7 Genomics and Transcriptomics to Analyze Fruiting Body Development

Minou Nowrousian[1]

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I. Introduction

Fungal fruiting bodies are multicellular structures that are produced by filamentous ascomycetes and basidiomycetes for the generation, protection, and dispersal of sexual spores (Pöggeler et al. 2006a; Wösten and Wessels 2006). Fruiting body formation involves the spatiotemporal coordination of the differentiation of specialized cell types, many of which are exclusively found in fruiting bodies (Moore 1998; Bistis et al. 2003). These drastic morphogenetic changes, compared to the vegetative mycelium, most likely depend on changes in activity of a large number of genes, similar to developmental processes in animals and plants in which coordinated patterns of gene expression orchestrate the spatiotemporal sequence of events that leads to embryonic or organ development (Guitton and Berger 2005; Scheres 2007). However, as the last common ancestor of fungi and animals was most likely unicellular (Lang et al. 2002; King 2004), multicellular development in fungi evolved independently of other groups; therefore, regulatory genes and networks that govern animal development are not necessarily similar in fungi.

The genetic and molecular basis of fruiting body development has been under investigation for several decades, and much of the progress in this area of research has been made through the analysis of developmental mutants and their corresponding individual genes (Moore 1998; Pöggeler et al. 2006a; Wösten and Wessels 2006). However, in the last approximately 15 years, a number of high-throughput techniques, so-called -omics techniques, have been established in quick succession, enabling the increasingly detailed analysis of complete genomes and their activity, especially at the level of the transcriptome. These technologies include, for example, EST (expressed sequence tag) sequencing, SAGE (serial analysis of gene expression), microarray hybridizations, and

[1]Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Bochum 44780, Germany; e-mail: minou.nowrousian@rub.de

a multitude of applications made possible through next-generation sequencing (NGS) techniques (Velculescu et al. 1995; Churchill 2002; Nowrousian et al. 2004; Ehrenreich 2006; Nowrousian 2007, 2010; Shendure and Li 2008). These technologies have led to a rapid increase in the number of sequenced fungal genomes, as well as genome-wide expression studies to analyze different aspects of fungal biology, including fruiting body development. In addition, the generation of fungal strains with reduced rates of nonhomologous end joining has greatly facilitated targeted gene deletions in many fungal species (Ninomiya et al. 2004; Kück and Hoff 2010), thereby enabling large-scale knockout projects ranging from deletions of all genes from a gene family to genome-wide knockout libraries (see Sect. II.B). Thus, we are now at a point where, in principle, high-throughput expression studies can rapidly identify candidate genes with interesting expression patterns during fruiting body development, and these genes can be characterized in more detail through libraries of deletion mutants. Mutants themselves can also be used for expression studies, thereby establishing molecular phenotypes to characterize mutant strains at the molecular level and to unravel the regulatory networks that govern differentiation processes. While knockout libraries are currently available for only a few model organisms, high-throughput expression studies can be performed also in nonmodel species (Nowrousian 2010). These data can be used for comparative studies to identify evolutionary conserved expression patterns, which in turn are useful for choosing candidate genes for downstream analysis because evolutionary conservation of expression is a powerful criterion for functional significance (Stuart et al. 2003). Thus, high-throughput techniques have made possible the characterization of select model organisms at an unprecedented depth as well as the analysis of a much broader range of nonmodel organisms, both on a genome-wide scale. In this review, I give an overview of how genomics and transcriptomics studies have increased our understanding of fruiting body development and point out some possible future directions in this field.

II. From Genome to Function

Fungi have always been at the forefront of genome-sequencing efforts, with the first sequenced eukaryotic genome that of the yeast *Saccharomyces cerevisiae* (Goffeau et al. 1996), with the first genome sequence of a filamentous fungus, namely that of *Neurospora crassa*, following some years later (Galagan et al. 2003). Because fungal genomes are usually less than 100 Mb in size and therefore smaller and easier to sequence than genomes of many other eukaryotes, fungi (together with animals) are the eukaryotic group with the highest number of sequenced genomes to date (Table 7.1). This also applies to the current rise in the number of genomes sequenced by NGS techniques, where fungi were (again) among the first eukaryotes whose genomes were assembled from NGS sequence reads (DiGuistini et al. 2009; Nowrousian et al. 2010).

The genome sequences of filamentous fungi not only allow downstream analyses like large-scale knockout studies and mutant genome-sequencing projects that yield more insights into fungal development (see Sects. II.B and II.C) but also were the basis for efforts to induce fruiting body formation in supposedly asexual fungi as described in the next section.

A. Sexual Development in "Asexual" Fungi

For an estimated 20 % of described fungal species, no sexual stage is known (Dyer and O'Gorman 2011), and these species were historically grouped together as **deuteromycetes**. First hints that supposedly asexual fungi might have "cryptic" sexual development came from a population genetic study of the pathogenic ascomycete *Coccidioides immitis* and from the isolation of a mating-type gene from the asexual fungus *Bipolaris sacchari* (Burt et al. 1996; Sharon et al. 1996); however, it was not clear at that time whether these findings were the exception or the rule. Therefore, it

Table 7.1. Genome assemblies in the National Center for Biotechnology Information (NCBI) Genome database

Group	Chromosome assemblies[a]	Contig/scaffold assemblies[a]	Total[a]
Fungi	40	165	205
Animals	43	162	205
Plants	19	32	51
Protists	20	53	73
Others	6	0	6
Total	128	412	540

[a]The number of genomes from different eukaryotic groups for which complete chromosomes or assembled contigs or scaffolds are available was calculated from data from http://www.ncbi.nlm.nih.gov/genome/browse/ in October 2012. For each species, only one sequenced genome was counted

came as quite a surprise that virtually all sequenced genomes from supposedly asexual ascomycetes had a full complement of genes known to be involved in sexual differentiation, including **mating-type genes** that were shown to be essential for sexual development in other fungi. This includes, for example, Aspergillus and Penicillium species (Pöggeler 2002; Galagan et al. 2005; Paoletti et al. 2005; Pel et al. 2007; Hoff et al. 2008; van den Berg et al. 2008). These results led to renewed efforts to induce fruiting body formation in supposedly asexual species or strains under laboratory conditions, and the development of sexual structures could be induced, for example, for *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Penicillium chrysogenum*, and the *Trichoderma reesei* strain QM6a (Horn et al. 2009; O'Gorman et al. 2009; Seidl et al. 2009; Böhm et al. 2013).

One key finding from genome sequencing was that the mating-type loci of the sequenced asexual strains indicated that the corresponding species might be heterothallic, that is, not self-fertile. In heterothallic fungi, sexual development can only take place when two strains with different mating types interact (Pöggeler et al. 2006a). Based on this information, researchers screened natural isolates for their mating type and combined strains with different mating types (Horn et al. 2009; O'Gorman et al. 2009; Seidl et al. 2009; Böhm et al. 2013).

The demonstration of sexual development in former deuteromycetes is not only important to increase our knowledge about fungal biology but also can be used as a valuable tool for basic and applied research with these fungi because the ability to perform genetic crosses is a major advantage for any organism under study.

This is especially interesting for species that are used in industrial production because strain improvement based on genetic recombination offers a much wider scope than one based solely on conventional mutagenesis or genetic engineering.

B. Large-Scale Knockout Studies

One of the most powerful approaches to analyze gene functions is the inactivation of the gene under investigation and the analysis of the resulting phenotype. Targeted gene deletions in fungi are usually accomplished by **homologous recombination** of a knockout construct containing sequences upstream and downstream of the Open Reading Frame (ORF) flanking a selection marker. The knockout construct is transformed into the wild type or a recipient strain deficient in nonhomologous end joining, and transformants are screened for those where the deletion cassette has replaced the original gene sequence (Ninomiya et al. 2004; Kück and Hoff 2010). The availability of complete genome sequences allows such **forward genetic studies** at a much greater scale because the necessary sequence information to generate gene deletion constructs is readily available for all predicted genes of a sequenced species. *Saccharomyces cerevisiae* was the first eukaryote for which a knockout project for all predicted genes was initiated (Winzeler et al. 1999), and the genome-wide knockout project for the filamentous fungus *N. crassa* followed shortly after its genome was sequenced (Colot et al. 2006; Dunlap et al. 2007). Deletion strains for most *N. crassa* genes are now available

through the Fungal Genetics Stock Center (FGSC) (McCluskey and Wiest 2011), and morphological screens for basic characteristics, including sexual development, are under way. The results are made available through the *Neurospora crassa* Database (http://www.broadinstitute.org/annotation/genome/ neurospora/ MultiHome.html). In a pilot study, knockout strains for about 100 transcription factor genes were generated and characterized, and 15 strains displayed phenotypes related to sexual development (Colot et al. 2006). An even higher fraction of serine-threonine protein kinase mutants (32 of 77) had defects in sexual differentiation in a study by Park et al. (2011).

Knockout screens of mutants for certain classes of genes have also been performed with *Gibberella zeae* (anamorph *Fusarium graminearum*). Based on the genome sequence (Cuomo et al. 2007), 15 polyketide synthase genes were identified and disrupted (Gaffoor et al. 2005). All of the mutants were still fertile; however, one of them produced nonpigmented fruiting bodies instead of the usual black ones. A recent study of 657 deletion mutants for predicted transcription factor genes showed that 105 of them were involved in sexual development (Son et al. 2011). Many of these mutants also had other phenotypes, mainly influencing growth, virulence, and toxin production. Generally, transcription factor genes associated with sexual development tended to be conserved throughout the fungal kingdom.

Mutants from such large-scale knockout screens can now be used for detailed functional characterization of the corresponding genes. Furthermore, these strains are also available as marker strains in genetic crosses e.g., to finely map unknown mutations in strains that were generated by classical mutagenesis (Hammond et al. 2011). Such mapping strategies can be used in combination with whole-genome sequencing to identify mutated genes as described in the next section.

C. Genome Sequencing to Identify Mutations

Prior to the possibility of generating knockout strains, many mutagenesis efforts centered around classical radiation or chemical mutagenesis, thereby generating random mutations. For many species, large **mutant collections** are available, and often such mutants have phenotypes different from knockout strains, when the function of the derived protein is not completely lost, but modified. Thus, even in the days of large-scale knockout projects, the identification of the mutated genes in such strains is an important tool for molecular and genetic analysis. The most common ways to identify mutated genes used to be genetic mapping, complementation by transformation, or a combination of both. However, these strategies are often laborious and time consuming. In recent years, the advent of NGS techniques has made the sequencing of mutant genomes and the identification of mutations by subsequent bioinformatics analysis a viable alternative. In filamentous fungi, this approach has been used to identify mutated genes in classical mutants of the ascomycetes *N. crassa* and *Sordaria macrospora* (McCluskey et al. 2011; Pomraning et al. 2011; Nowrousian et al. 2012).

Pomraning and coworkers (2011) crossed a mutant with a wild type with different genetic background, sequenced pools of DNA from individual progeny from the cross, and identified a region where sequence variants from the mutant background accumulated. The mutation causing cell cycle defects could be narrowed down to one of the genes in this genomic region.

In a study by McCluskey et al. (2011), 18 classical mutants for which the mutations had previously been mapped to genomic regions between 80 kb and 4 Mb were sequenced, and candidate genes or regions were identified for 17 of these strains. Six of the mutants for which mutated candidate genes were found are defective in sexual development as they were male sterile (mb-2, mb-3), female sterile (ff-1, fs-n), or producing underpigmented ascospores (ts, perithecial-1). A similar study in *S. macrospora* made use of pooled DNA from progeny of a cross from a mutant with an isogenic wild type, and in this case, causative mutations could be identified from the sequence data without prior genetic mapping (Nowrousian et al. 2012). The three mutants that were studied are all defective in sexual development, with two of

them unable to develop mature fruiting bodies, while the third produces light-brown instead of black ascospores. One of the sterile mutants is allelic to the pro41 mutant carrying a deletion of a gene encoding an endoplasmic reticulum (ER) membrane protein (Nowrousian et al. 2007a), while the second sterile mutant contains a point mutation in a transcription factor gene (*pro44*), and the spore color mutant fus has a defective melanin biosynthesis gene. Thus, mutant sequencing is a quick and efficient method to identify developmental genes from mutants generated by classical mutagenesis.

III. Transcriptomics: The Genome in Action

As mentioned in the Introduction, the profound morphogenetic changes that a mycelium undergoes during fruiting body development depend on corresponding changes in activity of large numbers of genes, and many of these changes are thought to be implemented at the level of transcription. Therefore, transcriptomics approaches (i.e., the analysis of transcript levels for many or all genes in a genome under certain conditions or in different cell types, organs, etc.) are especially suited for the analysis of fruiting body development. A range of transcriptomics methods has been applied in a number of asco- and basidiomycetes to study sexual differentiation (Table 7.2). These approaches have the advantage of allowing a bias-free insight into transcriptional expression because they do not access only a selected subset of genes, but rather yield a global picture. On the other hand, they also allow hypothesis-driven research, for example, the analysis of epistatic relationships or regulatory networks through comparing molecular phenotypes of single and double mutants. In the following sections, results from transcriptomics analyses to study gene expression throughout development or to characterize the molecular phenotype of developmental mutants are summarized.

A. Transcriptomics to Identify Global Spatiotemporal Expression Patterns During Development

Transcriptomics started with EST analyses (Adams et al. 1991) but soon afterward diversified through a number of sequencing- or hybridization-based methods, including SAGE (Velculescu et al. 1995), microarrays (Schena et al. 1995; DeRisi et al. 1997; Breakspear and Momany 2007), and RNA-seq (Nagalakshmi et al. 2008; Wilhelm et al. 2008). All of these methods have by now been applied to analyze fruiting body development (Table 7.2). These studies have not only improved our understanding of the processes that occur during developmental transitions, but also were instrumental in identifying a number of differentially expressed genes whose functions during fruiting body development were determined in subsequent studies.

1. Transcriptomics of Different Developmental Stages

The first transcriptomics approach to learn more about gene expression at different developmental stages was an **EST analysis** of fruiting bodies (perithecia), germinating asexual spores (conidia), and vegetative mycelium from *N. crassa* (Nelson et al. 1997). Nearly 60 % of the ESTs in the mycelial and perithecial libraries showed no match to proteins in public databases, whereas for germinating conidia, this was true for less than 40 %. One reason for this might be that the ESTs from the conidial library contained more genes with putative roles in protein biosynthesis, and that this group of genes was already well characterized, whereas not many genes involved in fungal-specific activities like fruiting body development were known at this time. However, even 15 years later, it is a recurring theme in many expression analyses that among the genes expressed during sexual development are relatively fewer involved in basic cellular functions and metabolism than in nonreproductive mycelia, and that the genes expressed during sexual development have more diverse functions or no characterized function yet. This was found in analyses of the ascomycetes

Table 7.2. Transcriptomics analyses to study sexual development in filamentous ascomycetes and basidiomycetes

Species	Analysis[a]	Subject	References[b]
Ascomycetes			
Pezizomycetes			
Pyronema confluens	E, D, R	Vegetative mycelium and sexually developing mycelium	[1, 2, 48]
Tuber borchii	E, M	Fruiting bodies versus vegetative mycelium	[3, 34]
Tuber melanosporum	E, M, R	Fruiting bodies versus free-living or ectomycorrhizal mycelia	[4, 5]
Eurotiomycetes			
Aspergillus nidulans	M	Wild-type and sterile mutant, vegetative and sexual development	[6]
Sordariomycetes			
Cryphonectria parasitica	M	Virus infected versus noninfected	[7]
	M	wild type versus sterile mutant	[42]
Gibberella fujikuroi	D, M	Wild-type and mating-type gene mutant	[8]
	R	Time course of perithecial development	[9]
Gibberella zeae	E	Vegetative mycelium and perithecia	[39]
	M, R	Time course of perithecial development	[9–11]
	D, M	Wild-type and mating-type gene mutant	[40]
	M	Wild-type versus sterile mutant	[12, 13]
Hypocrea jecorina	M	Sexually potent versus impotent mycelia	[14]
Neurospora crassa	E	Vegetative hyphae, conidia, and perithecia	[15]
	M	Wild-type versus sterile mutants	[41]
	M	Wild-type mat a and mat A mycelia under vegetative conditions	[16]
	M	Time course of perithecial development on two media	[17]
Neurospora intermedia	E, M	Vegetative and sexual mycelium	[46]
Ophiostoma novo-ulmi	D	SSH of perithecia versus synnemata	[18]
Podospora anserina	M	Wild-type mat+ and mat- mycelia, and mating-type gene mutants	[19]
	M	Wild-type and developmental mutants during vegetative growth	[45]
Sordaria macrospora	M	Wild-type versus sterile mutants	[20]
	M	Wild-type versus mating type gene mutants	[21, 22]
	M	Wild-type versus sterile mutants, and vegetative mycelium versus sexually developing mycelium	[23]
	R	Protoperithecia of wild-type versus sterile mutant and vegetative mycelium versus sexually developing mycelium	[24]
Basidiomycetes			
Agaricomycetes			
Agaricus bisporus	E, M	Different stages of fruiting body development	[37, 44]
Coprinopsis cinerea	S, M	Fruiting bodies versus vegetative mycelium	[47]
Ganoderma lucidum	R	Fruiting bodies versus vegetative mycelium	[25]
Laccaria bicolor	M	Fruiting bodies, vegetative mycelium, mycorrhizal root tips	[43]
Lentinula edodes	D	RDA of vegetative mycelium and stages of fruiting body development	[26]
	S	Dikaryotic mycelium and fruiting body primordium	[27]
	D	SSH of fresh versus harvested fruiting bodies	[28]
	R, S	Fruiting bodies before and after sporulation	[29]
Pleurotus ostreatus	E	Different stages of fruiting body development	[30, 35, 36]

(continued)

Table 7.2. (continued)

Species	Analysis[a]	Subject	References[b]
Schizophyllum commune	MP	Time course of fruiting body development	[31]
	M	Monokaryons and compatible and incompatible dikaryotic interactions	[32]
	R	Wild-type versus developmental mutants	[33, 38]

[a]Type of analysis: *E* EST (expressed sequence tag) sequencing, *D* differential hybridization (including *RDA* representational difference analysis and *SSH* suppression subtractive hybridization), *M* microarray analysis including cross-species array hybridizations, *MP* massively parallel signature sequencing, *R* RNA-seq, *S* SAGE (serial analysis of gene expression)

[b]References are as follows: [1] Nowrousian and Kück (2006), [2] Gesing et al. (2013), [3] Gabella et al. (2005), [4] Martin et al. (2010), [5] Tisserant et al. (2011), [6] Nahlik et al. (2010), [7] Allen et al. (2003), [8] Keszthelyi et al. (2006), [9] Sikhakolli et al. (2012), [10] Qi et al. (2006), [11] Hallen et al. (2007), [12] Lee et al. (2010a), [13] Lysoe et al. (2011), [14] Chen et al. (2012a), [15] Nelson et al. (1997), [16] Wang et al. (2012a), [17] Wang et al. (2012b), [18] Jacobi et al. (2010), [19] Bidard et al. (2011), [20] Nowrousian et al. (2005), [21] Pöggeler et al. (2006b), [22] Klix et al. (2010), [23] Nowrousian et al. (2007a), [24] Teichert et al. (2012), [25] Yu et al. (2012), [26] Miyazaki et al. (2005), [27] Chum et al. (2008), [28] Sakamoto et al. (2009), [29] Chum et al. (2011), [30] Joh et al. (2007), [31] Ohm et al. (2010), [32] Erdmann et al. (2012), [33] Ohm et al. (2013), [34] Lacourt et al. (2002), [35] Park et al. (2006), [36] Lee et al. (2002), [37] Ospina-Giraldo et al. (2000), [38] Ohm et al. (2011), [39] Trail et al. (2003), [40] Lee et al. (2006), [41] Li et al. (2005), [42] Deng et al. (2007), [43] Martin et al. (2008), [44] Morin et al. (2012), [45] Bidard et al. (2012), [46] Nygren et al. (2012), [47] Cheng et al. (2013), [48] Traeger et al. (2013)

G. zeae, *Gibberella fujikuroi*, and *Pyronema confluens* as well as in the basidiomycetes *Agaricus bisporus* and *Lentinula edodes* (Ospina-Giraldo et al. 2000; Trail et al. 2003; Miyazaki et al. 2005; Keszthelyi et al. 2006; Nowrousian and Kück 2006; Qi et al. 2006; Gesing et al. 2013; Morin et al. 2012; Sikhakolli et al. 2012). This might indicate that during vegetative growth, a set of well-characterized eukaryotic core genes is active to maintain basic cellular functions, whereas the higher morphological diversity that is achieved during fruiting body development requires the activity of a number of specialized, perhaps less-conserved (or at least less-well-characterized) or even **lineage-specific genes**. This is consistent with a long-standing hypothesis that the vegetative mycelium gathers and stores nutrients until a stage of "competence" is reached, and afterward the energetically costly production of fruiting bodies is supported by transfer of nutrients from the nonreproductive mycelium (Wessels 1993; Pöggeler et al. 2006a). It also fits with a hypothesis that sex-associated genes evolve more rapidly than genes involved in other functions. This has been shown in animals and plants (Swanson and Vacquier 2002; Clark et al. 2006; Brawand et al. 2011), and similar findings have been made for mating-type and pheromone signaling genes in filamentous asco-

mycetes (Pöggeler 1999; Karlsson et al. 2008). It is also supported by a recent EST analysis of *Neurospora intermedia* and comparison with other *Neurospora* species (Nygren et al. 2012). This study indicated that sex-associated genes (i.e., those genes that are preferentially expressed during sexual development) are also rapidly evolving in fungi. Further support for this hypothesis came from a transcriptomics analysis of *P. confluens* (Traeger et al. 2013). The authors showed at a genome-wide level that the highest percentage of genes upregulated during sexual development was found among the *P. confluens* orphan genes, that is, genes without detectable homolog in the public sequence databases.

Another, related finding is that transcriptional profiles of nonreproductive mycelia in many fungi are different from those of developing fruiting bodies. This was found, for example, in *Tuber borchii*, for which an analysis of two fruiting body stages and vegetative mycelium showed that the differences between the two fruiting body stages were smaller than between either stage of sexual development and vegetative mycelium (Gabella et al. 2005). Even an array of complementary DNAs (cDNAs) derived from vegetative mycelium of *T. borchii* showed large differences when hybridized with targets from vegetative mycelium

versus fruiting body, although strictly fruiting body-specific genes would not even be represented on this array (Lacourt et al. 2002). Interestingly, though, expression studies in *Tuber melanosporum*, a close relative of *T. borchii*, suggested that less than 10 % of all genes are differentially expressed in fruiting bodies of mycorrhizal tissue compared to free-living mycelium (Martin et al. 2010). However, studies in other fungi rather support a hypothesis of **transcriptional reprogramming during sexual development**: Using a genome-wide microarray to analyze a time course of *G. zeae* fruiting body differentiation, Hallen and coworkers found that 12 % of transcripts were specific to sexual development (Hallen et al. 2007). In a recent RNA-seq study of young fruiting bodies of *S. macrospora* that were extracted from surrounding nonreproductive hyphae by laser microdissection, it was found that the transcriptional profile of fruiting body precursors was drastically different from that of nonreproductive hyphae (Teichert et al. 2012), and similar results were obtained in serial analysis of gene expression studies of vegetative mycelium and fruiting body primordia from the basidiomycete *Coprinopsis cinerea* (Cheng et al. 2013).

In an analysis of fruiting body development of *N. crassa* on two different media, Wang and coworkers found that expression of metabolism-related genes was different, as expected, but that known development-specific genes were expressed similarly independent of the growth medium (Wang et al. 2012b). This indicates that expression of developmental genes is robust even in different environments. Overall, the analyses described here already offered first insights into the coordinated patterns of gene expression that underlie developmental transitions in time and space. Several of the genes that are upregulated during sexual development were already characterized in more detail; these are described in Sect. III.C.

2. Comparative Transcriptomics to Identify Evolutionary Conserved Expression Patterns

The rising number of expression studies conducted with different fungal species not only offers insights into the development of single species, but also opens up the possibility of comparing gene expression patterns between species at similar developmental stages. This is important not only to follow **evolutionary trajectories of gene expression** but also as a practical means to narrow down lists of differentially expressed genes for promising candidates for further studies. The necessity for narrowing down such lists has become apparent in the course of a number of expression studies, in which it was found that genes strongly upregulated during a condition of interest are not necessarily involved in the process under investigation (Nowrousian and Cebula 2005; Nowrousian et al. 2007b). Comparative transcriptomics analyses can help focus on genes with a higher likelihood of playing a significant role in the analyzed biological phenomenon because evolutionary conservation of expression is a strong indicator for functional significance (Stuart et al. 2003). This is especially the case if the species under investigation are only distantly related because comparing closely related species might give a high number of false positives because there is insufficient time for the divergence of expression patterns (Romero et al. 2012). Furthermore, comparative analyses can also be used to identify species-specific expression patterns that might help to explain morphological or physiological differences between species (Brawand et al. 2011; Romero et al. 2012).

Comparative expression analyses of fungal development have been performed for several ascomycetes. A small-scale pilot study was based on ESTs from *P. confluens*, whose expression was compared to the corresponding homologs from *S. macrospora* (Nowrousian and Kück 2006). These two ascomycetes are only distantly related, with *P. confluens* positioned at the base of the filamentous ascomycetes, forming apothecia as fruiting bodies, while *S. macrospora* is a derived ascomycete and develops the more complex perithecia.

Genes with conserved expression across the large evolutionary distance between these two species might therefore be among a set of core genes for ascomycete fruiting body formation. Even though this study encompassed only a few genes, it already indicated that there might be a significant amount of overlap in development-specific expression even in distantly related ascomycetes. These analyses were followed up by a comparison of developmentally regulated genes from *P. confluens* identified by suppression subtractive hybridization (SSH) with homologs in *S. macrospora* and *N. crassa* (Gesing et al. 2013). Interestingly, expression tendencies were more conserved between *S. macrospora* and *P. confluens* than between the much more closely related *S. macrospora* and *N. crassa*, both of which are members of the Sordariomycetes and form perithecia. One reason for this might be that *N. crassa* is heterothallic (self-sterile), in contrast to the homothallic (self-fertile) *S. macrospora* and *P. confluens*; therefore, *N. crassa* requires an additional fertilization step for fruiting body formation (Claussen 1912; Davis and deSerres 1970; Pöggeler et al. 2006a). Furthermore, *N. crassa* produces two different types of asexual spores (micro- and macroconidia) in addition to the sexual ascospores that are produced in the fruiting bodies, whereas neither *S. macrospora* nor *P. confluens* produces any asexual spores. Therefore, some differences in gene expression patterns between *N. crassa* and the other two species might be caused by additional differentiation processes in *N. crassa* that occur in parallel to fruiting body formation (Gesing et al. 2013). The recent sequencing of the *P. confluens* genome and several development-dependent transcriptomes enabled a genome-wide comparison of transcriptome data between *P. confluens* and *S. macrospora* (Traeger et al. 2013). Interestingly, expression patterns in vegetative or total mycelia in these two fungi were more similar to each other than those of the corresponding mycelia from *S. macrospora* to expression patterns in young fruiting bodies from this species. This suggests that similar tissues or developmental stages in different species are more similar in expression than different tissues or stages from the same species.

Another case for which the differences outweigh the similarities of expression was found in a comparison of genes that are differentially expressed in **mutants of mating-type genes** from *Podospora anserina*, *S. macrospora*, and *G. fujikuroi* (Bidard et al. 2011). The authors compared microarray hybridization results for mutants in the mating-type genes *FMR1* (*MAT1-1-1*) and *FPR1* (*MAT1-2-1*) from *P. anserina* with results from mutants in the corresponding genes *SmtA-1* (*MAT1-1-1*) and *Smta-1* (*MAT1-2-1*) of *S. macrospora* and with results from the *FRP1* homolog *MAT1-2-1* of *G. fujikuroi* from previous studies (Keszthelyi et al. 2006; Pöggeler et al. 2006b; Klix et al. 2010). They found between 16 and 57 orthologous gene pairs among the regulated genes, but the direction of regulation showed little or no correlation between the respective species (Bidard et al. 2011). However, these findings might have several explanations. One caveat for the interpretation is the fact that, in the case of *S. macrospora*, the microarray hybridizations were performed using *N. crassa* microarrays (cross-species microarray hybridizations), which result in fewer genes yielding significant hybridization results. Especially, signals from weakly expressed genes might have been lost in the background noise; therefore, the lists of differentially regulated genes might not be complete (Pöggeler et al. 2006b; Klix et al. 2010). This is also the case for *G. fujikuroi*, for which cDNA macroarrays were used for the analysis that did not cover all potential genes of this fungus (Keszthelyi et al. 2006). Other reasons for the observed differences might be rooted in the biology of the fungi because *S. macrospora* is homothallic, *G. fujikuroi* is heterothallic, and *P. anserina* is pseudohomothallic, but for the analysis by Bidard et al., homokaryotic strains were used that are effectively heterothallic. The mycelia used for RNA extraction were from vegetative, fertilization-competent mycelia in the case of *G. fujikuroi* and *P. anserina* and from already sexually differentiating mycelia in the case of *S. macrospora*. Thus, the analysis for *G. fujikuroi* and *P. anserina* focused on genes that are influenced by the mating-type factors during vegetative growth prior to fertilization

(Keszthelyi et al. 2006; Bidard et al. 2011), while in the case of *S. macrospora*, the role of mating-type genes was studied during sexual development (Pöggeler et al. 2006b; Klix et al. 2010). Furthermore, an additional difference between the three species is that *S. macrospora* does not produce any asexual spores, *P. anserina* produces spores that act only as male spermatia, while *G. fujikuroi* produces asexual conidia in parallel to sexual differentiation.

The previously described studies might indicate that it is important to compare similar stages of fruiting body development, if possible without interference of unrelated differentiation processes, to identify genes with conserved expression patterns. This was done in RNA-seq analyses of six stages of perithecial development in *G. zeae* and *G. fujikuroi* (Sikhakolli et al. 2012). The authors found large overall similarities in expression patterns; for example, a high number of genes encoding proteins involved in transcription are strongly expressed during the time of ascus formation. Expression differences were largely explained by morphological differences (e.g., the different pigmentation of sexually developing cultures in these two species). However, it has to be taken into account that the two species that were compared in this case were both from the genus *Gibberella* and therefore closely related. However, a comparison of gene expression patterns during fruiting body development between *S. macrospora* and *G. zeae* also revealed a number of similarly regulated genes, with functional categories of metabolism and energy overrepresented among the down-regulated genes, and transcription/RNA processing; protein activity regulation; cell fate (differentiation, apoptosis); and cell wall biogenesis overrepresented among the upregulated genes in both species (Gesing et al. 2012). Thus, expression patterns might be to some extent conserved during similar morphological stages, at least among filamentous ascomycetes.

In basidiomycetes, **gene expression during mushroom formation** has been compared between *Schizophyllum commune* and *Laccaria bicolor* (Ohm et al. 2010) and later between these two species and the button mushroom *A. bisporus* (Morin et al. 2012). In the first

comparison, it was found that several groups of genes (represented by the same gene ontology terms or other classification methods) were positively correlated with respect to their expression during mushroom differentiation. Among these groups were, for example, metabolic pathways or transcriptional regulation by transcription factors, indicating that regulatory processes might be to some degree conserved during basidiomycete fruiting body development. In the second study, it was found that only 22 and 35 genes were significantly upregulated during development in both *A. bisporus/S. commune* and *A. bisporus/L. bicolor*, respectively. Further studies including more species, and more distinct stages of development, will be necessary to determine the extent of expression conservation versus species-specific expression during fruiting body development.

B. Transcriptomics to Characterize Developmental Genes and Mutants

While the analysis of overt morphological phenotypes of mutant strains is a valuable tool for the identification of genes that are essential for fruiting body development, this does not necessarily give any information about the molecular actions that the corresponding gene product is performing to ensure the correct progression of differentiation. To understand the molecular mechanisms that are the basis of fruiting body development, a more detailed analysis of gene activities is necessary, and the genome-wide gene expression patterns of a mutant can serve as a molecular phenotype to establish the position of the mutated gene in a regulatory network. Furthermore, genes that are deregulated in a mutant strain are directly or indirectly dependent on the mutated gene for correct expression; therefore, transcriptomics studies of mutants are widely used to identify target gene candidates of transcription factors or regulatory factors.
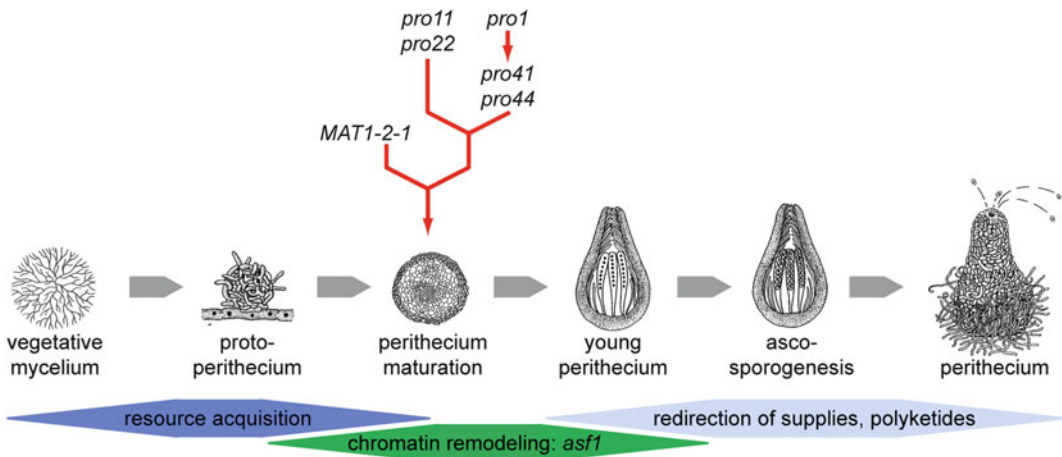
Fig. 7.1. Model for the regulation of fruiting body development in *S. macrospora* as determined through transcriptomics analyses. During vegetative growth, the mycelium gathers nutrients until a stage of competence is reached that allows fruiting body development. Maturation of protoperithecia to perithecia requires the action of several genes that act in a regulatory network that was elucidated by microarray and RNA-seq analysis of the corresponding mutants. Among others, the transcription factors PRO1 and PRO44 are necessary for the transition to perithecia, and the involvement of the histone chaperone ASF1 indicates that, in addition to gene-specific transcriptional changes, extensive chromatin remodeling might play a role in development. In later stages, nutrients are redistributed to the developing fruiting bodies, and polyketides (e.g., melanins) are necessary for timely differentiation of mature perithecia and ascospores. For more information, see text (Pictures of developmental stages are from Kück et al. 2009)

1. Transcriptomics to Analyze Mating-Type-Dependent Gene Expression

Among the first genetic loci that were identified as crucial for sexual development in fungi were the mating-type loci. While the number of loci per fungal genome and the number of genes per locus varies between different fungal groups and species, one common theme is the presence of at least one transcription factor gene among the mating-type genes in each mating system analyzed so far (Casselton 2008; Lee et al. 2010b). The best-studied mating system is that of the yeast *S. cerevisiae*, for which it was shown that the mating-type-encoded transcription factors activate or repress transcription of developmental genes during different stages of sexual differentiation (Galgoczy et al. 2004). In filamentous fungi, several mating-type genes were shown to be involved in fruiting body development; in a number of species, transcriptomics analyses were used to characterize mating-type-dependent gene expression. Most of these studies were performed with Sordariomy-cetes, but recently a study was carried out with the basidiomycete *S. commune* (Table 7.2).

The earliest large-scale analyses of mating-type-dependent gene expression in filamentous fungi involved comparisons between the wild type and mutants in the **mating-type gene** *MAT1-2-1* in *G. zeae*, *G. fujikuroi*, and *S. macrospora* (Keszthelyi et al. 2006; Lee et al. 2006; Pöggeler et al. 2006b). In all three analyses, up- as well as downregulated genes were found in the mating-type mutants, indicating that the *MAT1-2-1* gene acts both as repressor and as activator, and similar results were found later in the corresponding mutant of *P. anserina* (Bidard et al. 2011). In the yeast *S. cerevisiae*, the mating-type proteins, either individually or as dimers, directly regulate the expression of only about 30 genes, most of which are involved in sexual development themselves (Galgoczy et al. 2004). The number of differentially expressed genes in mating-type mutants of filamentous ascomycetes is higher; however, whether these effects are caused by direct targeting of the respective genes by the

mating-type proteins remains to be elucidated, as the transcriptomics analyses identify both direct and indirect target genes. In the case of *S. macrospora*, expression profiles of the MAT1-2-1 mutant were also compared to those of three other mutants with a block at the same developmental stage, but where the defective genes are not mating type related (Pöggeler et al. 2006b). Interestingly, even though all four mutants had similar phenotypes, the transcription profile of the MAT1-2-1 mutant was significantly different from those of the other mutants, indicating that the mating-type genes might regulate distinct developmental pathways (Fig. 7.1). MAT1-1-1 mutants were also analyzed in *S. macrospora* and *P. anserina* (Klix et al. 2010; Bidard et al. 2011); the comparative analysis was described in previous Sect. III.A.2.

Mating-type genes not only influence sexual development but also can have an effect during the asexual growth phase, as was shown in a microarray hybridization study of *N. crassa* (Wang et al. 2012a). In this fungus, the mat A (MAT1-1) mating type had an overall higher expression of many genes compared to the mat a (MAT1-2) mating type. These differences might be correlated with mating-type-dependent efficiency in fruiting body production (Dettman et al. 2003), possibly because the vegetative growth phase prior to fertilization is important for differentiation of fruiting body precursors (protoperithecia).

While the known mating systems from ascomycetes are bipolar (i.e., there exists only one mating-type locus per genome), many basidiomycetes possess tetrapolar mating systems with two independent mating-type loci in a genome (Casselton and Kües 1994; Kämper et al. 1994). For compatible interactions that lead to fruiting body formation in basidiomycetes with tetrapolar mating systems, alleles at both mating-type loci must be different in the mating partners. Mating-type-dependent gene expression in basidiomycetes was first studied at a genome-wide level in the plant-pathogenic *Ustilago maydis* (Wahl et al. 2010); however, this fungus does not produce fruiting bodies. The first mating-type-dependent transcriptome of a mushroom-forming basidiomycete was analyzed in *S. commune* (Erdmann et al. 2012). Here, it was shown

that the **mating-type loci, A and B**, regulate the expression of distinct sets of genes as well as several genes that are under control of both mating-type loci. Interestingly, more genes are differentially regulated by the pheromone/receptor system-encoding B mating-type locus than the transcription factor-encoding A mating-type locus. However, more studies with basidiomycetes will be needed to determine the extent of regulatory influence of different mating-type loci, especially with respect to spatiotemporal developmental effects.

2. Transcriptomics of Photoreceptor Mutants

Light has an effect on fruiting body development in many asco- and basidiomycetes, although the effects of light are species specific, ranging from completely light-dependent development to inhibition of fruiting body differentiation in constant light (Claussen 1912; Perkins 1969; Moore-Landecker 1979; Harding and Melles 1983; Kües 2000; Pöggeler et al. 2006a; Fischer 2008; Chen et al. 2012a). Furthermore, the wavelengths that affect fruiting body development can vary; for example, **blue light** affects differentiation in the ascomycetes *N. crassa* and *Hypocrea jecorina*, as well as the basidiomycete *S. commune*, whereas **red light** represses sexual development in the ascomycete *Aspergillus nidulans*. These effects are mediated by photoreceptors, in the case of blue light by the White Collar Complex (WCC) in *N. crassa* and its homologs in other fungi and the phytochrome FphA for the red light responses of *A. nidulans* (Oda and Hasunuma 1997; Froehlich et al. 2002; Blumenstein et al. 2005; Chen et al. 2012a; Ohm et al. 2013).

The White Collar proteins WC-1 and WC-2 that form the WCC are transcription factors as well as photoreceptors (Ballario et al. 1996; Crosthwaite et al. 1997; Linden and Macino 1997; Talora et al. 1999), and transcriptomics analyses have been performed with several fungi to identify genes that are dependent on the White Collar genes for correct developmental expression.

In *N. crassa*, microarray analyses were performed to identify light-responsive genes by comparing the wild type with several photoreceptor mutants, among them

wc-1 and *wc-2* mutants (Chen et al. 2009). While the conditions under which the mycelia were grown in this case allowed only vegetative development, it is interesting to note that one of the targets of the WCC that was identified is the *sub-1* gene. It encodes a GATA-type transcription factor that is essential for fruiting body development in *N. crassa*, and its orthologs are also essential for sexual development in *A. nidulans* and *S. macrospora* (Han et al. 2001; Colot et al. 2006; Nowrousian et al. 2012). In *N. crassa*, *sub-1* is required for most late light responses (Chen et al. 2009), and one might speculate that the light-dependent orientation of fruiting body necks in this fungus is mediated by *sub-1*.

In *H. jecorina*, it was shown recently that constant light inhibits fruiting body formation, and this effect is mediated by the White Collar homologs *blr1* and *blr2* (Chen et al. 2012a). Light effects are dampened by the *env1* gene, a homolog to the *N. crassa* *vvd* gene that was shown previously to be involved in modulating light effects in this fungus (Heintzen et al. 2001). Microarray analyses of the wild type and a Δenv1 mutant grown under different light regimes indicated that ENV1 might be involved in balancing expression of genes required for vegetative growth, asexual conidiation, and sexual development (Chen et al. 2012a).

White Collar homologs are also involved in the regulation of sexual development in the basidiomycete *S. commune* (Ohm et al. 2013). In this fungus, fruiting body formation is light dependent, and dikaryotic deletion strains of *wc-1* or *wc-2* are unable to form fruiting bodies, indicating that the White Collar proteins mediate the light responses that are needed for sexual development. RNA-seq analyses of the wild type and a Δwc-2Δwc-2 dikaryon identified a number of genes that are differentially expressed in the mutant. Among the genes downregulated in the mutant were several hydrophobin genes and two transcription factors that were shown previously to be involved in fruiting body formation (Ohm et al. 2011, 2013). So far, transcriptomics data have shown that light effects are integrated with developmental decisions, and that at least to some extent this is mediated at the level of transcription.

3. Transcriptomics of Other Developmental Mutants

Over the last decades, many developmental genes have been identified in several filamentous fungi, among them, for example, genes encoding transcription factors, signaling proteins, and others. A number of the corresponding mutant strains have been subjected to transcriptomics analysis to characterize the molecular phenotype of the mutants on a genome-wide basis. With a complex phenotype like multicellular differentiation, this approach is especially useful to cover the diverse effects of mutations in central developmental genes.

Most of these studies have been performed with Sordariomycetes (Table 7.2). In *N. crassa*, a microarray analysis was conducted with mutants in *mak-2* and *pp-1*, encoding homologs of the yeast mitogen-activated protein (MAP) kinase Fus3p and transcription factor Ste12p, respectively, both of which are part of a signal transduction cascade that is essential for sexual development in yeast (Li et al. 2005). Both genes are essential for fruiting body formation in *N. crassa*, and most genes that are differentially expressed in the mutants compared to the wild type show the same pattern of deregulation in both mutants, confirming that the two genes also might be part of the same signal transduction cascade in *N. crassa*. Interestingly, the pheromone-like gene *poi-2* that was identified in a previous EST analysis (Nelson et al. 1997) is downregulated in both mutants, while the true pheromone gene *ccg-4* is upregulated. Upregulation of pheromone genes was also found in microarray analyses of three different developmental mutants from *S. macrospora* (Nowrousian et al. 2005). It can be hypothesized that in all these cases, the failure to progress through certain developmental stages prevents downregulation of genes like the pheromone genes, something that would occur during normal development in the wild type.

Gene expression patterns constitute **molecular phenotypes** and can therefore be used to determine the position of genes within regulatory networks by the analysis of transcriptomes from single and double mutants. This

was done, for example, for the *Sordaria macrospora* genes *pro1* and *pro41*, which encode a transcription factor and ER membrane protein, respectively, both of which are required for fruiting body formation (Masloff et al. 1999; Nowrousian et al. 2007a). Microarray analysis of single and double mutants and clustering of the mutant strains according to their gene expression patterns revealed that *pro1* acts upstream of *pro41* in a genetic network of development (Fig. 7.1). An even more detailed analysis of gene expression in the mutant *pro1* was performed by combining laser microdissection and RNA-seq to analyze the transcriptome specifically in young fruiting bodies (protoperithecia) because the mutant is able to differentiate these, but cannot progress beyond this step (Teichert et al. 2012). On the one hand, these analyses showed that gene expression in protoperithecia is drastically different from that in nonreproductive hyphae; on the other hand, a number of genes were identified that depend on the transcription factor gene *pro1* for correct expression in young fruiting bodies. One example is the transcription factor gene *pro44* that is essential for fruiting body development (Nowrousian et al. 2012) and strongly expressed in wild-type protoperithecia, but not in pro1 protoperithecia, and therefore probably acts downstream of *pro1* in a regulatory network (Fig. 7.1).

The effect of a transcription factor mutation on the transcriptome was also studied in the sterile mutant of the *FgStuA* gene from *G. zeae* (Lysoe et al. 2011). However, the growth conditions used in these experiments did not support sexual differentiation; therefore, conclusions on the role of FgStuA in sexual development will require additional data.

In *G. zeae*, several microarray analyses were conducted to analyze genes that might be involved in signaling during development (Hallen and Trail 2008; Lee et al. 2010a). The *cch1* gene encodes a calcium channel that is necessary for ascospore discharge from mature perithecia. Transcriptomes were recorded at three developmental stages, and several hundred genes were differentially regulated in the mutant compared to the wild type at each stage, with little overlap between stages (Hallen and Trail 2008). This indicates that the calcium channel CCH1 has a significant impact on the physiological state of the mycelium, and that this effect might be stage specific during development. Microarray hybridizations were also used to compare transcriptomes between the wild type and a mutant of the G protein alpha subunit gene *gpa1* (Lee et al. 2010a). The mutant is sterile, and it was found that several hundred genes are up- or downregulated in the mutant strain 3 days after induction of development. Detailed investigation of 100 downregulated genes showed that the majority of these were also upregulated in the wild type during sexual development compared to vegetative growth. Deletion of 57 of these genes served to identify 11 genes involved in sexual differentiation because the corresponding mutants have phenotypes ranging from sterility to delayed fruiting body maturation. Thus, the signal transduction cascade that contains *gpa1* regulates a number of genes that are necessary for fruiting body formation themselves.

An interesting case is fruiting body development in the chestnut blight fungus *Cryphonectria parasitica*. In this fungus, sexual differentiation (among other traits) is impaired in strains that are infected with certain hypoviruses (Dawe and Nuss 2001). Microarray analysis showed that about 13 % of all genes represented on the arrays were differentially expressed in hypovirus-infected versus noninfected strains (Allen et al. 2003). Thus, hypovirus infection causes a broad reprogramming of the host transcriptome. Interestingly, one of the genes that are downregulated during hypovirus infection is *CpST12*, encoding a homolog of the yeast transcription factor Ste12p. It was shown in subsequent analyses that this gene is also essential for female fertility in *C. parasitica* (Deng et al. 2007).

While most transcriptomics analyses of developmental mutants were conducted with Sordariomycetes, there have also been some reports from other fungal groups (Table 7.2). In the Eurotiomycete *A. nidulans*, a combined transcriptomics/proteomics/metabolomics study was carried out to analyze the genome-wide effects of the deletion of *csnE*, a gene encoding a subunit of the COP9 signalosome (Nahlik et al. 2010). The COP9 signalosome is a multisubunit protein complex that regulates

ubiquitin ligase activity and is conserved in higher eukaryotes. Deletion of individual subunits results in identical phenotypes in *A. nidulans* because the complex can only be assembled when all required subunits are present (Busch et al. 2007). The multilevel gene expression analysis of the csnE mutant showed that during vegetative growth, the COP9 signalosome is involved in protection against oxidative stress and the regulation of hormone levels, whereas later during development, it activates genes for secondary metabolism and cell wall restructuring (Nahlik et al. 2010).

In basidiomycetes, not many developmental mutants have been characterized at the molecular level yet; however, a transcriptome analysis by RNA-seq was performed recently to analyze the effect of transcription factor deletions on gene expression during fruiting body formation in *S. commune* (Ohm et al. 2011). Mutants in the transcription factor genes *fts4* and *hom2* are blocked early during mushroom development, and transcriptomes were analyzed for an early developmental stage in the wild type and the two mutants. At this stage, expression profiles of the mutants were more similar to each other than to the wild type. Interestingly, two other transcription factor genes that are also involved in fruiting body formation, *hom1* and *c2h2*, were downregulated in both mutants. *hom1* regulates number and size of mushrooms, with the mutant forming more, but smaller fruiting bodies, while the c2h2 mutant is arrested during development (Ohm et al. 2011). The transcriptomics data together with information about mutant phenotypes could thus be integrated in a model of the transcription factor network that regulates mushroom differentiation.

C. Detailed Analysis of Genes Differentially Expressed During Fruiting Body Development

Apart from establishing genome-wide transcriptional patterns, gene expression data can also be used to identify target genes for further detailed analyses. By now, a number of genes that are differentially expressed during fruiting

body development in one or more fungi have already been analyzed in more detail to determine whether they play a role in this process.

As mentioned in Sect. III.A.1, one of the first transcriptomics analyses in a filamentous fungus was carried out in *N. crassa* (Nelson et al. 1997), and two of the genes, *poi-1* (*mfa-1*) and *poi-2*, that were found to be strongly expressed in starved mycelia and perithecia, but not in conidia, were later shown to be essential for correct fruiting body development (Kim et al. 2002; Kim and Nelson 2005). While *poi-1* encodes a pheromone gene and was therefore later renamed *mfa-1* (*mating factor α-1*), *poi-2* has some structural similarities to fungal pheromones but seems to be involved in a mating response signaling pathway rather than acting as a pheromone itself.

In *S. macrospora*, a number of genes that are regulated differentially in developmental mutants were analyzed in more detail. The first two genes were *tap1* and *app*, encoding a putative lectin and a protein that turned out to be among the most abundant in mature perithecia, respectively (Nowrousian and Cebula 2005; Nowrousian et al. 2007b). Somewhat surprisingly at that time, none of the corresponding deletion mutants showed any developmental phenotype, at least under laboratory conditions. This led to the conclusion that more stringent or specific criteria were needed to identify promising candidate genes from expression studies. Several criteria can be envisioned, among them a comparative approach in which candidate genes are selected from those with evolutionary conserved expression patterns (see also Sect. III.A.2) or a selection of genes upregulated only in morphological structures that are specific to sexual development. The latter criterion requires the analysis of transcriptomes from fruiting body stages only, in contrast to the analyses of total mycelia containing fruiting bodies and vegetative mycelia in varying proportions. Both selection criteria were successfully used in subsequent analyses. Comparative expression analysis of a **cluster of differentially regulated polyketide biosynthesis genes** in *S. macrospora* and *N. crassa* was the basis for the identification of the *fbm1* gene, which was

shown to be required for timely fruiting body maturation in both fungi (Nowrousian 2009) (Fig. 7.1). Comparative analysis of microarray data from *S. macrospora* and *G. zeae* identified the histone chaperone gene *asf1*, and a deletion mutant of the *S. macrospora asf1* turned out to be sterile (Gesing et al. 2012). The second strategy for selection of candidate genes, namely, from expression data for specific structures, is also promising, as indicated by an RNA-seq analysis of young fruiting bodies that were isolated by **laser microdissection** from *S. macrospora* (Teichert et al. 2012). Among the genes that are strongly expressed or upregulated in these structures are the pheromone precursor genes *ppg1* and *ppg2* and the transcription factor genes *mcm1* and *pro44*, all of which were shown previously to be involved in fruiting body formation (Mayrhofer et al. 2006; Nolting and Pöggeler 2006; Nowrousian et al. 2012). Microscopic analysis of *ppg1* expression using promoter-*gfp* fusion constructs confirmed the organ-specific expression of this pheromone precursor gene and revealed a distinct expression pattern within hyphae of the outer layer of developing fruiting bodies (Teichert et al. 2012).

The identification of candidate genes can be significantly improved by the availability of large mutant collections (see Sect. II.B). The collections can be used to quickly screen mutants of differentially expressed genes for interesting phenotypes (e.g., in the case of genes with conserved expression patterns). For example, the corresponding knockout mutants from *N. crassa* were used to screen for sterile phenotypes among homologs to genes that were differentially regulated in *S. macrospora* or *P. confluens*, identifying a polyketide synthase and a putative transporter required for fertility in *N. crassa* (Nowrousian 2009; Gesing et al. 2013). The ability to generate and screen a large number of targeted deletion mutants can also be used to overcome less-than-stringent selection criteria for candidate genes. For example, in an analysis of *G. zeae*, deletion mutants were generated for 57 genes downregulated in a sterile mutant, and of these,

11 showed a development-related phenotype (Lee et al. 2010a).

IV. Other "-Omics" Approaches

Apart from transcriptomics, several other techniques have been established to survey gene expression across a large number of genes. Among these are proteomics and metabolomics, which analyze the protein content and metabolite content, respectively. Because of the chemical diversity of proteins and metabolites, these methods can usually capture only subsets of the complete proteome or metabolome of a cell or organ, in contrast to transcriptomics, by which, at least in principle, it is possible to analyze more or less all transcripts produced from a genome. Nevertheless, proteomics and metabolomics analyses can contribute valuable insights into genome activity because transcript levels often, but not always, correlate with protein levels, and when additional layers of regulation are in place, transcriptomics approaches can miss them. However, with respect to fruiting body formation, not many proteomics or metabolomics approaches have been conducted. Examples are the combined **transcriptomics/proteomics/metabolomics** study of the *csnE* mutant in *A. nidulans* described in Sect. III.B.3 (Nahlik et al. 2010); proteomics analyses of fruiting bodies from *T. borchii*, *Sparassis crispa*, and *Hericium erinaceum* (Pierleoni et al. 2004; Horie et al. 2008); and a study of cell wall proteins from different developmental stages of *Pleurotus tuber-regium* (Chen et al. 2012b). However, further combined "-omics" studies of different stages of fruiting body formation are needed to integrate such data into models of developmental regulation.

In addition to methods that measure gene expression at different levels, a number of techniques have been established that access the chromatin state (e.g., for the analysis of DNA binding sites of transcription factors and other DNA binding proteins, distribution of DNA modifications, or long-range interactions

between different genomic regions) (Lieberman-Aiden et al. 2009; Park 2009; Laird 2010; Nowrousian 2010). In combination with transcriptomics studies, these can be used, for example, to differentiate between direct and indirect targets of transcription factors by analyzing both the genome-wide transcript levels and DNA binding sites. This approach was, for example, applied to unravel the regulatory network of light signaling in *N. crassa* (Chen et al. 2009; Smith et al. 2010); however, similar studies have yet to be performed to analyze sexual development in filamentous fungi.

V. Conclusions and Outlook

Since the first high-throughput studies of fungi by the end of the 1990s, -omics in filamentous fungi has come a long way. Milestones along the way were the sequencing of fungal genomes (Sect. II), EST sequencing and microarrays for filamentous fungi (Sect. III), the development of efficient gene deletion strategies (Sect. II), and the application of NGS to address questions of fungal biology (Sects. II and III). All these advances have already been used to learn more about fruiting body differentiation, for example, to identify genes that are involved in this process and to understand the coordinated patterns of gene expression that underlie developmental events. It has become clear that morphological changes during development are mediated by drastic changes in gene expression patterns, and one of the future challenges will be to refine these analyses by improving the spatiotemporal resolution to analyze regulatory principles that govern the transitions from vegetative mycelium to mature fruiting body. In addition, it is important to study more species from different branches of the phylogenetic tree to establish both core developmental events as well as species-specific aspects of regulation. Currently, a 1,000-genome project for fungi is under way (http://1000.fungalgenomes.org), and among the sequenced genomes will be a number from species that produce fruiting bodies. A corresponding transcriptomics project to survey transcriptomes from different developmental stages for each species would greatly increase our understanding of the evolution of developmental transitions and the diversity of fungal multicellular structures. Thus, looking both deeper (in individual species) and wider (across the phylogenetic spectrum) can lead to a unified theory that explains common traits as well as differences in fruiting body development.

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