggeler 2000; Bobrowicz et al. 2002; Coppin et al. 2005; Paoletti et al. 2005; Hoff et al. 2008; Kim et al. 2008; Schmoll et al. 2010b; Böhm et al. 2013).

Filamentous ascomycetes also encode two types of pheromone receptors, which are related to the Ste2 and Ste3 GPCRs of *S. cerevisiae* (Pöggeler 2011). Unlike in basidiomycetes, which encode only Ste3-like receptors, the two types of pheromone precursor and receptor genes are present in the same nucleus and are not part of the mating-type locus (Raudaskoski and Kothe 2010; Kües et al. 2011; Pöggeler 2011). In many heterothallic ascomycetes, mating-type encoded transcription factors directly control expression of pheromone precursor and receptor genes (Herskowitz 1989; Zhang et al. 1998; Shen et al. 1999; Bobrowicz et al. 2002; Coppin et al. 2005; Kim et al. 2012), while in others such as *H. jecorina* expression of pheromone genes does not depend on mating-type genes. However, these fungi showed enhanced expression of pheromone and receptor genes in the cognate mating type (Schmoll et al. 2010b; Seibel et al. 2012b).

In self-fertile, homothallic ascomycetes, spermatia and trichogynes are absent, and conidia are often missing. Therefore, recognition between a female and male cell of opposite mating type and a **pheromone/receptor system** do not seem to be necessary for sensing a mating partner or initializing fertilization events in these fungi. Nevertheless, they transcriptionally express pheromone and receptor genes (Pöggeler 2000; Pöggeler and Kück 2001; Paoletti et al. 2007; Kim et al. 2008; Lee et al. 2008).

Similar to the *S. cerevisiae* α-factor, one of the precursor genes, termed *ppg1* or *ppgA* in filamentous ascomycetes, encodes a polypeptide containing a signal sequence for secretion and multiple repeats of a putative pheromone sequence bordered by protease processing sites (Fig. 6a). A Ste2-like pheromone receptor termed PRE2 or PREB is able to sense the mature peptide pheromone. The other pheromone gene, *ppg2* or *ppgB*, encodes a short polypeptide similar to the *S. cerevisiae* **a**-factor precursor with a C-terminal CAAX (C = cysteine, a = aliphatic, and X = any amino acid residue) motif expected to produce a mature pheromone with a C-terminal carboxymethyl isoprenylated cysteine (Fig. 6b) (Jones and Bennett 2011). The mature lipopeptide is sensed by the Ste3-like pheromone receptor PRE1 (PREA).

Common to all α-like pheromone precursors are 2–15 repeats of a presumed mature pheromone. Within a given precursor, the length of the repeated sequence bordered by the processing sites varies between 9 and 12 aa with an 8-aa consensus motif (CR[RW]PGQPC). Three-dimensional structure determination of the ten-amino acid pheromone from *Fusarium oxysporum* revealed the presence of a central β-turn similar to its *S. cerevisiae* counterpart (Fig. 7). Structure-activity relationship of the α-like *F. oxysporum* pheromone demonstrated that the conserved central GQ is crucial for its chemoattractive activity (Naider and Becker 2004; Vitale et al. 2017).

Usually, the length of the repeated sequence is constant within a peptide pheromone precursor. However, some positions may vary in the repeated sequence (Martin et al. 2011; Pöggeler 2011). An N-terminal signal sequence is predicted for all of the PPG1/PPGA precursors. Usually maturation signals for endopeptidase (KR for KEX2), carboxypeptidases (KEX1 for removal of basic residues, e.g., KR, KE, KV, or KA after KEX2 cleavage), and aminopeptidases (XA, XP) surround the repeated sequences of the pheromone precursors (Fig. 6a). Phylogenetic analysis of repeats from PPG1/A pheromones revealed that repeated sequences undergo a rapid evolution that might contribute to speciation in ascomycetes (Martin et al. 2011).

Similar to **a**-factor-like precursors of ascomycete yeasts and basidiomycetes, the PPG2/PPGB precursors from filamentous ascomycetes are short with only 21–32 aa and harbor

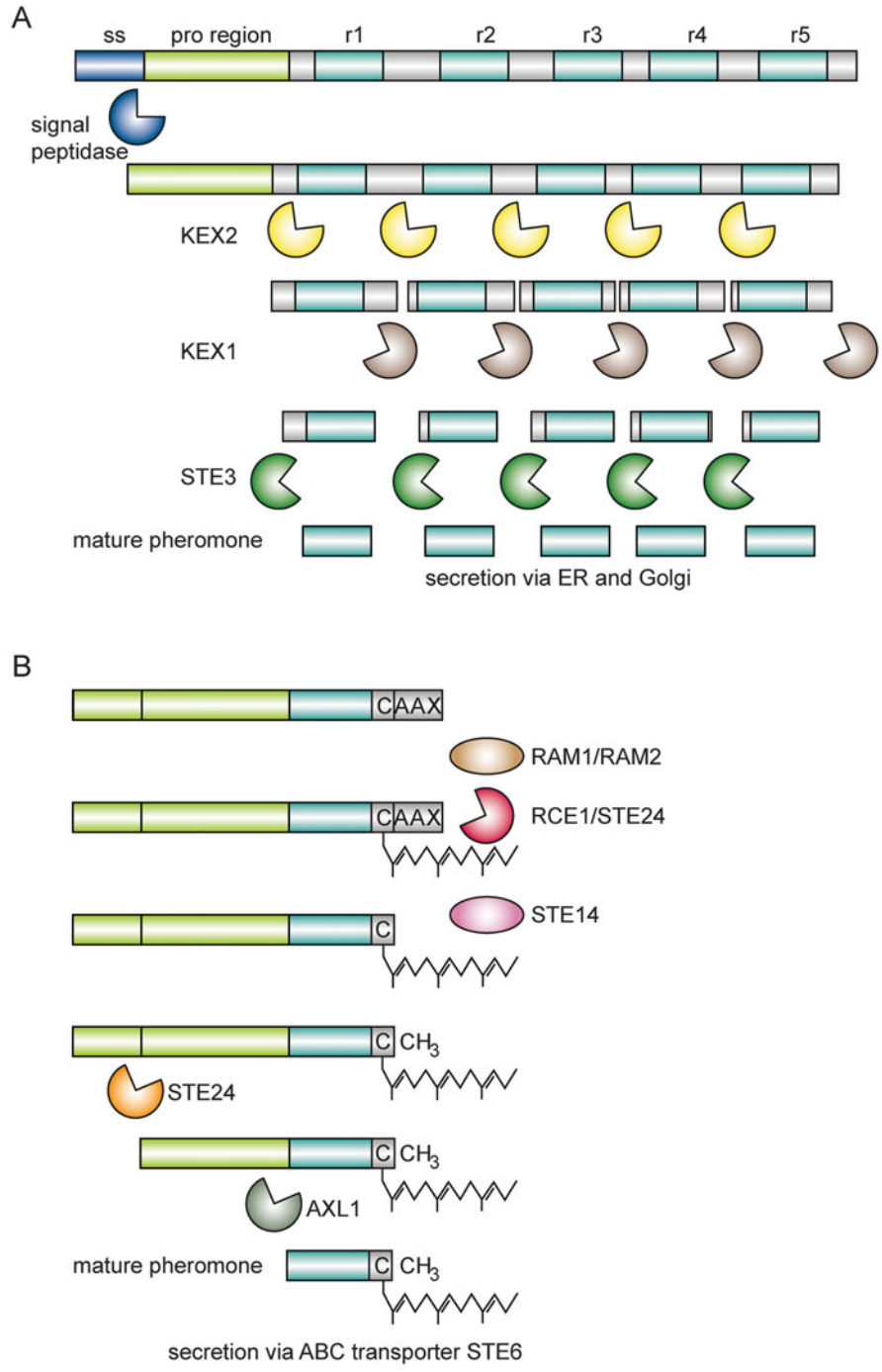

Fig. 6 Processing of PPG1/PPGA and PPG2/PPGB mating pheromones in analogy to α-factor and **a**-factor processing of *S. cerevisiae*. (**a**) Processing of the pre-pro peptide pheromone precursor PPG1/PPGA. The secretion signal (ss, blue) is cleaved by the signal peptidase, KEX2 endopeptidase removes the pro-region (green), and carboxypeptidase KEX1 and aminopeptidase STE13 digest the connecting regions of the repeated copies (r1–r5). The mature peptide pheromone is exported via the ER/Golgi pathway. (**b**) Processing of the lipopeptide pheromone precursor PPG2/PPGB. Farnesylation at the conserved cysteine residue

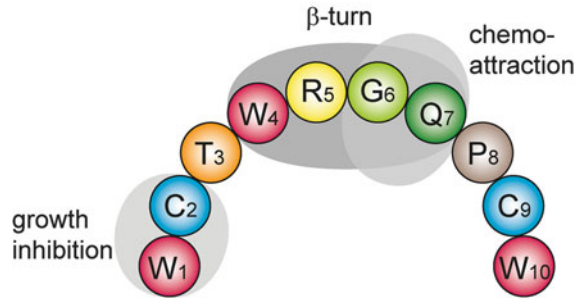

Fig. 7 Cartoon representing functional and structural segmentation of the *F. oxysporum* PPG1 pheromone according to Vitale et al. (2017). Shaded in light gray are those residues that are functional in growth inhibition and chemoattraction, while the β-turn is indicated by dark gray shading

no signal sequence for secretion. They have a 9-aa conserved motif comprising the CAAX motif for farnesylation by farnesyltransferase RAM1/RAM2 and five preceding residues. In addition to the invariant C four amino acids from the end, in filamentous ascomycetes, the last residue of the CAAX motif is an invariant methionine and the penultimate residue an aliphatic amino acid (Pöggeler 2011). After farnesylation, methylation, and N-terminal processing, the mature lipopeptide pheromone is predicted to be exported from the hyphae using an ATB-binding cassette (ABC) transporter (Fig. 6b).

TBLASTN searches of genomes from many filamentous ascomycetes, e.g., from species of the genera *Aspergillus*, *Paracoccidioides*, and *Penicillium*, identified only the α-factor-like pheromone gene *ppg1/ppgA*, but failed to identify a *ppg2/ppgB* homolog (Dyer et al. 2003; Hoff et al. 2008; Gomes-Rezende et al. 2012). This may be explained by the small size and poor conservation of **a**-factor-like pheromone precursors or by the absence of a hydrophobic pheromone gene in these species (Pöggeler 2011). However, the conserved coding capacity for the Ste3-like receptor PRE1/PREA argues against the absence of **a**-like pheromones in these species.

Species of the order *Hypocreales*, including *F. oxysporum*, *Fusarium verticillioides*, *H. jecorina*, and *N. haematococca*, encode a novel class of pheromone precursors (Schmoll et al. 2010b). This new type of pheromone precursor has characteristics of both PPG1/PPGA and PPG2/PPGB pheromone precursors. It was therefore termed h-type (hybrid) pheromone precursor. H-type pheromone precursors contain the CPAX motif at the C-terminus (with a proline replacing one aliphatic residue of the CAAX motif) or copies of this motif and putative KEX2 processing sites. Similar to PPG2/PPGB pheromone precursors, h-type pheromone precursors are predicted to contain no signal sequence and are therefore suggested to be secreted via a nonclassical ABC-transporter-mediated secretion pathway (Schmoll et al. 2010b). Martin et al. (2011) considered this gene structure to be a variation of the **a**-class precursor, as opposed to an entirely distinct precursor class.

In filamentous ascomycetes, pheromones and their cognate pheromone receptors have two main functions: (1) recognition between male and female cells of opposite mating types in heterothallic filamentous ascomycetes and (2) the regulation of postfertilization events, which are equally important for homothallic and heterothallic fungi. These latter include processes such as nuclear migration, maintenance of the dikaryotic state, cell fusion, and meiosis (Spellig et al. 1994; Willer et al. 1995; Casselton 2002).

Male and female fertility of heterothallic ascomycetes depend on the specific interaction

Fig. 6 (continued) of the CAAX motif by farnesyltransferases RAM1/RAM2 is followed by proteolysis of the three most C-terminal amino acids AAX by RCE1 and STE24. Carboxymethylation is performed by the methyltransferase STE14. Further proteolytic events remove amino acids from the N-terminus. The mature lipophilic pheromone is exported by an ABC transporter

of pheromones with their cognate receptors. Deletion of pheromone genes results in male sterility, but does not lead to defects in vegetative development and female fertility. Spermatia or macroconidia of pheromone mutants are unable to attract their female partners, and heterologous expression of a pheromone gene enables male cells to direct the chemotropic growth of trichogynes from an otherwise incompatible mating-type background. However, pheromone-deleted mutants are not affected in fusion of vegetative hyphae (Kim et al. 2002a, 2012; Turina et al. 2003; Coppin et al. 2005; Kim and Borkovich 2006). In *N. crassa*, co-expression of the Ste2-like receptor gene *pre2 and* the cognate pheromone gene *ppg1* in a MAT1-1 instead of a MAT1-2- background leads to self-attraction and development of barren perithecia without ascospores. Forced heterokaryons of opposite mating-type strains expressing one receptor gene and the compatible pheromone gene are able to form mature perithecia. Thus, the presence of one receptor and its compatible pheromone is necessary and sufficient for perithecial development and ascospore production (Kim et al. 2012).

In the heterothallic *N. crassa* and the pseudohomothallic *P. anserina*, **pheromone precursor genes** are highly expressed under conditions that favor sexual development (Bobrowicz et al. 2002; Kim et al. 2002a; Coppin et al. 2005; Bidard et al. 2011; Wang et al. 2014b). Recently, ChIP-seq and in vivo binding assays verified direct binding of the MAT1-1-1 a domain transcription factor to the *ppg1* gene promoter and *pre1* receptor gene promoter in *P. chrysogenum* (Becker et al. 2015).

In the homothallic *S. macrospora*, both pheromone genes are positively controlled by MAT1-1-1, while *ppg2* is controlled by MAT1-2-1 (Pöggeler et al. 2006b; Klix et al. 2010). Moreover, laser microdissection and RNA-seq analysis revealed that pheromone genes *ppg1* and *ppg2* are strongly upregulated in prefruiting bodies of *S. macrospora* (Teichert et al. 2012).

The expression of pheromone receptors is not significantly altered in *MAT* deletion strains of *S. macrospora* (Pöggeler et al. 2006b; Klix et al. 2010), while in the homothallic *Sclerotinia sclerotiorum*, all *MAT* genes are involved in the expression of both pheromone and receptor genes (Doughan and Rollins 2016). In *F. graminearum*, expression of *ppg2* is under control of the MAT1-2 locus, and *pre1* is regulated by the MAT1-1 locus (Kim et al. 2015), whereas in *A. nidulans* expression of *ppgA* and the two pheromone receptor genes is not regulated by *MAT* genes, although upregulated during sexual development (Paoletti et al. 2007).

In homothallic filamentous ascomycetes, deletion of any single pheromone or receptor gene does not impair vegetative growth or fruiting-body development (Seo et al. 2004; Mayrhofer and Pöggeler 2005; Mayrhofer et al. 2006; Kim et al. 2008; Lee et al. 2008). However, double-deletion strains without any compatible pheromone receptor pair showed a reduced number of fruiting bodies and ascospores. The most drastic negative effects occurred in receptor double-deletion mutants. In *A. nidulans* and *S. macrospora*, these mutants are unable to form fruiting bodies and ascospores (Seo et al. 2004; Mayrhofer et al. 2006). In *F. graminearum*, only deletion combinations that include either the *ppg1* pheromone gene or the *pre2* receptor gene cause increased numbers of immature perithecia and display reduced fertility in self-fertilization tests (Kim et al. 2008; Lee et al. 2008). However, even a quadruple mutant of *F. graminearum* without any pheromone and receptor genes produces fruiting bodies and ascospores. The pheromone/receptor system seems to play a nonessential role in the sexual development in *F. graminearum* (Kim et al. 2008). Thus, similar to heterothallic ascomycetes, in some homothallic species, at least one pheromone/receptor system is required for later stages of the sexual development such as nuclear migration, crozier, and ascogenous hyphae formation, while in others these processes seem to proceed independently of a functional pheromone/receptor system. Differences in the regulation of the expression and functions of the pheromone/receptor system might be the consequence of an independent adaptation to the homothallic lifestyle.

Studies in the asexual root-infecting ascomycete *F. oxysporum* recently revealed that

germinating conidiospores use the peptide pheromone receptor PRE2 to sense gradients of sugars, amino acids, pheromones, and plant root exudates to redirect their growth toward these chemoattractants. Interestingly, synthetic peptide pheromones from *S. cerevisiae* and *F. oxysporum* as well as root peroxidase can induce polarized growth of the germ tubes equally well (Turrà et al. 2015, 2016). Moreover, the *F. oxysporum* PPG1 pheromone inhibits cell division of germ tubes in a STE2-independent way (Vitale et al. 2017).

These examples show that during adaptation to different lifestyles, the fungal pheromone/receptor system can be used or converted to sense and respond to various environmental cues. Therefore, pheromones and receptors might have more functions than so far assumed.

IV. Regulatory Networks

The formation of three-dimensional fruiting bodies is a highly controlled and complex process. Development in filamentous ascomycetes is orchestrated by signal transduction pathways, protein degradation systems, as well as transcriptional regulation.

A. Signal Transduction Pathways

Sexual development in ascomycetes requires a tight regulation of sending, responding, and processing signals. These signals contribute to, e.g., mating partner recognition, cell-to-cell communication, induction of sexual development, and finally fruiting-body formation. The communication processes are conducted by several signal transduction pathways, in which reversible protein phosphorylation is often the key signal. Remarkably, many of the signaling components are highly conserved in eukaryotes, and a detailed knowledge on their molecular mechanisms not only contributes to our understanding of fungal fruiting-body formation but also to our understanding of signal transduction pathways in general.

1. MAP Kinase Signaling

Many **signal transduction pathways** rely on the transfer of protein phosphorylation on mainly S, T, or Y amino acid residues. The enzymes catalyzing the transfer of a phosphate to one of these residues are protein kinases. Fungal kinomes, the entirety of all protein kinases encoded in a genome, contain approximately 90 protein kinases (Park et al. 2011; De Souza et al. 2013a). In *N. crassa*, 77 viable deletion mutants of these kinase genes are available, out of which 33 exhibit defects in sexual development (Park et al. 2011). Strikingly, all kinases involved in mitogen-activated protein kinase (MAPK) cascades are necessary for sexual development in *N. crassa* (Park et al. 2011; Lichius et al. 2012). MAPK cascades are highly conserved signaling modules that transmit signals from the cell surface to nuclei and consist of three kinases. The MAPKKK phosphorylates the MAPKK, which phosphorylates the MAPK that activates downstream targets. Many of these targets are nuclear proteins and regulate transcription. In most filamentous ascomycetes, three different MAPK cascades are present (Fig. 8), which regulate **cell wall integrity (CWI)**, **pheromone signaling (PR)**, and **osmotic stress (HOG)** (Irniger et al. 2016). The corresponding subunits in several model ascomycetes are given in Table 1.

The **CWI pathway** in filamentous ascomycetes is homologous to the *S. cerevisiae* Bck1p, Mkk1/Mkk2, and Slt2 MAP kinase pathway (Borkovich et al. 2004). This kinase module is crucial for fruiting-body development, since the corresponding *N. crassa* and *S. macrospora* deletion strains are unable to generate mature fruiting bodies, exhibit defects in hyphal fusion, and are sensitive to cell wall stress (Maerz et al. 2008; Park et al. 2008; Teichert et al. 2014b). In *S. macrospora*, the kinases of the CWI pathway, MIK1, MEK1, and MAK1 are crucial for sexual development, hyphal growth, and vegetative cell fusion. Extensive mass spectrometry, yeast two-hybrid, and phosphorylation studies showed that the developmental protein PRO40, the homolog of *N. crassa* SOFT (SO), is the scaffold protein for the CWI pathway (Teichert et al. 2014b). Further CWI components were

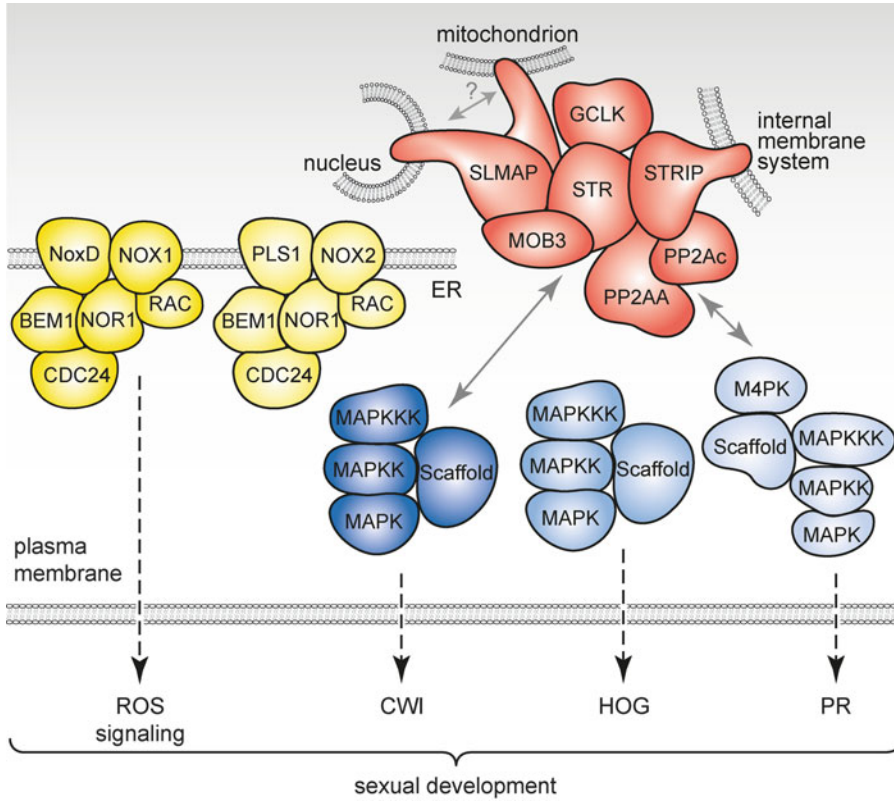

Fig. 8 Schematic overview on signal transduction pathways controlling fruiting-body development in filamentous ascomycetes. The NOX complex exists in two different compositions with either NoxD (synonymous to PRO41) and NOX1 or PLS1 and NOX2. NOX1 is the NADPH oxidase involved in sexual development. The three MAPK cascades are equally important for fruiting-body development with a high likelihood of interdependence. Presumably, the connection to other signaling pathways has also a fundamental impact on sexual development. The STRIPAK complex is a major complex of developmental proteins, which are connected to the CWI and the PR pathway (double arrows). This interconnection might be the key link for controlled development

Table 1 Overview of MAPK cascade components in *S. cerevisiae* (*Sc*), *N. crassa (Nc)*, *S. macrospora (Sm)*, *P. anserina (Pa)*, *A. nidulans (An)*, *and B. cinerea (Bc)*

		Sc	Nc	Sm	Pa	An	Bc
PR	MAPKKK	Ste11	NRC-1	MIK2	PaTLK2	SteC	BcSte11
	MAPKK	Ste7	MEK-2	MEK2	PaMKK2	Ste7	BcSte7
	MAPK	Fus3	MAK-2	MAK2	PaMPK2	MpkB	BcBmp1
	Scaffold	Ste5	HAM-5	HAM5	IDC1	hypoth.	Ste50
CWI	MAPKKK	Bck1	MIK-1	MIK1	PaASK1	BckA	BcBCK1
	MAPKK	Mkk1/2	MEK-1	MEK1	PaMKK1	MkkA	BcMKK1
	MAPK	Slt2	MAK-1	MAK1	PaMPK1	MpkA	Bmp3
	Scaffold	–	SOFT	PRO40	PaSO	hypoth.	hypoth.
HOG	MAPKKK	Ste11	OS-4	OS4	hypoth.	SskB	Bos4
	MAPKK	Pbs2	OS-5	OS5	hypoth.	PbsB	Bos5
	MAPK	Hog1	OS-2	OS2	hypoth.	HogA/MpkC	BcSak1
	Scaffold	Pbs2	–	–	–	–	–

CWI cell wall integrity pathway, *HOG* high-osmolarity glycerol pathway, *PR* pheromone response pathway

identified in *S. macrospora*, namely, the essential upstream activator protein kinase C (PKC1) and the small GTPase RHO1, which has been functionally described in *N. crassa* (Richthammer et al. 2012; Teichert et al. 2014b). The deletion strains of the CWI cascade components are female sterile and are involved in germling fusion in *N. crassa*. This process is highly regulated and requires communication between the tips of two germlings (Fleissner et al. 2009). Especially, the scaffold protein SO is required for communication between germling tips, and SO and the MAPK of the PR pathway, MAK-2, oscillate to opposing germling tips during fusion (Fleissner et al. 2009).

Accumulating evidence points toward a genetic and direct interaction of the PR and the CWI pathway during the formation of fruiting bodies and the regulation of cell wall integrity (Maerz et al. 2008; Dettmann et al. 2013; Kamei et al. 2016). The **PR pathway** in filamentous ascomycetes is homologous to the *S. cerevisiae* PR pathway Ste11-Ste7-Fus3, which has been the paradigm for understanding signaling upon pheromone response in ascomycetes. In *N. crassa*, the PR kinases NRC-1, MEK-2, and MAK-2 and their scaffold protein HAM-5 are important for early colony development, all types of cell fusion, female fertility, and cell-cell communication (Dettmann et al. 2014; Jonkers et al. 2014, 2016). The *A. nidulans* PR pathway is also a major regulator of sexual development, while it is also crucial for proper formation and germination of conidia (Kang et al. 2013; Irniger et al. 2016).

A conidiation defect is present in some deletion strains of another developmental protein complex, the multi-subunit velvet complex, which is a major regulator of secondary metabolism (Bayram and Braus 2012). One subunit, the velvet protein VeA discussed later, is phosphorylated by the PR MAPK FUS3, which is mandatory for the proper ratios of different proteins in the velvet complex (Bayram et al. 2012). Thus, the PR pathway has an indirect impact on secondary metabolism by influencing the phosphorylation status of the velvet component VeA. Further, the PR pathway most likely includes the upstream G proteins and the aforementioned pheromone receptors (Li et al. 2007). Even though the actual inducing signal of the PR pathway, such as pheromones, remains to be determined, it seems likely that GPCRs are involved in sensing in filamentous fungi, as described in the pheromone section above (Sect. III.B.2). GPCRs transmit signals via heterotrimeric G proteins that consists of Gα and Gβγ. Fungal genomes mostly encode for three Gα subunits, and single Gβ and Gγ subunits (Mayrhofer and Pöggeler 2005; Li et al. 2007; Kamerewerd et al. 2008). Mostly, the Gα subunit determines the target pathway, and in *N. crassa* and *S. macrospora*, the Gα subunits GNA-1 and GSA-1 are major regulators of sexual development (Ivey et al. 1996; Kamerewerd et al. 2008).

There are strong links that both other subunits, Gβ and Gγ, contribute to fruiting-body development, but their many functions are also related to other developmental processes, such as asexual development and carbon sensing (Deka et al. 2016). Like the *N. crassa gna-1* deletion strain, the deletion strains of Gβ and Gγ are female sterile but male fertile (Krystofova and Borkovich 2005). Strikingly, none of the *A. nidulans* Gα subunits has been linked to sexual development; however, deletion strains of Gβ and Gγ are sterile (Rosen et al. 1999; Seo et al. 2005). Besides the aforementioned downstream signaling through MAPK cascades, stimulated G proteins activate cyclic AMP (cAMP)-dependent signaling by inducing soluble adenylyl cyclases. These enzymes produce cAMP from ATP as a second messenger, which is involved in several important developmental processes ranging from nutrient sensing, stress response, metabolism, and pathogenicity to sexual development (Lengeler et al. 2000; D'Souza and Heitman 2001). A possible outcome of second messenger signaling via cAMP is also a stimulation of MAPK cascade signaling.

The third MAPK pathway in filamentous ascomycetes is homologous to the yeast **HOG MAPK pathway**, which is required for the adaptation to stress (Hohmann 2009). A plethora of stressors can activate this pathway ranging from heat stress to high osmolarity and oxidative stress. In *N. crassa*, the HOG kinases OS-2,OS-4, and OS-5 influence the development of protoperithecia and the generation of fruiting

bodies (Park et al. 2011; Lichius et al. 2012). In contrast to other filamentous ascomycetes, the aspergilli genomes encode two HOG MAPKs, SakA and MpkC (May et al. 2005). Mutants lacking SakA show premature cleistothecia formation, and SakA is involved in light signaling (see Sect. III.A.1) and represses expression of *noxA*, encoding a NOX pathway component (see Sect. IV.A.3) (Kawasaki et al. 2002; Lara-Ortiz et al. 2003).

2. STRIPAK

The **striatin-interacting phosphatase and kinase (STRIPAK) complex** is conserved from yeast to human (Kück et al. 2016), while only a few subunits were detected in plants so far (Rahikainen et al. 2016). The mammalian STRIPAK encompasses at least the protein phosphatase 2A (PP2A) scaffolding and catalytic subunits, striatins, striatin-interacting proteins STRIP1 and STRIP2, the monopolar spindle one-binder (Mob) protein Mob3, the cerebral cavernous malformation 3 protein (CCM3), and associated germinal center kinases (Hwang and Pallas 2014). In yeasts, the STRIPAK-like *S. cerevisiae* FAR (factor arrest) complex regulates pheromone-induced cell cycle arrest and antagonizes TORC2 signaling (Kemp and Sprague 2003; Pracheil and Liu 2013), while the *S. pombe* SIP (septation initiation network (SIN) inhibitory protein complex) controls coordination of mitosis and septation (Singh et al. 2011). The first hint of a highly conserved signaling complex in filamentous fungi, as depicted in Fig. 8, came from the discovery that the sterile phenotype of the *S. macrospora* mutant pro11 was restored to wild type by the mouse striatin cDNA (Pöggeler and Kück 2004). Concomitant studies identified several developmental proteins that are homologous to human STRIPAK complex subunits, namely, the STRIP1/2 homolog PRO22, the MOB3 homolog SmMOB3, PRO45, PP2AA, PP2Ac1, as well as the kinases SmKin24 and SmKin3 (Kück et al. 2016). The characterization of the mutant pro22 led to the initial discovery of the STRIPAK in ascomycetes (Bloemendal et al. 2010, 2012). The fungal STRIPAK complex is a regulator of fruiting-body development, vegetative growth, hyphal fusion, and asexual development (Kück et al. 2016). In *S. macrospora*, the STRIPAK complex is a key factor of sexual development, and all available deletion mutants show similar defects, producing only nonpigmented protoperithecia (Bernhards and Pöggeler 2011; Bloemendal et al. 2012; Nordzieke et al. 2015). Further, the strains are impaired in hyphal growth and fusion in vegetative mycelium. Strikingly, PRO22 and PP2Ac1 appear to regulate septation of the ascogonial coil, which might interfere with proper coordination of meiosis (Bloemendal et al. 2010; Beier et al. 2016). In *N. crassa*, most deletion mutants lacking genes for STRIPAK are female sterile and exhibit a defect in germling fusion (Fu et al. 2011; Dettmann et al. 2013). In both, *S. macrospora* and *N. crassa*, most mutants lacking MAPK subunits of CWI and PR resemble the STRIPAK deletion mutant phenotype. This similarity and data from protein-protein interaction studies indicate a functional relationship between MAPK cascade and STRIPAK signaling during the formation of fruiting bodies (Dettmann et al. 2013; Kück et al. 2016). Even the nuclear localization of MAK-1 is partially affected by STRIPAK (Dettmann et al. 2013). Many STRIPAK subunits are localized to the ER and the nuclear envelope, while some evidence indicates additional localizations at mitochondria and tubular vacuoles in *S. macrospora* (Bloemendal et al. 2012; Nordzieke et al. 2015). Interestingly, data from *S. cerevisiae* indicate that the PRO22 homolog Far11 is a target of MAPKs, while PP2A might negatively regulate the CWI pathway (Junttila et al. 2007; Lisa-Santamaría et al. 2012; Sacristán-Reviriego et al. 2015). Homologs of STRIPAK subunits are present in all ascomycetes and have well-conserved functions. For example, striatin homologs in *A. nidulans*, *F. graminearum*, and *F. verticillioides* are key regulators of polar growth, sexual development, and conidiation (Shim et al. 2006; Wang et al. 2010a). Besides sexual development, the STRIPAK complex appears to influence pathogenic and symbiotic interactions with plants in diverse fungi (Shim et al. 2006; Green et al. 2016).

3. NOX Complexes

The production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, was long believed to be an inevitable and damaging byproduct of several metabolic processes (Halliwell and Gutteridge 2015). Accumulating data from plant, animal, and fungal species revealed the importance of the controlled production of **ROS by NADPH oxidases (NOX)** for multiple signaling pathways (Kaur et al. 2014; Marschall and Tudzynski 2016; Gao and Schöttker 2017). The mechanisms of ROS perception and signaling functions remain to be determined in all species.

In filamentous ascomycetes, the NOX enzymes regulate several developmental processes like fruiting-body formation, hyphal fusion, and ascospore germination. In fungi, three enzymes of the NOX family have been described. NOX1 and NOX2 (also referred to as NOXA and NOXB) are present in most ascomycetes, while NOX3 has been identified only in *M. grisea*, *P. anserina*, and several *Fusarium* species (Aguirre et al. 2005; Scott and Eaton 2008; Brun et al. 2009; Dirschnabel et al. 2014). As depicted in Fig. 8, two fungal NOX complexes exist, containing the common regulator NOR1 (NOX regulating), associated with the small GTPase RAC1, CDC24, and BEM1. This complex can be either associated with NOX1 and NoxD (PRO41) or with NOX2 and PLS1 (Marschall and Tudzynski 2016).

Especially NOX1 and NOR1 are indispensable for the formation of proper fruiting bodies in filamentous ascomycetes. The *A. nidulans noxA* deletion strain is unable to form mature cleistothecia, the *S. macrospora nox1 and nor1* deletion strains form only protoperithecia, and *P. anserina* and *N. crassa nox1* deletion strains are female sterile (Lara-Ortiz et al. 2003; Malagnac et al. 2004; Cano-Domínguez et al. 2008; Dirschnabel et al. 2014). Strikingly, sterility of *P. anserina* and *S. macrospora nox1* mutants can be surpassed by serial passaging to nutrient-rich medium (Malagnac et al. 2004; Dirschnabel et al. 2014). This result indicates a link between sterility and availability of nutritional factors or ROS scavenging and signaling molecules (Malagnac et al. 2004; Dirschnabel et al. 2014). Cytochemical analysis in *A. nidulans* revealed that NoxA generates ROS in young primordial, peridial, and Hülle cells. Presumably, these ROS function as a second messenger in the regulation of developing ascogenous and peridial tissues (Lara-Ortiz et al. 2003). Besides regulating fruiting-body formation, NOX1 and NOR1 are major regulators of conidial anastomosis tube (CAT) fusion and hyphal fusion in vegetative mycelium in *N. crassa*, *B. cinerea*, *E. festucae*, and *S. macrospora* (Read et al. 2012; Roca et al. 2012; Kayano et al. 2013; Dirschnabel et al. 2014). Often, sexual differentiation and hyphal fusion seem to be co-regulated, although this is not always the case (Dirschnabel et al. 2014; Lichius and Lord 2014). Additionally, transcriptional data from *S. macrospora* and phenotypic analysis from *N. crassa* indicate a functional relationship between F-actin organization and regulation through NOX1 (Roca et al. 2012; Dirschnabel et al. 2014).

B. Protein Degradation

Development of the multicellular fruiting bodies relies on ongoing changes and remodeling of the proteome. Disused proteins have to be removed when specialized cells are formed. As in all other eukaryotes, two major degradation systems handle protein degradation in fungi, autophagy and the ubiquitin-proteasome system (UPS).

Autophagy ("self-eating") delivers cytoplasm and whole organelles to the vacuole for their degradation. In addition, it protects the cell from harm by dangerous protein aggregates or dysfunctional and superfluous organelles (Yin et al. 2016). The UPS is primarily responsible for proteolytic degradation of short-lived, misfolded, and damaged proteins which are marked for degradation via the 26S proteasome by ubiquitination (Ciechanover 1994; Doherty et al. 2002). Protein degradation by the UPS is highly specific and precisely regulated by E3 ubiquitin ligases (Buetow and Huang 2016).

1. Autophagy

Autophagy is conserved in all eukaryotes and essential for the delivery of cytosolic cargoes to the vacuole for their degradation (Feng et al. 2014). The recycled building blocks can be reused to survive starvation or stress conditions and to drive cellular remodeling during development. Proteins involved in autophagy are termed autophagy-related (ATG) proteins (Klionsky et al. 2003). At least 41 ATG proteins have been identified by genetic experiments in yeasts, and most of them are conserved in filamentous ascomycetes (Araki et al. 2013; Reggiori and Klionsky 2013; Voigt and Pöggeler 2013b; Nazarko et al. 2014; Mochida et al. 2015; Yao et al. 2015). About half of the proteins (core ATG proteins) are required for all autophagy-related pathways, while others are only involved in selective types of autophagy.

There are three distinct types of **autophagy: macroautophagy**, **microautophagy**, and **chaperone-mediated autophagy (CMA)**. Macro- and microautophagy take place in all eukaryotes, while CMA is mammalian specific and does not rely on *atg* genes (Okamoto 2014). Microautophagy describes the direct engulfment by the vacuolar membrane (Li et al. 2012). However, this type has not yet been described in filamentous ascomycetes (Voigt and Pöggeler 2013b).

Macroautophagy (hereafter autophagy) is the best-characterized and most prominent type and can be either selective or nonselective. It involves sequestering of cytosolic cargoes by a double-membrane phagophore that by expansion and closure becomes a double-layered autophagosome. By fusion of the outer membrane of the autophagosomes with the vacuole, the inner vesicle, termed autophagic body, is released into the lumen of the vacuole. Vacuolar hydrolases degrade the membrane of the vesicle and the cargo. The resulting macromolecules are delivered into the cytoplasm via permeases (Fig. 9).

The phagophore assembly site (PAS) is the initiation site for the forming of autophagosomes. The majority of ATG proteins are recruited in a hierarchical manner to the PAS (Suzuki et al. 2007). The ATG1 kinase complex consisting of the serine/threonine kinase ATG1, its regulatory subunit ATG13, and other associated proteins is the autophagy initiation complex. Upon starvation this complex is required for recruitment of further ATG proteins and membrane sources such as ATG9 vesicles (Stanley et al. 2014). The origin of the autophagosomal membranes is still discussed. Membranes originating from different sources seem to be involved (Ge et al. 2014). The phosphatidylinositol (PtdIns) 3-kinase complex produces phosphatidylinositol 3-phosphate (PI3P) at the PAS, which is necessary to recruit proteins that function in autophagosomes formation (Reggiori and Klionsky 2013). Expansion of the phagophore requires the ubiquitin-like (UBL) proteins ATG8 and ATG12, which are conjugated by a UBL machinery. ATG8 conjugated to the lipid phosphatidylethanolamine (PE) is a major component of autophagosomal membranes. ATG8-PE covers both sides of the phagophore. During later stages, the protease ATG4 acts as deconjugating enzyme to release ATG8 from PE on the outer membrane for reuse. ATG8-PE conjugates from the inner membrane are not cleaved off and are therefore degraded by hydrolytic enzymes in the vacuole together with the autophagic body and the cargos (Reggiori and Klionsky 2013). Permeases release the degradation products back into the cytoplasm for reuse (Yin et al. 2016) (Fig. 9).

Autophagy can be further divided into nonselective and selective processes. The latter are specific for the degradation of superfluous or damaged organelles like peroxisomes (pexophagy), mitochondria (mitophagy), nuclei (nucleophagy), endoplasmatic reticulum (reticulophagy), and ribosomes (ribophagy) (Anding and Baehrecke 2017).

In *S. cerevisiae*, a specific type of selective autophagy is the cytoplasm to vacuole targeting (Cvt) pathway. This pathway utilizes the core machinery of autophagy and specific receptor proteins to deliver hydrolases such as peptidases and mannosidases the vacuole (Yamasaki and Noda 2017).

The detailed mechanisms of selective autophagy remain to be characterized; however, common principles are arising. A receptor able to interact with ATG8 at the convex site of

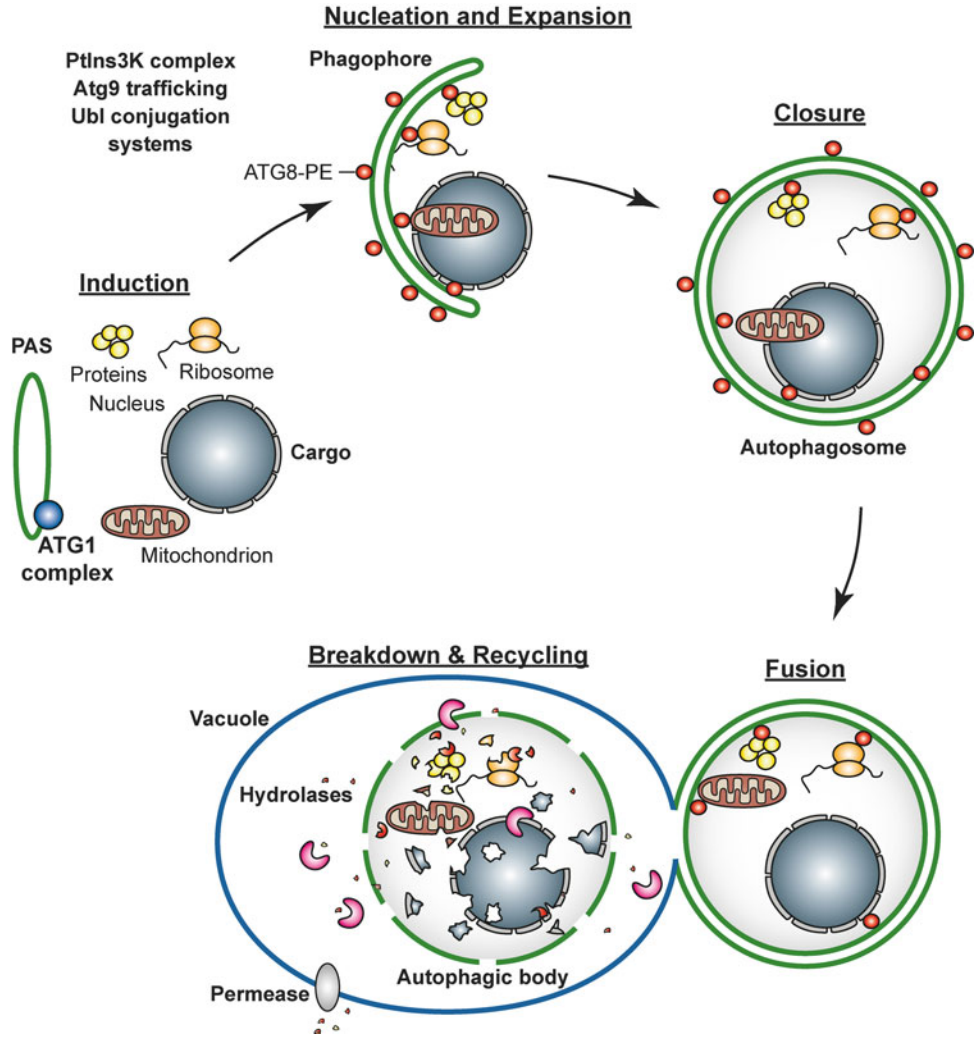

Fig. 9 Sequential steps of autophagy in *S. cerevisiae*. The initiation of autophagy requires the ATG1 complex at the PAS, which recruits other ATG proteins. Expansion and curvature of the phagophore relies on the PtdIns 3-kinase complex, the transmembrane protein ATG9, and two UBL conjugation systems. ATG8-PE participates in cargo recognition during selective autophagy and is important for expansion of the phagophore. After vesicle closure, the protease ATG4 deconjugates ATG8 from the outer membrane of the autophagosomes. The resulting vesicle fuses with the vacuolar membrane resulting in the release of an autophagic body surrounded by the inner autophagosomal membrane. The membrane of the autophagic body is lysed by the lipase ATG15, and the sequestered cargoes are degraded by hydrolases. Finally, the breakdown products are exported into the cytoplasm via permeases

the growing phagophore recognizes and recruits specific cargoes. During selective autophagy, core ATG proteins are required and often ubiquitin acts as a signaling molecule (Khaminets et al. 2016).

Molecular analyses of autophagy have been mostly performed in the unicellular budding yeast *S. cerevisiae* and in *Pichia pastoris* (Ohsumi 2014; Harnett et al. 2017). The main physiological role for autophagy in yeasts is to maximize survival under stress and starvation conditions, to generate nutrients for ascospore formation, and to deliver hydrolytic enzymes into the vacuole via the Cvt pathway (Reggiori

and Klionsky 2013; Yamasaki and Noda 2017). However, in filamentous ascomycetes, autophagy is involved in pathogenicity, production of secondary metabolites, and asexual and sexual development even under non-starvation conditions (Palmer et al. 2008; Pollack et al. 2009; Bartoszewska and Kiel 2010; Khan et al. 2012; Voigt and Pöggeler 2013b).

With regard to fruiting-body development, autophagy has been investigated in *Podospora anserina*, *S. macrospora*, and *N. crassa* as well as in the plant pathogens *Magnaporthe oryzae* and *Fusarium graminearum*.

In *P. anserina*, *atg* genes (*idi* genes) have been initially identified as genes induced during heterokaryon incompatibility, a cell death reaction after fusion of cells of dissimilar genotype (Pinan-Lucarré et al. 2003). Among the genes upregulated during heterokaryon incompatibility were the vacuolar protease gene *idi-6*/*pspA* and *idi-7*/*atg8* encoding the UBL autophagosomal membrane protein ATG8. Deletion of both genes causes differentiation defects such as a lower density of aerial hyphae, decreased pigmentation of the mycelium, and the absence of female reproductive structures (Pinan-Lucarré et al. 2003). Similarly, deletion of the serine threonine kinase gene *atg1*, the UBL genes *atg8* and *atg12*, and the protease gene *atg4* resulted in sterility in the homothallic *F. graminearum* and *S. macrospora*, in the heterothallic *N. crassa*, and in the plant pathogen *M. oryzae*. In addition to defects in sexual development, autophagy mutants of all fungi displayed a reduced vegetative growth rate (Pinan-Lucarré et al. 2005; Liu et al. 2010; Park et al. 2011; Josefsen et al. 2012; Voigt and Pöggeler 2013a; Chinnici et al. 2014; Werner et al. 2016). In *S. macrospora*, cross-species microarray experiments revealed that the bZIP transcription factor gene *Smjlb1* was downregulated in the sterile fruiting-body mutants pro1, pro11, and pro22, as well as in the mating-type mutant ΔSmta-1 (Nowrousian et al. 2005; Pöggeler et al. 2006b) (see Sects. IV.A.2 and IV.C.1). In *P. anserina*, expression of the *Smjlb1* ortholog *idi-4* is assumed to be involved in the regulation of *atg* genes (Dementhon et al. 2004; Dementhon and Saupe 2005). Generation of the homokaryotic *S. macrospora* deletion mutant demonstrated that *Smjlb1* is required for fruiting-body development and proper vegetative growth. Quantitative real-time PCR experiments suggest that SmJLB1 acts as a repressor on gene expression of *atg8* and *atg4* (Voigt et al. 2013).

Careful microscopic examination of *N. crassa* and *S. macrospora* autophagy mutants revealed that they initiate female development and are able to produce ascogonia and small protoperithecia, indicating that they are unable to fully support perithecia development (Voigt and Pöggeler 2013a; Chinnici et al. 2014; Werner et al. 2016). Protoperithecia grafting experiments in *N. crassa* have demonstrated that autophagy is required within the vegetative hyphal network, as fertilized autophagy mutant perithecia are able to complete perithecia development and to produce ascospores when grafted onto a wild-type host (Chinnici et al. 2014). In filamentous ascomycetes, autophagy seems to be an essential and constitutively active process to sustain high energy levels for filamentous growth and multicellular development. Autophagy mutants of filamentous fungi seem to be affected in fruiting-body development because the underlying vegetative mycelium is unable to provide an adequate supply of nutrients to the developing fruiting body.

In contrast to *N. crassa*, where it has been shown that a homokaryotic knockout mutant of *atg7*, encoding the common E1-like enzyme of the ATG2 and ATG8 UBL conjugation systems, is female sterile, the ortholog of *S. macrospora*, *Smatg7*, was shown to be required for viability (Nolting et al. 2009; Chinnici et al. 2014). Interestingly, a heterokaryotic ΔSmatg7/*Smatg7 S. macrospora* strain and transformants generated by RNA interference showed considerable morphological phenotypes during fruiting-body development and an increased number of double-neck perithecia. In addition, these mutants displayed a significantly reduced vegetative growth rate and ascospore germination efficiency (Nolting et al. 2009). These results indicate that core autophagic genes might have species-specific relevance for vegetative growth, sexual development, and viability.

2. Proteasomal Degradation

Besides protein degradation via autophagy, the **UPS protein degradation pathway** is essential for the degradation of a broad array of intracellular proteins (Sontag et al. 2014; Cohen-Kaplan et al. 2016). Among the proteins tagged for degradation by ubiquitin are damaged, misfolded, and regulatory proteins such as transcription factors or time-limited cell cycle proteins (Geng et al. 2012; Genschik et al. 2014). Therefore, the UPS plays important roles during developmental processes, and misregulation of the UPS degradation pathway is associated with defects in development (Chung and Dellaire 2015). Two reversible posttranslation modifications are important for the UPS pathway: ubiquitination and neddylation. Ubiquitin is conserved in all eukaryotes and consists of 76 amino acids. It is attached to target proteins in an enzymatic cascade, which involves three successive enzymatic steps by E1, E2, and E3 enzymes (Fig. 10). Successive rounds of E1-E2-E3 cascades assemble polyubiquitin chains at the substrate protein (Glickman and Ciechanover 2002). Proteins covalently linked to at last four ubiquitin molecules are recognized and degraded by the 26S proteasome, and ubiquitin is recycled from the target protein (Bhattacharyya et al. 2014).

E1 and E2 enzymes are characterized by a conserved domain containing a cysteine residue as an acceptor for ubiquitin, whereas two types of E3 ligases are present in eukaryotes. They are defined by a HECT domain (homologous to E6-AP carboxyl terminus) or a RING (really interesting new gene) motif. RING E3 ligases are the largest class of E3 ligases. They facilitate the direct transfer of ubiquitin from an E2-ubiquitin conjugate to a substrate protein (Vittal et al. 2015). The best-characterized group of RING E3 ligases is the cullin-based RING ligases (CRLs), which are multi-protein complexes with cullin as a central scaffold (Fig. 10b). Fungi possess the minimal eukaryotic set of cullins which are the three cullins CUL1, CUL3, and CUL4 (Braus et al. 2010). The prototype of CRL is termed SCF (SKP1/CUL1/F-box) ligase. In SCFs, cullin binds at its C-terminal domain the RING-domain protein RBX1 (Ring box protein 1) that functions as an adaptor for the E2 enzyme and at its N-terminal domain the adaptor protein SKP1 (S-phase kinase-associated protein 1) and an F-box protein (Lee and Diehl 2014). The F-box proteins, carrying an F-box domain and variable protein-protein interaction domains, are the substrate-specifying factors that recruit the substrate to be ubiquitinated to SCF ligases.

Approximately 70 different proteins with F-box domains and variable protein interaction domains were identified in *A. nidulans* (Draht et al. 2007), whereas in plants around 700 F-box domain proteins are described (Gagne et al. 2002). Despite their central role in protein turnover, only few F-box proteins have been characterized in filamentous fungi (Jonkers and Rep 2009). The F-box gene *grrA* was identified among genes that are transcriptionally upregulated during fruiting-body formation in *A. nidulans*. *A. nidulans* Δ*grrA* mutants resemble the wild type in hyphal growth, asexual sporulation, Hülle cell formation, and development of asci-containing cleistothecia, but they are unable to produce mature ascospores due to a block in meiosis (Krappmann et al. 2006). Deletion of the F-Box gene *fbx15* in *A. nidulans* results in reduced asexual and sexual development, whereas a *fbx23* mutant forms cleistothecia during conditions favoring asexual development (von Zeska Kress et al. 2012). Recently, it was demonstrated that FBX15 is required for an appropriate oxidative stress response in *A. fumigatus* (Jöhnk et al. 2016).

The covalent attachment of another UBL protein, the NEDD8 protein, to a conserved lysine residue of cullin is essential for the function of CRLs, because neddylation stabilizes the CRL complex (Bornstein and Grossman 2015).

Among all UBLs, NEDD8 is the most identical to ubiquitin with 60% amino acid and high structural identity. It consists of 81 amino acids including a lysine at position K48R (Kumar et al. 1993; Jones et al. 2008). As in most eukaryotes, NEDD8 is essential in *A. nidulans* (Kerscher et al. 2006; Rabut and Peter 2008; von Zeska Kress et al. 2012).

Removal of NEDD8 from cullins results in the disassembly of the CRL complex and dissociation of the substrate recognition unit SKP1/F-Box. Since F-box proteins determine the specificity of CRLs, repeating

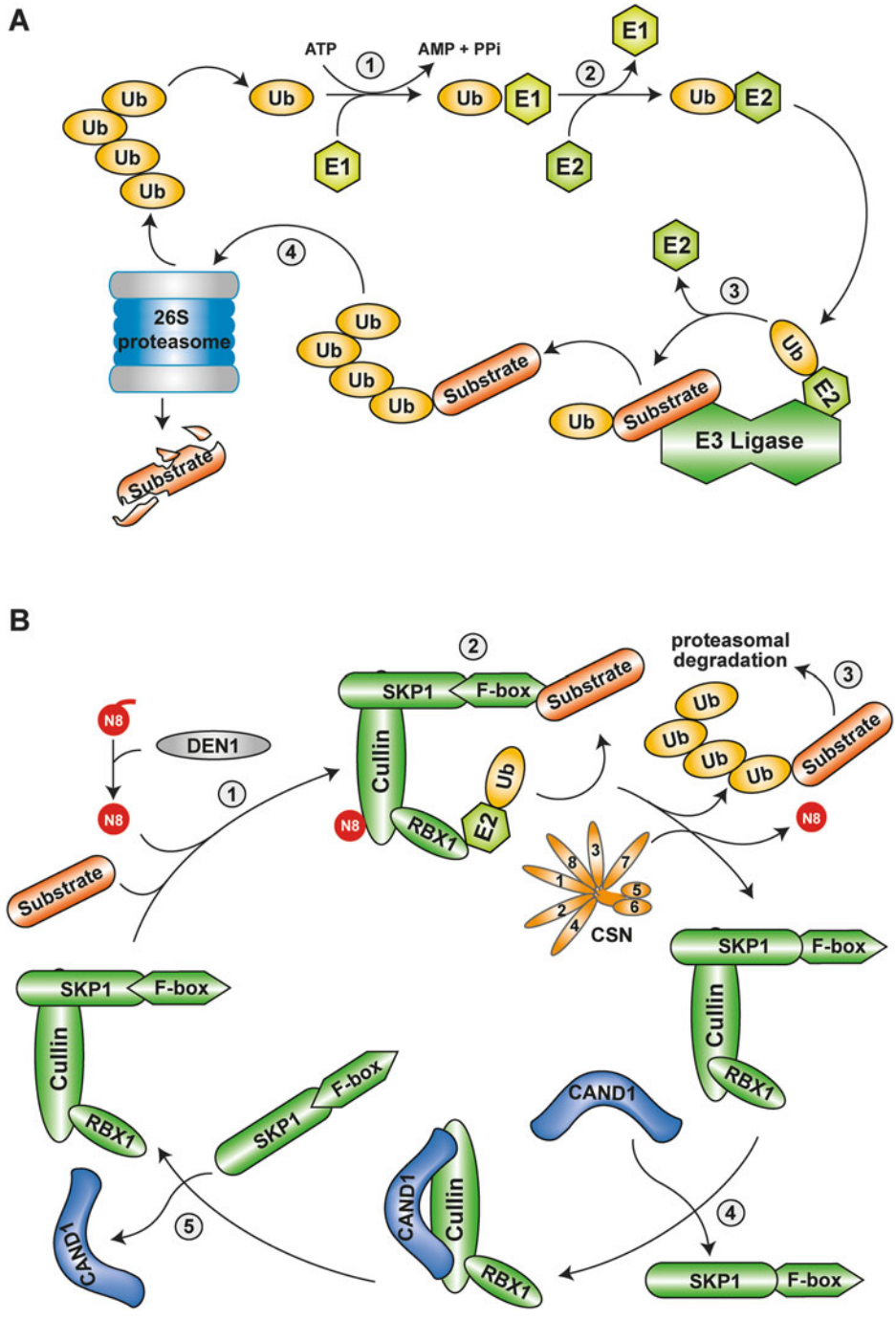

Fig. 10 The ubiquitin-proteasome system (UPS). (**a**) Ubiquitin-dependent proteasomal degradation of a substrate protein. (1) Free monomeric ubiquitin (Ub) is activated and bound to the E1 ubiquitin-activating enzyme in an ATP-dependent manner. (2) Ubiquitin is transferred to an internal cysteine of the ubiquitin-conjugating enzyme E2. (3) The substrate protein and the E2 with the activated ubiquitin are bound by the E3 ubiquitin ligase, which catalyzes the transfer of ubiquitin to a lysine residue of the substrate. (4) Repeated rounds of ubiquitination create a polyubiquitin chain that labels the substrate for degradation via the 26S proteasome, which degrades the substrate and recycles the ubiquitin. (**b**) Regulation of E3 cullin-RING ligase

cycles of neddylation and deneddylation allow binding of new F-box proteins.

The *deneddylase COP9* (constitutive photomorphogenesis 9) signalosome (CSN) hydrolyzes cullin-NEDD8 conjugates. The CSN is conserved from fungi to human, and the prototype harbors eight subunits (CSN1–CSN8). In filamentous ascomycetes, the CSN has been intensively studied in *A. nidulans* and *N. crassa*. As plants and animals, *A. nidulans* possess an eight-subunit CSN, whereas the CSN of *N. crassa* consists of seven subunits lacking CSN8 (Busch et al. 2003, 2007; He et al. 2005; Braus et al. 2010; Wang et al. 2010b). The CSN complex shares structural similarities with the lid of the 26S proteasome and with the eukaryotic translation initiation factor eIF3. Subunit CSN5 is a metalloprotease and acts as a deneddylase (Zhou et al. 2012; Beckmann et al. 2015; Meister et al. 2016). In higher eukaryotes, deletion of CSN subunits results in embryonic lethality. *A. nidulans* and *N. crassa* CSN mutants are viable but show defects in sexual fruiting-body development, a misregulated secondary metabolism, and defects in vegetative growth, in conidiation, as well as in circadian rhythm (Busch et al. 2003, 2007; He et al. 2005; Wang et al. 2010b; Gerke et al. 2012; Beckmann et al. 2015). Unexpectedly, a *csn3* deletion mutant of *N. crassa* had a wild-type-like phenotype (Wang et al. 2010b).

Combined transcriptome, proteome, and metabolome analysis of *A. nidulans* Δcsn5 mutants revealed that the CSN affects transcription of at least 15% of genes during development, including numerous oxidoreductases, and leads to changes in the fungal proteome indicating impaired redox regulation and hypersensitivity to oxidative stress. More than 100 metabolites, including orsellinic acid derivatives, accumulate in the Δcsn5 mutant. These results suggest different roles for CSN during development. During early development, the CSN is required for protection against oxidative stress and later essential for control of the secondary metabolism and cell wall rearrangement (Nahlik et al. 2010). A comprehensive genome-wide analysis of gene expression throughout the first 144 h of perithecial development of *N. crassa* revealed that expression of *csn* genes is low at early stages during fruiting-body development and increases dramatically after 48 h (Wang et al. 2014b). In contrast to CSN, which is necessary for sexual development, the second deneddylase DEN1 is required for asexual development in *A. nidulans*. DEN1 is able to interact with CSN, which targets DEN1 for protein degradation. An increased amount of DEN1 partially compensates the lack of a functional CSN. Thus, a deneddylase disequilibrium impairs multicellular development, which suggests that control of deneddylase activity is important for multicellular development (Christmann et al. 2013; Schinke et al. 2016).

Binding of the exchange factor CAND1 (cullin-asociated-NEDD8-disocciated 1) locks the unneddylated CRL in an inactive state. The N-terminal domain of CAND1 blocks the cullin neddylation site, whereas the C-terminal domain inhibits cullin adaptor interaction (Mergner and Schwechheimer 2014).

In most fungi CAND1 is encoded by a single gene. However, in aspergilli the *cand1* homolog is divided into two separate genes, into *candA-N* encoding the smaller Cand1 N-terminus and *candA-C* for the larger C-terminal part. Either deletion results in an identical

Fig. 10 (continued) (CRL) by NEDD8 (N8), the deneddylase DEN1, and the COP9 signalosome (CSN) as well as by the cullin-associated NEDD8 dissociated protein CAND1. (1) Cullin-RING ligases (CRLs) bind the substrate protein via their substrate recognition unit consisting of the adaptor protein SKP1 and a specific F-box protein. The deneddylase DEN1 processes the NEDD8 precursor (N8), which then binds the CRL E3 ligase resulting in its activation. (2) The NEDD8-activated CRL catalyzes ubiquitination of the bound substrate. (3) The ubiquitinated substrate is recognized and degraded by the 26 proteasome (see A). The CSN removes NEDD8 from the CRL E3 ligase, which then is unable to bind E2-Ub but has a high affinity for CAND1. (4) Binding of CAND1 blocks the neddylation site and leads to disassembly of SKP1 and the F-box protein (5). Recruitment of a new substrate recognition unit (SKP1 and a specific F-box) results in CAND1 release. The newly assembled CRL is again activated by NEDD8 and binds new substrates [according to Lydeard et al. (2013) and Pierce et al. (2013)]

developmental and secondary metabolism phenotype in *A. nidulans*, which resembles *csn* mutants deficient in the CSN deneddylase (Helmstaedt et al. 2011), whereas deletion of *cand1* in *N. crassa* had little effect on conidial development and the circadian clock (Zhou et al. 2012).

In summary, proper targeting of regulatory proteins to the 26S proteasome is required for fruiting-body development in filamentous ascomycetes.

C. Transcriptional Regulation

Fruiting-body development in ascomycetes requires differentiation of many cell types that are specific to this process (Bistis et al. 2003; Han 2009; Lord and Read 2011; Dyer and O'Gorman 2012) and thus leads to drastic morphological changes compared to vegetative mycelium. These changes are reflected in vastly different transcriptomes of vegetative cells versus developmental stages (see Sect. IV.C.2), and these transcriptional changes are thought to organize the accompanying morphological changes. Transcription is regulated through several mechanisms including specific transcription factors as well as chromatin modifiers. A number of genes encoding such factors have already been identified as essential for fruiting-body development in different filamentous ascomycetes. The following sections give an overview of transcription factors, transcriptional changes, and the emerging role of chromatin modifiers in fruiting-body development.

1. Transcription Factors

The genomes of filamentous ascomycetes encode on average 450 transcription factor genes per genome (Todd et al. 2014). High-throughput screens of deletion mutants in *N. crassa* and *F. graminearum* showed that about 15–19% of transcription factor mutants had a defect in sexual development (Colot et al. 2006; Son et al. 2011b; Carrillo et al. 2017). Many **transcription factors** have already been characterized in detail with respect to their role in fruiting-body formation (Table 2). This section gives an overview of the varying processes that are controlled by transcription factors during sexual morphogenesis.

Among the first transcription factors that were identified as essential for fruiting-body formation in filamentous ascomycetes are several genes encoded by the mating-type (MAT) loci (Glass et al. 1990; Staben and Yanofsky 1990) (Table 2; see also Sect. I). Even though the gene content of the MAT loci varies, most species contain at least two *MAT* genes, *MAT1-1-1* and *MAT1-2-1*, either in one individual in case of homothallic species or in different individuals in case of heterothallic species. *MAT1-1-1* encodes an α domain transcription factor and *MAT1-2-1* a transcription factor with an HMG domain (Turgeon and Yoder 2000; Bennett and Turgeon 2016). Both genes were shown to be essential for fruiting-body formation in a number of species covering the phylogenetic range of filamentous ascomycetes (Table 2). The α domain was recently shown to bear structural similarity to the HMG domain, thus strengthening the hypothesis that ascomycete *MAT* genes are derived from an ancient MAT locus containing (a) HMG domain gene(s) (Idnurm et al. 2008; Martin et al. 2010). The involvement of HMG domain proteins in sexual development may be a conserved feature in fungi and metazoa, as the MAT1-2-1 (MatA) HMG box protein of *A. nidulans* can be functionally substituted by the human SRY (sex-determining region Y) protein (Czaja et al. 2014).

However, some variability exists with respect to *MAT* gene function. For example, it was shown in the homothallic *S. macrospora* that while *MAT1-2-1* is required for sexual development, *MAT1-1-1* is not (Pöggeler et al. 2006b; Klix et al. 2010). Surprisingly, it turned out that the *MAT1-1-2* gene, which does not encode any known DNA-binding domain, is essential for fruiting-body formation in this species. This result is in contrast to its close relative *N. crassa*, where *MAT1-1-1*, but not *MAT1-1-2*, is required for sexual development (Ferreira et al. 1998). Another example of a lineage-specific gene that does not encode a transcription factor but is present in a MAT locus and essential for sexual development is *MAT1-1-5* in *S. sclerotiorum* (Doughan and Rollins 2016). Thus, gene content and function at MAT loci varies to some extent in filamentous ascomycetes, but so far, at least two *MAT* genes per species,

Table 2 Transcription factor genes involved in fruiting-body development

Gene name(s)	Species	Class	References
Transcription factors encoded by mating-type genes			
MAT-1-1-1	*N. crassa*, *P. anserina*, *C. heterostrophus*, *A. nidulans*, *A. fumigatus*, *F. graminearum*, *D. zeae-maydis*, *S. sclerotiorum*	α domain	Glass et al. (1990), Debuchy and Coppin (1992), Turgeon et al. (1993), Saupe et al. (1996), Wirsel et al. (1998), Paoletti et al. (2007), Szewczyk and Krappmann (2010), Yun et al. (2013), Zheng et al. (2013), Doughan and Rollins (2016)
MAT-1-1-3	*F. graminearum*, *P. anserina*	HMG	Debuchy et al. (1993), Zheng et al. (2013)
MAT-1-2-1	*N. crassa*, *P. anserina*, *C. heterostrophus*, *S. macrospora*, *A. nidulans*, *A. fumigatus*, *F. graminearum*, *D. zeae-maydis*, *S. sclerotiorum*	HMG	Staben and Yanofsky (1990), Debuchy and Coppin (1992), Turgeon et al. (1993), Wirsel et al. (1998), Pöggeler et al. (2006b), Paoletti et al. (2007), Szewczyk and Krappmann (2010), Czaja et al. (2011), Yun et al. (2013), Zheng et al. (2013), Doughan and Rollins (2016)
Other transcription factors			
stuA, *asm-1*	*A. nidulans*, *N. crassa*, *F. graminearum*, *A. benhamiae*	APSES domain	Miller et al. (1992), Aramayo et al. (1996), Wu and Miller (1997), Lysoe et al. (2011), Kröber et al. (2017)
devR	*A. nidulans*	bHLH	Tüncher et al. (2004)
urdA	*A. nidulans*	bHLH	Oiartzabal-Arano et al. (2015)
atfA	*A. nidulans*	bZIP	Lara-Rojas et al. (2011)
cpcA	*A. nidulans*	bZIP	Hoffmann et al. (2000)
napA	*A. nidulans*	bZIP	Yin et al. (2013)
rsmA	*A. nidulans*	bZIP	Yin et al. (2013)
zipA	*A. nidulans*	bZIP	Yin et al. (2013)
zif1	*F. graminearum*, *M. oryzae*	bZIP	Wang et al. (2011)
asl-2, *ts*	*N. crassa*	bZIP	Colot et al. (2006), McCluskey et al. (2011)
Smjlb1	*S. macrospora*	bZIP	Voigt et al. (2013)
flbC	*A. nidulans*	C_2H_2	Kwon et al. (2010)
mtfA	*A. nidulans*	C_2H_2	Ramamoorthy et al. (2013)
nsdC	*A. nidulans*	C_2H_2	Kim et al. (2009)
sltA	*A. nidulans*	C_2H_2	Ramamoorthy et al. (2013)
pcs1	*F. graminearum*	C_2H_2	Jung et al. (2014)
VpCRZ1	*V. pyri*	C_2H_2	He et al. (2016)
fhpA	*A. nidulans*	FKH box	Lee et al. (2005)
FoxE2	*S. sclerotiorum*	FKH box	Wang et al. (2016a)
SsFKH1	*S. sclerotiorum*	FKH box	Fan et al. (2016)

(continued)

Table 2 (continued)

Gene name(s)	Species	Class	References
nsdD, *sub-1*, *pro44*, *bcltf1*	*A. nidulans*, *N. crassa*, *A. fumigatus*, *S. macrospora*, *B. cinerea*	GATA	Han et al. (2001), Colot et al. (2006), Szewczyk and Krappmann (2010), Nowrousian et al. (2012), Schumacher et al. (2014)
Cmwc-1	*C. militaris*	GATA	Yang et al. (2016)
asd4	*N. crassa*	GATA	Feng et al. (2000)
steA, *pp-1*, *ste12*, *cpst12*	*A. nidulans*, *N. crassa*, *S. macrospora*, *C. parasitica*, *A. benhamiae*	HD	Vallim et al. (2000), Li et al. (2005), Nolting and Pöggeler (2006b), Deng et al. (2007), Kröber et al. (2017)
pah2	*P. anserina*	HD	Coppin et al. (2012)
pah5	*P. anserina*	HD	Coppin et al. (2012)
fmf-1	*N. crassa*	HMG	Iyer et al. (2009)
PaHMG5, *FGSG_01366*	*P. anserina*, *F. graminearum*	HMG	Ait Benkhali et al. (2013), Kim et al. (2015)
PaHMG6	*P. anserina*	HMG	Ait Benkhali et al. (2013)
PaHMG8	*P. anserina*	HMG	Ait Benkhali et al. (2013)
PaHMG9	*P. anserina*	HMG	Ait Benkhali et al. (2013)
fsd-1	*N. crassa*	IgG-fold	Hutchinson and Glass (2010)
vib-1	*N. crassa*	IgG-fold	Hutchinson and Glass (2010)
Fvmads2, *Bcmads1*	*F. verticillioides*, *B. cinerea*	MADS box	Ortiz and Shim (2013), Zhang et al. (2016)
mcm1, *Fvmcm1*, *Fgmcm1*, *mcmA*	*S. macrospora*, *F. verticillioides*, *F. graminearum*, *A. nidulans*	MADS box	Nolting and Pöggeler (2006a), Ortiz and Shim (2013), Yang et al. (2015), Zhang et al. (2016)
flbD	*A. nidulans*	Myb domain	Arratia-Quijada et al. (2012)
myt1	*F. graminearum*	Myb domain	Lin et al. (2011)
myt2	*F. graminearum*	Myb domain	Lin et al. (2012)
myt3	*F. graminearum*	Myb domain	Kim et al. (2014)
veA, *vel1*	*A. nidulans*, *C. heterostrophus*, *T. reesei*	velvet domain	Kim et al. (2002b), Bayram et al. (2008b), Wu et al. (2012), Bazafkan et al. (2015))
velB, *vel2*	*A. nidulans*, *C. heterostrophus*	velvet domain	Bayram et al. (2008b), Wang et al. (2014a)
velC	*A. nidulans*	velvet domain	Park et al. (2014)
vosA, *vos1*	*A. nidulans*, *C. heterostrophus*	velvet domain	Ni and Yu (2007), Ahmed et al. (2013), Wang et al. (2014a)
rosA	*A. nidulans*	$Zn(II)_2Cys_6$	Vienken et al. (2005)
pro1, *adv-1*, *nosA*	*S. macrospora*, *N. crassa*, *A. nidulans*, *C. parasitica*	$Zn(II)_2Cys_6$	Masloff et al. (1999), Colot et al. (2006), Vienken and Fischer (2006), Sun et al. (2009)

Genes are sorted according to encoded transcription factor class and within class according to species name. This table does not contain transcription factor genes that were identified in large-scale screens of *N. crassa* (Colot et al. 2006; Chinnici et al. 2014; Carrillo et al. 2017) and *F. graminearum* (Son et al. 2011b; Kim et al. 2015) deletion strains
bHLH basic helix-loop-helix, *C_2H_2* C_2H_2 zinc finger, *FKH box* forkhead box, *HD* homeodomain, *HMG*high-mobility group

with at least one of them encoding one of the core transcription factors MAT1-1-1 or MAT1-2-1, were found to be essential for sexual development in all species that were investigated.

Apart from the mating-type genes, a number of other transcription factor genes were shown to be required for fruiting-body formation in different species (Table 2). Interestingly, $Zn(II)_2Cys_6$-encoding genes seem to be underrepresented among the characterized transcription factors compared to their prevalence in *Pezizomycotina* genomes, where they constitute the largest transcription factor class with more than one-quarter of the encoded transcription factors (Shelest 2008; Todd et al. 2014). Indeed, in an analysis of deletion mutants of 657 of the predicted 693 transcription factors of *F. graminearum*, $Zn(II)_2Cys_6$ transcription factors made up 45% of the deleted genes but only 17% of those genes with a phenotype in sexual development (Son et al. 2011b). A different result was obtained in a deletion analysis of 99 transcription factor genes of *N. crassa*, where $Zn(II)_2Cys_6$ transcription factors contributed 71% of the deleted genes and 66% of genes with a phenotype in sexual development (Colot et al. 2006). However, a recent study of 242 transcription factor deletion mutants of *N. crassa* analyzed 101 $Zn(II)_2Cys_6$ transcription factor genes, of which 7 showed a phenotype related to sexual development. These comprise only 15% of transcription factor genes with a sexual development-related phenotype, whereas the $Zn(II)_2Cys_6$ family makes up 42% of all predicted transcription factors in *N. crassa* (Carrillo et al. 2017). Thus, the available data suggest that $Zn(II)_2Cys_6$ proteins might be underrepresented among the transcription factors regulating development. The $Zn(II)_2Cys_6$ transcription factors are a fungal-specific class of proteins that expanded most strongly in the filamentous ascomycetes (Shelest 2008; Todd et al. 2014). It is possible that fruiting-body development evolved preferentially based on evolutionary older regulatory pathways or that the expansion of the $Zn(II)_2Cys_6$ class occurred after core developmental pathways evolved. However, more large-scale studies of species outside of the *Sordariomycetes* will be needed to address this question comprehensively.

Several transcription factors were shown to be involved in fruiting-body formation in only distantly related species, e.g., in *Sordariomycetes* and *Eurotiomycetes* (Table 2). Among these are homologs of the *A. nidulans* transcription factor StuA, which was first studied with respect to its role in asexual development but in addition is required for cleistothecia formation (Miller et al. 1992). A mutant of the *N. crassa* homolog ASM-1 has a similar phenotype, as the mutant does not form protoperithecia, and conidia are formed on stunted conidiophores (Aramayo et al. 1996). The *N. crassa asm-1* gene can complement the conidiation defects, but not the sexual development phenotype of the *A. nidulans* stuA mutant (Chung et al. 2015). However, it was shown previously that different levels of *stuA* transcript are required for correct progression of different developmental pathways in *A. nidulans* (Wu and Miller 1997); therefore failure of *asm-1* to complement the lack of fruiting-body formation in the stuA mutant might be related to non-wild-type-like expression. StuA homologs were also analyzed in two additional species from the *Sordariomycetes* and *Eurotiomycetes*, respectively, namely, *F. graminearum* and *Arthroderma benhamiae* (Lysoe et al. 2011; Kröber et al. 2017). In both species, the corresponding homologs are required for sexual development and additionally have phenotypes related to species-specific environments; the *F. graminearum* mutant is no longer pathogenic, whereas the dermatophyte *A. benhamiae* requires *stuA* for growth on keratin-containing substrates. Overall, the role of *stuA* in sexual development seems to be conserved across a wide range of filamentous ascomycetes.

Another transcription factor with a (partially) conserved role in sexual development is Ste12. This gene was first identified as an essential gene for sexual differentiation in the yeast *S. cerevisiae* (Johnson 1995). Subsequent studies in several filamentous ascomycetes showed a role for *ste12* homologs in all species that were analyzed (Table 2); however, the developmental phenotypes of ste12 mutants vary widely. The first *ste12* homolog that was analyzed in filamentous ascomycetes was *steA* from *A. nidulans*

(Vallim et al. 2000). The corresponding mutant makes Hülle cells, but no cleistothecia, ascogenous hyphae, or ascospores. A similar phenotype was observed in the *steA* mutant of another eurotiomycete, *A. benhamiae* (Kröber et al. 2017). A mutant in the *N. crassa* homolog *pp-1* is also unable to form fruiting bodies or even fruiting-body precursors, and in addition, ascospores from outcrossing the pp-1 mutant are ascospore lethal when carrying the mutant allele (Li et al. 2005). However, in *S. macrospora*, which is closely related to *N. crassa*, *ste12* deletion still allows fruiting-body formation, but ascospore development is impaired (Nolting and Pöggeler 2006b). Interestingly, despite different morphological outcomes, parts of the signaling cascades involving *ste12* appear to be conserved from yeast to filamentous ascomycetes. For example, it was shown in *S. macrospora* that STE12 interacts with the transcription factors Mcm1 and MAT1-1-1, similar to its counterpart in yeast (Nolting and Pöggeler 2006b). Mcm1 itself is another example of a transcription factor with a conserved role in sexual development in different ascomycetes (Nolting and Pöggeler 2006a; Ortiz and Shim 2013; Yang et al. 2015). In *A. nidulans*, SteA acts downstream of a MAPK cascade that controls the balance of sexual development, asexual development, and secondary metabolism in response to external signals and comprises elements that form a similar MAPK cascade regulating sexual development in yeast (Bayram et al. 2012). Thus, core regulatory pathways might be conserved, but it is likely that input and output pathways were rewired extensively during the evolution of fruiting-body development. A special case of such rewiring might be the interaction between hypovirus infection, virulence, and sexual development in *Cryphonectria parasitica* (Deng et al. 2007). In this sordariomycete, deletion of the *ste12* homolog *CpST12* has the same effects as hypovirus infection, namely, female sterility and reduced virulence. Furthermore, many genes that are differentially regulated in the CpST12 mutant are also differentially regulated upon hypovirus infection (see Sect. IV.C.2). Thus, hypovirus infection seems to interfere with pathways regulating sexual development in this species. Yet another example for this interference is the role of the *pro1* homolog in *C. parasitica*. *pro1* was first identified as a transcription factor essential for sexual development in *S. macrospora* (Masloff et al. 1999), whereas in *C. parasitica*, *pro1* is not only required for female fertility but also for stable hypovirus maintenance (Sun et al. 2009). Additional roles for *pro1* orthologs were also found in *N. crassa*, where deletion of the ortholog *adv-1* leads to a pleiotropic phenotype with defects in vegetative growth, conidiation, and protoperithecia formation (Colot et al. 2006). In *A. nidulans*, there are two *pro1* homologs, *nosA* and *rosA*, with *nosA* being more closely related to *pro1* than *rosA* (Vienken et al. 2005; Vienken and Fischer 2006). Both genes are involved in fruiting-body formation, but with opposite roles; while *nosA* is an activator required for sexual development, *rosA* is a repressor of fruiting-body formation.

A conserved role in fruiting-body development was also found for the GATA-type transcription factor NsdD. It was first identified in *A. nidulans*, where *nsdD* mutants make no cleistothecia or Hülle cells under standard conditions, whereas overexpression of *nsdD* leads to inappropriate formation of sexual structures, e.g., when the mutant is grown submerged in liquid medium (Han et al. 2001). In *A. nidulans*, *nsdD* is not only required for sexual development but is part of the regulatory circuits that control the balance between sexual and asexual morphogenesis. Deletion of *nsdD* leads to increased conidiation, and NsdD binds the promotor of the conidiation activator *brlA* as a repressor (Lee et al. 2014, 2016). The orthologs of *nsdD* in *Sordariomycetes* are also required for fruiting-body formation as was shown for *N. crassa sub-1* and *S. macrospora pro44* (Colot et al. 2006; Nowrousian et al. 2012). In addition to being blocked at the transition from protoperithecia to perithecia, the corresponding mutants show the additional phenotype of protoperithecia that are submerged in the agar medium. Thus, *sub-1*/*pro44* is not only involved in the development as such but also in the correct spatial placement of fruiting bodies within the mycelium. *nsdD*/*sub-1*/*pro44* orthologs are not only required for fruiting-body formation in the *Eurotiomycetes* and *Sordariomycetes* but

also in the *Leotiomycetes*. This was shown for *Botrytis cinerea* where the corresponding ortholog *bcltf1* is unable to make sclerotia, the structures from which fruiting bodies are initiated (Schumacher et al. 2014). A *nsdD/sub-1/pro44* ortholog is also present in the early-diverging lineage of *Pezizomycetes*, and the corresponding gene from the pezizomycete *P. confluens* can complement the *S. macrospora pro44* mutant, suggesting a conserved role for this gene for fruiting-body development in the ancestor of filamentous ascomycetes (Traeger et al. 2013).

However, a fruiting-body-related role is not conserved in all transcription factors that were investigated. One example to the contrary is the ortholog pair ASD4/AreB from *N. crassa* and *A. nidulans*, respectively (Feng et al. 2000; Wong et al. 2009). While *asd4* is involved in the formation of asci and ascospores in *N. crassa*, its ortholog *areB* in *A. nidulans* is involved in nitrogen metabolism and asexual differentiation, but not in sexual development. Similarly, the WC-1 homolog in *Cordyceps militaris* is essential for fruiting-body formation (Yang et al. 2016), whereas *wc-1* mutants of *N. crassa* are impaired in protoperithecia formation but still fertile (Degli-Innocenti and Russo 1984). One explanation in this case is that fruiting-body formation in *C. militaris* is light dependent, which is not the case in *N. crassa*, and generally rare in filamentous ascomycetes (see Sect. III.A.1). Therefore, it is possible that preexisting regulatory circuits for light regulation were recruited into regulating sexual development in those species where light influences this process.

Analysis of species that are able to form conidia in addition to undergoing sexual development has revealed that many transcription factors involved in sexual development are in fact regulating the balance between the two different morphogenetic pathways. This might make sense in the light of limited resources, e.g., nutrients, because both processes require a considerable investment of such resources until mature spores can be produced, and depending on the conditions, commitment to one or the other might be the most efficient way toward successful propagation. The regulation of this balance was most intensively studied in the homothallic *A. nidulans*, where in principle conidiation and fruiting-body formation can be carried out by a single individual. These studies have revealed a role for a number of transcription factors in regulating not only the balance between sexual and asexual development, but also secondary metabolism, another resource-intensive process that is tightly linked to environmental conditions. Apart from the GATA transcription factor NsdD mentioned above, the transcription factors FlbC, NapA, NsdC, RsmA, UrdA, and ZipA are part of this extensive regulatory network (Kim et al. 2009; Kwon et al. 2010; Yin et al. 2013; Oiartzabal-Arano et al. 2015). However, the best-studied player in this process might be the velvet protein VeA. The *veA1* mutant allele has been present for many years in a number of laboratory strains, used because it preferentially forms conidia (Käfer 1965). *veA* was identified as a gene required for fruiting-body formation in 2002 (Kim et al. 2002b) and has been studied intensively since (Yin and Keller 2011; Gerke and Braus 2014) (see Sect. III.A.1). The novel velvet domain present in VeA and several other proteins was only recently shown to be a DNA-binding domain (Ahmed et al. 2013). VeA was shown to participate in several protein complexes that shuttle between cytoplasm and nucleus depending on external signals. One such complex consists of VeA, a second velvet protein named VelB, and the putative methyltransferase LaeA. In darkness, VeA localizes to the nucleus and allows protein complex formation leading to sexual development and expression of a number of genes involved in secondary metabolism, whereas in light, VeA stays in the cytoplasm, thereby preventing formation of the nuclear complex (Bayram et al. 2008b). In addition, VeA can form an alternative complex with the methyltransferases VipC and VapB, which inhibits sexual development and instead promotes conidiation (Sarikaya-Bayram et al. 2014) (see Sect. IV.C.3). As part of the regulatory network controlling the balance between sexual and asexual development, the MAPK cascade that regulates SteA/Ste12 also regulates VeA through phosphorylation of VeA by the MAP kinase Fus3 (Bayram et al. 2012). By now, homologs of VeA and other velvet proteins have been identified as regulators of sexual development also in other ascomycetes (Table 2).

The transcription factors described so far mostly lead to a block at a certain developmental

stage when the corresponding genes are deleted. However, there are also transcription factors that are involved in modulating morphology or are required for morphogenesis of specific parts of the fruiting body. An example of the former is MYT2 in *F. graminearum*, deletion of which leads to larger perithecia, whereas overexpression leads to smaller perithecia (Lin et al. 2012). An interesting case is the transcription factor FlbD in *A. nidulans*. This protein was originally analyzed for its role in conidiophore differentiation but was also shown to be required for the formation of the fruiting-body peridium; mutants in *flbD* are able to produce ascospores without the surrounding peridium (Arratia-Quijada et al. 2012). Further analysis of factors like MYT2 or FlbD might help to elucidate the regulatory events involved in the morphogenesis of specific cell types during fruiting-body formation, a topic not much explored in fungi to date. What is also not well known is the spatial regulation of development, e.g., on which parts of the mycelium fruiting bodies are differentiated, and how the organization of different cell types within the fruiting body is regulated. It is reasonable to assume that transcription factors will play a role in these processes, similar to morphogenetic events in plants and animals. Future studies involving advanced microscopy techniques or single-cell transcriptomics might shed light on these questions.

2. Transcriptional Changes During Development

Fruiting-body development involves the differentiation of many cell types that are not present in the vegetative mycelium and consequently requires changes in the activity of a large number of genes compared to vegetative growth. This change is thought to occur to a large degree at the level of transcription, and consequently transcription factors are likely to play a role in this process. Therefore, **transcriptomics studies** have not only been used to compare transcriptomes of different developmental stages but also to analyze transcriptomes of developmental mutants, especially transcription factor mutants, which will be the focus of this section. For a review of transcriptomics of other developmental mutants, different growth conditions, and comparative transcriptomics approaches, see Nowrousian (2014).

Among the first transcription factor mutants that were analyzed using transcriptomics were *MAT* gene mutants. Studies using differential hybridization, microarrays, or RNA-seq have been performed for *MAT* gene mutants of *F. graminearum*, *G. fujikuroi*, *P. anserina*, *P. chrysogenum*, and *S. macrospora* (Lee et al. 2006; Pöggeler et al. 2006b; Keszthelyi et al. 2007; Klix et al. 2010; Bidard et al. 2011; Böhm et al. 2013; Kim et al. 2015). A recent study of *P. chrysogenum* used chromatin immunoprecipitation-sequencing (ChIP-seq) to identify direct target genes of the MAT1-1-1 protein (Becker et al. 2015). A recurrent finding in these studies was that there are many more genes regulated directly or indirectly by *MAT* genes than in the yeast *S. cerevisiae*, where the *MAT* genes directly regulate only about 30 genes (Galgoczy et al. 2004). Furthermore, most of the *MAT* target genes in yeast have roles in sexual development themselves, whereas function predictions of *MAT*-regulated genes in filamentous ascomycetes point to various roles outside of fruiting-body formation. Consequently, gene deletion studies of differentially regulated genes found that some but not all of the potential target genes are involved in sexual development (Kim et al. 2015). Thus, deletion and gene expression studies show that *MAT* genes in filamentous ascomycetes have acquired functions outside of sexual development in addition to their "classical" roles as sexual regulators. Furthermore, the regulatory networks surrounding the MAT genes might be only moderately conserved. For example, the HMG domain protein HMG5 in *P. anserina* was found to act upstream of *MAT1-1-1* (Ait Benkhali et al. 2013), whereas its ortholog *FGSG_01366* in *F. graminearum* is a potential target gene of MAT1-2-1 (Kim et al. 2015).

Transcriptomics analyses were also conducted for other transcription factor mutants. Ste12 mutants of *N. crassa* (*pp-1*) and *C. parasitica* (*CpST12*) were analyzed using

microarrays (Li et al. 2005; Deng et al. 2007). In *N. crassa*, it was shown that the expression patterns of the *pp-1* mutant were similar to those of a mutant of the *fus3*-homolog *mak-2*, indicating that similar to yeast, these two genes are involved in the same regulatory pathway. Many of the genes regulated differentially in the *C. parasitica* mutant are also regulated differentially upon hypovirus infection, confirming the influence of hypoviruses on the regulation of sexual development in this fungus.

The *stuA* mutant of *F. graminearum* was also used for transcriptome analysis. However, these studies were carried out under growth conditions that favor conidiation (Lysoe et al. 2011). In contrast, transcriptome studies were conducted for the pro1 mutant of *S. macrospora*, either using total mycelia under conditions allowing sexual development or using RNA from young fruiting bodies from the wild type and the pro1 mutant (Nowrousian et al. 2005, 2007; Teichert et al. 2012). In addition, ChIP-seq analyses were used to identify direct target genes of PRO1 in *S. macrospora* and its ortholog ADV-1 in *N. crassa* (Steffens et al. 2016; Dekhang et al. 2017). The transcriptomics analyses found large differences in expression patterns between the pro1 mutant and the wild type, including differential expression of a number of genes known to be involved in sexual development. Furthermore, the ChIP-seq analyses showed PRO1 binding to promoters of genes involved in pheromone signaling, ROS metabolism, and cell wall integrity. These pathways were shown previously to play a role in fruiting-body development (see Sects. III.B.2, IV.A.3, and IV.A.1), and it is possible that PRO1 acts as a master regulator controlling several important downstream pathways.

3. Chromatin Modifiers

Underlying the drastic morphological changes during fruiting-body development are corresponding genome-wide changes in gene expression. While specific transcription factors play a role in those expression changes, it seems likely that many of these are also facilitated or regulated at the level of chromatin. Therefore, **chromatin-modifying factors** are thought to play a role in fruiting-body formation. Chromatin modifications can include modification of the DNA itself, e.g., through methylation, positional changes of nucleosomes, incorporation of histone variants into nucleosomes, or a wide range of histone modifications (Zhang and Pugh 2011; Freitag 2014; Voss and Hager 2014). Several studies in recent years have uncovered a number of chromatin-modifying factors that are involved in fruiting-body formation and that cover a wide range of potential functions in chromatin modification.

Two such factors, HDF1 and FTL1, were studied in *F. graminearum* and are predicted to be homologs of members of the yeast Set3 complex that contains several histone deacetylase (HDAC) proteins and regulates HDAC activity (Ding et al. 2009; Li et al. 2011). In *S. cerevisiae*, the Set3 complex controls the progression through meiosis, with mutants showing accelerated meiosis and lower numbers of viable spores (Pijnappel et al. 2001). HDF1 from *F. graminearum* is an ortholog of the yeast HDAC Hos2, and the corresponding deletion mutant is no longer able to form perithecia under selfing conditions. Instead, the mutant forms conidia, indicating that HDF1 is required to maintain the correct balance between sexual and asexual development in *F. graminearum* (Li et al. 2011). FTL1 is homologous to the yeast Set3 complex protein Sif2, and similar to the HDF1 mutant, the FTL1 mutant is female sterile. Furthermore, HDAC activity in the mutant is reduced (Ding et al. 2009). These data suggest that chromatin regulation via histone modification plays an important role in priming the genome for sexual development.

Another chromatin modifier that is required for fruiting-body formation is the histone chaperone ASF1 in *S. macrospora* (Gesing et al. 2012). Histone chaperones comprise a heterogenic group of proteins that are characterized by their ability to handle non-nucleosomal histones in vivo and mediate the assembly of nucleosomes from isolated histones and DNA in vitro (Hammond et al. 2017). Histone chaperones are involved in essentially all processes involving chromatin, including DNA replication, repair, and transcription (Das et al. 2010). ASF1 is a conserved eukaryotic histone chaperone specific for

histones H3 and H4 and involved in a number of processes ranging from nucleosome assembly to regulation of transcription (Mousson et al. 2007). Interestingly, deletion of *asf1* is lethal in many organisms where it was investigated, and so far *S. macrospora* is the only multicellular organism where an *asf1* deletion mutant is viable. Apart from a slower vegetative growth rate, the mutant is unable to progress from immature to mature fruiting bodies (Gesing et al. 2012). A number of known developmental genes are transcriptionally deregulated in the *asf1* mutant, but the exact molecular mechanisms by which ASF1 causes changes in gene expression during fruiting-body formation are not clear yet.

Another player among chromatin modifiers involved in regulating sexual development is the protein complex comprising the methyltransferases VipC and VapB as well as the velvet protein VeA in *A. nidulans* (Sarikaya-Bayram et al. 2014). The methyltransferases were identified in a screen for VeA interaction partners. A mutant in *vipC* shows the opposite phenotype of the *veA* mutant, namely, the production of more fruiting bodies in the light. Depending on external signals, VipC and VapB either interact with a protein called VapA, which tethers the complex to the plasma membrane and prevents interaction with VeA, or VipC and VapB are released from VapA and translocate to the nucleus, where they can interact with VeA and inhibit sexual development and secondary metabolism (Sarikaya-Bayram et al. 2014). This is in contrast to the effect of VeA interacting with another putative methyltransferase, LaeA, in which case sexual development and secondary metabolism are promoted (Bok et al. 2005; Bayram et al. 2008b). However, in contrast to LaeA, for which the target molecules have not been identified yet, it is possible that VapB is a bona fide methyltransferase, as overexpression of *vapB* leads to a reduction in histone H3 lysine 9 trimethylation (H3K9me3) (Sarikaya-Bayram et al. 2014, 2015). Thus, it is possible that histone modifications play an important role in regulating the transition between sexual and asexual propagation. This is also supported by the finding that the white collar-1 homolog LreA interacts not only with the white collar-2 homolog LreB, which in turn interacts with the phytochrome FphA, which itself interacts with VeA (see Sect. III.A.1), but that LreA also interacts with the histone acetyltransferase GcnE and the deacetylase HdaA (Hedtke et al. 2015). An additional case in point comes from the analysis of another putative histone-modifying protein, RtfA, in *A. nidulans* (Ramamoorthy et al. 2012). *rtfA* was identified as a suppressor of ΔveA with respect to secondary metabolism. RtfA is homologous to the *S. cerevisiae* Rtf1 protein, which is a member of the Paf complex required for transcription-associated histone modifications (Warner et al. 2007). Deletion of *rtfA* in *A. nidulans* leads to loss of Hülle cells and cleistothecia but also to vegetative growth defects and the production of fewer conidia (Ramamoorthy et al. 2012). As *rtfA* was identified as a suppressor of ΔveA, it is possible that *veA* acts as a central hub for the control of chromatin landscape during different life phases of *A. nidulans* (Sarikaya-Bayram et al. 2015).

Overall, analysis of the role of chromatin modifications in regulating sexual development in filamentous ascomycetes is still in its infancy, but from results obtained so far, it seems likely that there are major regulatory events remaining to be discovered.

V. Conclusions

Filamentous ascomycetes generate multiple types of fruiting bodies and serve as model organisms to investigate eukaryotic multicellular development at the molecular level. Highly developed fungal genetic systems provide the experimental basis for forward and reverse genetic approaches to decipher components of cellular networks that direct and regulate the development of complex fruiting bodies. These experimental benefits of filamentous fungi are complemented by their relatively small genome size, and the ease of culturing makes them amenable to high-throughput screening. The data output of these technologies has led to new ideas about the mechanisms to build a eukaryotic multicellular structure. We just

begin to understand how genetic and environmental cues initiate and regulate fruiting-body formation at the molecular level.

While fruiting-body-dependent gene expression and its regulation is intensively studied, relatively little is known about signaling events that transmit outside-in signals and direct the coordinated formation of different cell types. Currently, we are aware of several multi-subunit protein complexes that are involved in fruiting-body development, and we begin to get a picture about how these complexes interact with each other and transduce signals that direct the initiation of the sexual cycle, and the subsequent formation of a dikaryon. However, the signaling events for maintenance of the dikaryon and the regulated entry into karyogamy remain obscure.

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