16 Fruiting-Body Development in Ascomycetes

S. Pöggeler[1], M. Nowrousian[1], U. Kück[1]

CONTENTS

[1] Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany

I. Introduction

The primary morphological character that distinguishes members of the phylum Ascomycota from all other fungi is the ascus. This sac-like meiosporangium contains the meiospores (ascospores) and is produced only during the sexual life cycle. The taxonomic classification of members of the phylum Ascomycota is complex, because these comprise a multitude of species showing high diversity in morphology, habitat and life history. However, the ability to produce a dikaryon separates members of the Ascomycota into two distinct groups: (1) the saccharomycetes, which are mostly unicellular, and (2) mycelial ascomycetes, the majority of which share several characteristics including certain cell wall constituents, septal pores with Woronin bodies, and a dikaryotic phase as part of their life cycle. Mycelial ascomycetes typically form fruiting bodies called ascomata or ascocarps (Alexopoulos et al. 1996; Barr 2001). These are highly complex, multicellular structures composed of many different cell types that surround the asci in a characteristic manner (Bistis et al. 2003). Whereas ascospores arise from dikaryotic hyphae after karyogamy and meiosis, all fruiting body-forming tissues rise from haploid, non-dikaryotic hyphae. This feature clearly distinguishes fruiting-body formation in ascomycetes from that in basidiomycetous fungi, in which dikaryotic hyphae are involved in both the meiotic cycle and fruiting-body formation (for review, see Moore 1998). Fruiting-body development in filamentous ascomycetes is a complex cellular differentiation process that requires special environmental conditions and is controlled by many developmentally regulated genes. This chapter gives a concise overview on the basics of fruiting-body development in hyphal ascomycetes, focusing on aspects relevant for taxonomy and morphology, and it summarizes environmental

The Mycota I
Growth, Differentation and Sexuality
Kües/Fischer (Eds.)

as well as intrinsic signals influencing ascocarp formation. Finally, we highlight recent molecular genetic datasets from forward and reverse genetic approaches, which give further insight into presumptive signal transduction pathways determining this multicellular differentiation process.

A. Induction of Sexual Development and Fruiting-Body Morphology

The sexual life cycle of ascomycetes can be either heterothallic (self-incompatible) or homothallic (self-compatible). Heterothallic fungi exist in two mating types, designated, for example, *A* and *a* (or + and −), and mating occurs only between sexual structures of opposite mating type. In a homothallic fungus, every strain is able to complete the sexual cycle without a mating partner.

Sexual reproduction is typically controlled by genes that reside in the mating-type locus. Mating type-encoded proteins are putative transcription factors that are thought to act as transcriptional regulators on pheromone and pheromone receptor genes (see Sect. III.C, and Debuchy and Turgeon, Chap. 15, this volume). In most cases, the single mating-type locus conferring mating behavior consists of dissimilar DNA sequences (idiomorphs) in the mating partners (Coppin et al. 1997; Kronstad and Staben 1997; Pöggeler 2001).

The first step in the sexual reproduction of mycelial fungi is to combine two compatible nuclei in the same cell. This occurs by fusion of morphologically similar or morphologically differentiated gametangia, the male antheridia and female ascogonia. In some species, the ascogonium is surrounded by sterile hyphae to form a pre-fruiting body. It then bears a specialized hypha, the trichogyne, which receives the male nucleus. A functional male gamete may be a uninucleate spermatium or microconidium or a multinucleate macroconidium, which can be formed on the mycelium or in specialized structures called spermogonia. Subsequent to contact with a male gamete, the trichogyne recruits a fertilizing nucleus from the male gamete, which migrates to the ascogonium (Bistis 1981). An alternative method of fertilization, referred to as somatogamy, involves the fusion of unspecialized somatic hyphae of two compatible mycelia. Fertilization is not immediately followed by karyogamy. The nuclei migrate in pairs to the developing, hook-shaped ascogenous hyphae (croizers), and divide mitotically in synchrony. The two nuclei remain in close association and undergo successive divisions that result in dikaryotic cells (ascogenous hyphae), in which each cell has two sexually compatible haploid nuclei. During the dikaryotic phase, a dikaryotic mycelium grows within, and draws nourishment from, the haploid ascoma tissue. Inside the ultimate branches of the dikaryotic hyphae (the young asci), of which there may be millions in larger ascomata, fusion of the male and female nucleus takes place. Immediately after karyogamy, the diploid nucleus of the zygote undergoes meiosis, which is in many species followed by a post-meiotic mitosis, resulting in the formation of eight nuclei that will become incorporated into eight ascospores (see Zickler, Chap. 20, this volume).

Depending on the number of post-meiotic mitoses and the degeneration of nuclei after meiosis, the number of ascospores within an ascus varies between one and many thousands. The sizes and shapes of ascospores and asci vary in a species-specific manner (Esser 1982; Alexopoulos et al. 1996). Based on light microscope studies, and irrespective of size and shape, different types of asci can be defined (Kirk et al. 2001). In a large number of hyphal ascomycetes, the spores are discharged from the ascus. Inside the ascoma (fruiting body), the asci may be arranged in a scattered fashion or within a definite layer, which is called the hymenium.

Four major types of the multicellular ascomata (fruiting bodies) can be distinguished: cleistothecia, perithecia, apothecia and pseudothecia (Fig. 16.1). These produce the asci, and act as the platforms from which the meiospores are launched. The cleistothecium is a completely closed fruiting body, whereas the perithecium is a more or less closed, globose or flask-like fruiting body with a pore (ostiole) through which the ascospores escape. The apothecium is a cup- or saucer-like ascoma in which the hymenium is exposed at maturity. In ascomycetes with a pseudothecium (or ascostroma), the fruiting body is initiated by the formation of a locule within a stroma, which is a matrix of vegetative hyphae, with or without tissue of a host. In contrast to cleistothecia, perithecia and apothecia, the sexual organs of fungi producing pseudothecia are formed from hyphae already within the developing ascocarp. In addition to asci, many fruiting bodies contain sterile hyphae of various types that are important taxonomic characters. Amongst these are paraphyses, elongated hy-

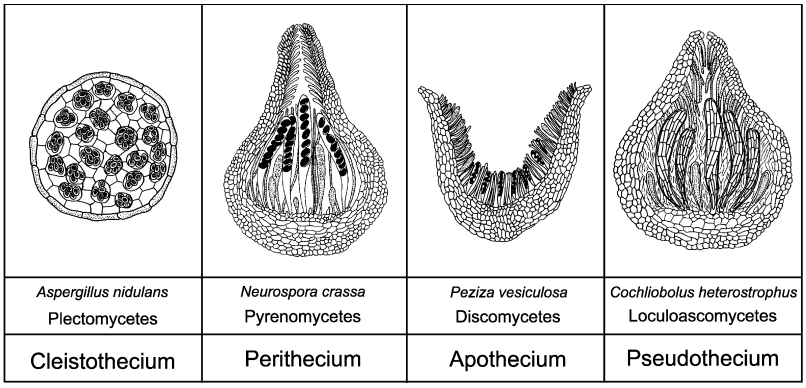

Fig. 16.1. Fruiting bodies of filamentous ascomycetes

phae originating from the base of an ascocarp, periphyses, short unbranched hyphae in the ostiolar canal of perithecia, and pseudoparaphyses, sterile hyphae originating above the level of asci in a pseudothecium (Alexopoulos et al. 1996).

Traditionally, fruiting-body morphology and the ascus structure have been used for a general taxonomic classification of the hyphal ascomycetes, which have thereby been grouped into four classes. The typical ascocarps of plectomycetes, pyrenomycetes, discomycetes and loculoascomycetes are cleistothecia, perithecia, apothecia and pseudothecia, respectively. However, this traditional classification has been questioned in recent years, due to the availability of molecular markers based on DNA sequencing data (Berbee et al. 2000). From this follows that some groups, such as the pyrenomycetes and plectomycetes, can indeed be defined by their ascocarp and ascus structure. However, others like the discomycetes and loculoascomycetes appear not to be monophyletic (Liu et al. 1999; Lumbsch 2000; Lumbsch et al. 2000; Lindenmuth et al. 2001).

B. Model Organisms to Study Fruiting-Body Development

In recent years, classical and molecular genetic approaches have been used to gain a detailed insight into the mechanism directing the cellular processes of fruiting-body development.

Classical genetic studies have demonstrated that fruiting-body development is under polygenic control. Much information has come from mutagenesis assessments in which mutants have been obtained that are blocked at one specific stage of development. Fungal model organisms used to study fruiting-body development are mainly the pyrenomycetes *Neurospora crassa* and *Sordaria macrospora*, and the plectomycete *Aspergillus nidulans*, which will be described in detail in the following sections. However, molecular genetic analyses of many other hyphal ascomycetes, including the pyrenomycetes *Podospora anserina*, *Magnaporthe grisea* and *Cryphonectria parasitica* as well as the loculoascomycete *Cochliobolus heterostrophus*, have also led to the isolation of fruiting-body developmental genes (Table 16.1).

1. *Neurospora crassa*

Since the 1940s, the pyrenomycete *N. crassa* has been used as model organism for genetic and biochemical analyses (Perkins and Davis 2000; Davis and Perkins 2002). *N. crassa* is heterothallic with two mating types, designated *mat a* and *mat A*, and is able to propagate by both sexual and asexual spores. Haploid strains are hermaphroditic but self-sterile. The formation of protoperithecia is induced by nitrogen limitation. However, the sexual cycle is initiated only when a protoperithecium of one mating type is fertilized via a trichogyne by a male cell of another mating type. In *N. crassa*, there are no specialized male cells that function exclusively as spermatia; rather, macroconidia, microconidia or hyphal fragments can fertilize the protoperithecia (see Glass and Fleißner, Chap. 7, this volume). Fertilized protoperithecia develop into perithecia within which asci with eight linearly ordered, homokaryotic ascospores are formed. Over 200 mutants that affect sexual development have been isolated in *N. crassa*. These include male-sterile mutants that are fertile as female parents, female-sterile mutants that are fertile as male parents, mutants that are both female- and male-sterile, and mutants that affect ascus development in various ways

Table. 16.1. Genes involved in fruiting-body development

Organisms	Gene	Gene product	Defect of mutants	Reference
Neurospora crassa	*asd-1*	Rhamnogalacturonase	No ascospores	Nelson et al. (1997b)
	ndk-1	Nucleoside diphosphate kinase	Light-dependent positioning of perithecia	Ogura et al. (2001)
	sod-1	Superoxid dismutase	Light-dependent positioning of perithecia	Yoshida and Hasunuma (2004)
	nuo12.3, nuo20.8, nuo30.4, nuo78, nuo51, nuo24, nuo2	Subunit of respiratory chain NADH dehydrogenase	No ascospores	Fecke et al. (1994), Duarte et al. (1998), Almeida et al. (1999), Duarte and Videira (2000)
	mfa-1	Hydrophobic peptide pheromone	Male sterility, aberrant female sexual development and ascospore production	Kim et al. (2002a)
	pre-1	GPRC, pheromone receptor	Female sterility	Kim and Borkovich (2004)
	gna-1	G protein α subunit	Female-sterile, no perithecia	Ivey et al. (1996), Yang and Borkovich (1999)
	gna-3	G protein α subunit	Smaller perithecia with no beaks, reduced number of ascospores	Kays et al. (2000)
	gnb-1	G protein β subunit	Female-sterile, no perithecia	Yang et al. (2002)
	gng-1	G protein ɣ subunit	Female-sterile, no perithecia	Krystofova and Borkovich (2005)
	krev-1	GTP/GDP-binding protein; member of the ras superfamily	No perithecia	Ito et al. (1997)
	cr-1	Adenylyl cyclase	Delayed perithecia and ascospore formation	Perkins et al. (1982), Ivey et al. (2002)
	mak-2	MAPK, related to *S. cerevisiae* Fus3p and Kss1p	Female-sterile, no protoperithecia	Pandey et al. (2004), Li et al. (2005)
	nrc-1	MAPKKK, similar to *S. cerevisiae* Ste11p	Female-sterile, no protoperithecia	Kothe and Free (1998)
	nrc-2	Serine-threonine protein kinase	Female-sterile, no protoperithecia	Kothe and Free (1998)
	rgb-1	B regulatory subunit of the type 2A Ser/Thr phosphatase	Female-sterile, no prothoperithecia	Yatzkan and Yarden (1999)
	ham-2	Putative transmembrane protein	Female-sterile, no protoperithecia	Xiang et al. (2002)
	eat-2	Highly similar to a domain present in the plasma membrane ATPase	Female-sterile, no prothoperithecia	Randall and Metzenberg (1998)
	cel-2	β subunit of fatty acid synthase	Fewer peritecia, rare ascospores	Goodrich-Tanrikulu et al. (1999)
	asm-1	Transcription factor with APSES domain	Female-sterile, no protoperithecia	Aramayo et al. (1996)
	vma-1	Subunit A of the V-ATPase	Female-sterile, no protoperithecia, can not donate nuclei in a cross	Bowman et al. (2000)
	cpc-2	WD-repeat protein required to repress general amino acid control, scaffold protein	Female-sterile, no protoperithecia	Müller et al. (1995)
	rco-1	Multidomain protein that mediates transcriptional repression	Female-sterile, no protoperithecia	Yamashiro et al. (1996)
	asd-4	GATA-type zinc finger transcription factor	No asci, no ascospores	Feng et al. (2000)
	wc-1	GATA-like zinc finger transcription factor	No phototropism of perithecial beaks	Ballario et al. (1996), Oda and Hasunuma (1997)

Table. 16.1. (continued)

Organisms	Gene	Gene product	Defect of mutants	Reference
	wc-2	GATA-like zinc finger transcription factor	No phototropism of perithecial beaks	Linden and Macino (1997), Oda and Hasunuma (1997)
	pp-1	Transcription factor homeodomain, C_2H_2 zinc finger, Ste12p homolog	No protoperithecia	Li et al. (2005)
Podospora anserina	*car1*	Peroxisomal membrane protein	Impaired karyogamy, no ascospores	Berteaux-Lecellier et al. (1995)
	cro1	Cytosolic protein with C_2H_2 zinc finger motif	Impaired meiosis, no ascospores	Berteaux-Lecellier et al. (1998)
	AS4	Translation-elongation factor EF-1α, eF1A	No ascospores, no perithecia	Silar et al. (2001)
	su1	Translation-termination factor eRF3	Female-sterile, no protoperithecia	Gagny and Silar (1998)
	su2	Translation-termination factor eRF1	Female-sterile, no protoperithecia	Gagny and Silar (1998)
	PaCox17	Chaperone targeting copper to cytochrome c	Delayed perithecial formation and reduced ascus formation	Stumpferl et al. (2004)
	rmp1	No homology, functions in nucleus–mitochondria cross-talk	Lethal or without mitochondrial targeting sequence, no ascospores	Contamine et al. (2004)
	PaNox1	NADPH oxidase	No perithecia	Malagnac et al. (2004)
	PaNox2	NADPH oxidase	No ascospore germination	Malagnac et al. (2004)
	mod-A	Proline-rich protein with SH3-binding motif	Female-sterile, no protoperithecia	Barreau et al. (1998)
	mod-E	Heat-shock protein HSP90	Sterile perithecia, no ascospores	Loubradou et al. (1997)
	mfp	Hydrophobic peptide pheromone	Male-sterile	Coppin et al. (2005)
	mfm	Peptide pheromone	Male-sterile	Coppin et al. (2005)
	mod-D	G protein α subunit	Female-sterile, no protoperithecia	Loubradou et al. (1999)
	PaAsk1	MAPKKK, similar to *S. cerevisiae* Bck1p	No perithecia	Kicka and Silar (2004)
	grisea	Copper-activated transcription factor, ortholog of the yeast transcription factor MAC1	Female-sterile, no protoperithecia	Osiewacz and Nuber (1996), Borghouts and Osiewacz (1998)
	fle1	Transcription factor, C_2H_2 zinc finger	Fewer microconidia, more abundant protoperithecia	Coppin (2002)
	pah1	Transcription factor, homeobox protein	Enhanced number of microconidia, delayed protoperithecia development	Arnaise et al. (2001)
	ami1	Homolog of *A. nidulans* APS-A	Male-sterile, delayed fruiting-body formation	Graia et al. (2000)
Sordaria macrospora	*pro11*	WD-repeat protein	No perithecia	Pöggeler and Kück (2004)
	pro4	Leucine biosynthesis	No perithecia	Kück (2005)
	acl1	Subunit of ATP-citrate-lyase	No ascospores	Nowrousian et al. (1999)
	spo76	Chromosomal protein	Impaired meiosis, few ascospores	van Heemst et al. (1999)
	pro1	C_6 zinc finger transcription factor	No perithecia	Masloff et al. (1999)
Aspergillus nidulans	*trpB*	Tryptophane biosynthesis	No cleistothecia	Eckert et al. (1999, 2000)
	hisB	Histidine biosynthesis	No cleistothecia	Busch et al. (2001)
	tubB	α-tubulin	No ascospores	Kirk and Morris (1991)
	uvsC	DNA-repair enzyme homolog of *S. cerevisiae* RAD51	No karyogamy, no ascospores	van Heemst et al. (1997)
	lsdA	No sequence similarity, unknown function	No inhibition of cleistothecia development under high salt conditions	Lee et al. (2001)

Table. 16.1. (continued)

Organisms	Gene	Gene product	Defect of mutants	Reference
	phoA	Cyclin-dependent kinase	Enhanced number of cleistothecia under phosphorus-limited conditions	Bussink and Osmani (1998)
	pho80	Putative cyclin-dependent kinase inhibitor	Promotes sexual development	Wu et al. (2004)
	csnD	Subunit of COP9 signalosome	No cleistothecia	Busch et al. (2003)
	veA	No homology, unknown	No cleistothecia	Kim et al. (2002b)
	noxA	NADPH oxidase	No cleistothecia	Lara-Ortíz et al. (2003)
	ppoA	Putative fatty acid dioxygenase	Increased ratio of asexual to sexual development	Tsitsigiannis et al. (2004)
	odeA	Δ-12 desaturase	Delayed ascosporogenesis	Calvo et al. (2001)
	gprA	GPRC, pheromone receptor	Fewer cleistothecia and ascospores	Seo et al. (2004)
	gprB	GPRC, pheromone receptor	Fewer cleistothecia and ascospores	Seo et al. (2004)
	gprD	GPRC	No cleistothecia	Han et al. (2004)
	fadA	G protein α subunit	No cleistothecia	Rosèn et al. (1999)
	sfdA	G protein β subunit	No cleistothecia, more Hülle cells	Rosèn et al. (1999)
	flbA	RGS protein	No cleistothecia	Han et al. (2001)
	sakA	MAPK, similar to *S. cerevisiae* Hog1p	Premature sexual development, more cleistothecia	Kawasaki et al. (2002)
	steC	MAPKKK, similar to *S. cerevisiae* Ste11p	No cleistothecia	Wei et al. (2003)
	steA	Transcription factor homeodomain, C_2H_2 zinc finger, Ste12p homolog	No cleistothecia, only Hülle cells	Vallim et al. (2000)
	nsdD	Transcription factor GATA-type	No cleistothecia, no Hülle cells	Han et al. (2001)
	stuA	Transcription factor with APSES domain	No cleistothecia, no Hülle cells	Wu and Miller (1997)
	dopA	Putative transcription factor, leucine zipper-like domains and similarity to C/EBP transcription factor	No cleistothecia, no Hülle cells	Pascon Castiglioni and Miller (2000)
	medA	Transcriptional regulator	No cleistothecia, only Hülle cells	Busby et al. (1996)
	cpcA	Transcription factor, c-Jun-like leucine zipper, required to activate general amino acid control	When overexpressed, no cleistothecia	Hoffmann et al. (2001b)
	cpcB	WD-repeat protein, required to repress general amino acid control, scaffold protein	No cleistothecia	Hoffmann et al. (2000)
Magnaporthe grisea	*magA*	G protein α subunit	No ascospores	Liu and Dean (1997)
	magC	G protein α subunit	No ascospores	Liu and Dean (1997)
	magB	G protein α subunit	Female-sterile, no perithecia	Liu and Dean (1997)
	mac-1	Adenylyl cyclase	Female-sterile, no perithecia	Choi and Dean (1997)
	mps-1	MAPK, similar to *S. cerevisiae* Sltp	Female-sterile, no perithecia	Xu et al. (1998)
Cryphonectria parasitica	*Mf2-2*	Hydrophobic peptide pheromone	Female sterility	Zhang et al. (1993)
	Mf1-1	Peptide pheromone	Male-sterile	Turina et al. (2003)
	cpg-1	G protein α subunit	Female-sterile	Gao and Nuss (1996)
	cpg-2	G protein α subunit	Enhanced perithecial development	Gao and Nuss (1996)
Fusarium graminearum	*mgv-1*	MAPK, similar to *S. cerevisiae* Sltp	Female-sterile, no perithecia	Hou et al. (2002)
Cochliobolus heterostrophus	*cga-1*	G protein α subunit	Female sterility	Horwitz et al. (1999)
	chk-1	MAPK, related to *S. cerevisiae* Fus3p and Kss1p	Female sterility	Lev et al. (1998)

(Raju 1992). When male-sterile mutants are used as fertilizing parent, perithecial development is initiated, but then arrested at an early stage. In *N. crassa*, numerous female-sterile mutants that do not form functional protoperithecia or display a reduced fertility have been described. Because of this high frequency of sterility in female strains, it was suggested that at least 400 genes are required for perithecium and ascospore development in *N. crassa* (Johnson 1978; Leslie and Raju 1985). However, many of these female-sterile mutants show abnormal vegetative growth, and thus this female sterility may be a consequence of a morphological defect, rather than a mutation in a gene specifically needed for fruiting-body differentiation (Raju 1992).

Effective molecular techniques developed for *N. crassa*, such as the transformation and creation of cosmid genomic libraries involving, for example, phenotypic complementation, have led to the cloning and functional characterization of regulatory genes that affect morphology (Bailey and Ebbole 1998). Other molecular genetic approaches used subtractive hybridization to isolate *N. crassa* sexual developmental genes.

With this attempt, Nelson and Metzenberg (1992) succeeded in identifying 14 genes transcribed only under nitrogen-depleting growth conditions. One of these, *asd-1*, has been shown to encode a putative rhamnogalacturonase necessary for ascus development (Nelson et al. 1997b). In large-scale analyses, fruiting body-specific expressed sequence tags (ESTs) from *N. crassa* were sequenced for further molecular characterization (Nelson et al. 1997a). In such experimental approaches, isolated candidate genes have to be inactivated to obtain detailed information about mutant phenotypes and gene function.

Inactivation of developmental genes in *N. crassa* was achieved either by homologous recombination or by gene silencing via repeat-induced point mutation (RIP). The *N. crassa* RIP process efficiently detects and mutates both copies of a sequence duplication. RIP acts during the dikaryotic stage of the sexual cycle, causing numerous C:G-to-T:A transitions within duplicated sequences, and is frequently used to inactivate genes in *N. crassa* (Galagan and Selker 2004). Finally, the whole genome sequence of *N. crassa* has become available, opening the opportunity for analyzing genes involved in fruiting-body development by means of reverse genetic approaches (Galagan et al. 2003; Borkovich et al. 2004).

2. *Sordaria macrospora*

In the heterothallic species *N. crassa*, mutations conferring male and/or female sterility can be detected directly because of their sterility effects in heterozygous crosses. However, recessive mutations that affect post-fertilization perithecial development will remain undetected in heterothallic species until the mutant allele is available in both mating types, thus allowing homozygous crosses. In contrast to *N. crassa*, the homothallic pyrenomycete *S. macrospora* is self-fertile, which means that recessive mutations can directly be tested for defects in fruiting-body development. Moreover, *S. macrospora* produces only meiotically derived ascospores, whereas asexual spores, such as conidia, are absent. Thus, there is no interference between two different developmental programs, which makes it easier, for example, to analyze differentially expressed genes involved in ascocarp development. Under laboratory conditions, perithecia and ascospores reach maturity within 7 days after ascospore germination. During this development, distinct reproductive structures, such as ascogonia, protoperithecia (young fruiting bodies), and perithecia, can be distinguished (Fig. 16.2). Because *S. macrospora* represents such a favorable genetic system for scientists, this homothallic pyrenomycete was used to generate numerous mutants that are blocked at various stages of perithecial development (Esser and Straub 1958; Masloff et al. 1999). Wild-type strains of *S. macrospora* are self-fertile and produce perithecia. However, fertile perithecia are also formed in crosses between sterile strains, when nuclei are interchanged by hyphal anastomoses at the contact zones of two sterile mycelia. These crosses facilitate the analysis of epistatic relationships among developmental mutants. The establishment of molecular tools provides the basis for studying fruiting-body development in *S. macrospora* (Walz and Kück 1995; Pöggeler et al. 1997).

3. *Aspergillus nidulans*

The plectomycete *Aspergillus nidulans* (teleomorph: *Emericella nidulans*) propagates by the formation of spores that can be either asexual or sexual, and has long served as a model system for understanding the genetic regulation of asexual development in ascomycetes (Adams et al. 1998). The asexual cycle is characterized by the produc-

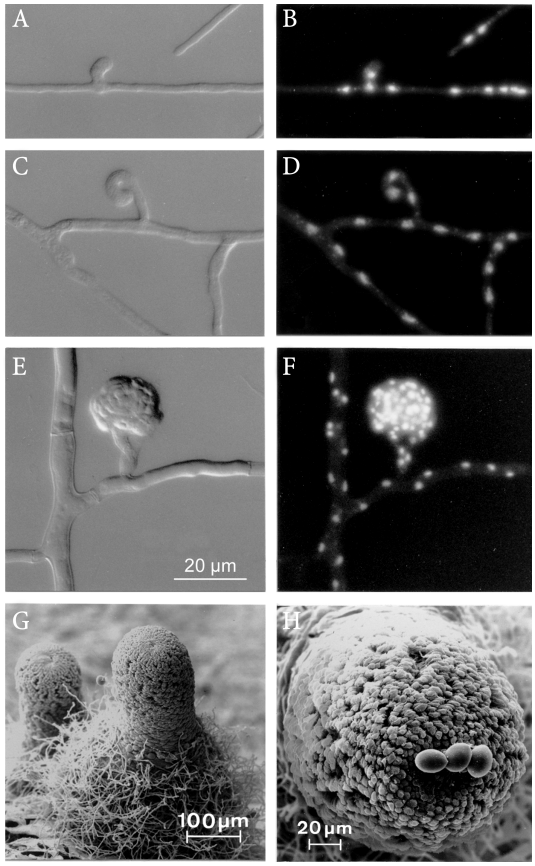

Fig. 16.2. Sexual development of *Sordaria macrospora*. **A**,**B** Young ascogonium; **C**,**D** ascogonium; **E**,**F** protoperithecium; **G** perithecium; **H** top view of perithecium, with ascospores. **A**,**C**,**E** Differential interference contrast light micrographs DIC. **B**,**D**,**F** Fluorescence micrographs of **A**,**C**, and **E** with DAPI staining of nuclei. **G**,**H** Scanning electron micrographs

tion of haploid conidiophores that bear asexual, single-celled spores called conidia (Fischer 2002, see Fischer and Kües, Chap. 14, this volume). Sexual development of *A. nidulans* starts after conidiophore differentiation. The conidiophores are produced by mitotic division in 3 days after germination, whereas the sexual ascospores are formed after at least 7 days (Pontecorvo 1953). *A. nidulans* is homothallic, and a single colony can produce cleistothecia filled with up to 1000 ascospores by self-fertilization.

In *A. nidulans*, no obvious antheridium or ascogonium structures can be observed (Benjamin 1955). It was, however, assumed by Kwon and Raper (1967) that in *Aspergillus heterothallicus*, a heterothallic relative of *A. nidulans*, a coiled structure equivalent to an ascogonium fuses to a second cell equivalent to an antheridium.

In contrast to pyrenomycetes, the presumptive ascogonium of *A. nidulans* is not surrounded by sterile hyphae, and pre-fruiting bodies are not formed. Only after fertilization is the ascogonium surrounded by growing, unordered hyphae, which form an increasingly packed "nest" and then differentiate globose, multinucleate Hülle cells, which support the development of cleistothecia (Ellis et al. 1973). The surrounding hyphae that form the "nest" later differentiate into the cleistothecial envelope. The developing spherical cleistothecia are filled with ascogenous hyphae that differentiate into asci. After karyogamy, the zygote stage is immediately followed by meiosis. Directly after meiosis, the four resulting nuclei pass through a first post-meiotic mitosis. The eight nuclei are then separated by membranes, and give rise to eight red-pigmented ascospores within each ascus. As second post-meiotic mitosis results in eight binucleate, mature ascospores (Braus et al. 2002). Similarly to the homothallic pyrenomycete *S. macrospora*, self-fertile *A. nidulans* strains can be used in crossing experiments. In this case, hyphae from two different strains can form anastomoses and exchange nuclei when growing sufficiently close to each other (Hoffmann et al. 2001a).

The coexistence of sexual and asexual reproduction within one and the same individual has made *A. nidulans* a popular genetic model organism to compare fitness effects of sexual and asexual reproduction. Because of its homothallism, the sexual and asexual offspring of *A. nidulans* have largely identical genotypes. As was shown by Bruggeman et al. (2003, 2004), slightly deleterious mutations accumulate at a lower rate in the sexual than in the asexual pathway.

In addition to classical genetics, various tools required for molecular biology have been developed, and recently the entire genome of *A. nidulans* has been sequenced (http://www.broad.mit.edu/annotation/fungi/aspergillus), thus making *A. nidulans* an excellent model for studying various biological questions, including the multicellular cleistothecium development.

For the genetic dissection of sexual sporulation in *A. nidulans*, a collection of ascospore-less mutants was isolated by Swart et al. (2001). To understand the sexual reproduction of *A. nidulans* in more detail, Han et al. (1990) identified numerous mutants that were defective in sexual development in a forward genetic screen. These were classified into two groups:

1. *nsd* (*n*ever in *s*exual *d*evelopment) mutants that are unable to form any sexual structures; and
2. *bsd* (*b*lock in *s*exual *d*evelopment) mutants that show differences in the amount or timing of sexual organ production, compared to the wild type.

Several *nsd* mutants were analyzed for their genetic and morphological characteristics (Han et al. 1994, 1998). Finally, the *nsdD* gene has been cloned and was shown to encode a GATA-type transcription factor (Han et al. 2001). Beside the *nsdD* gene, several other *A. nidulans* genes that affect sexual development have been cloned, not only by complementation of developmental mutants but also by reverse genetic approaches (Table 16.1).

II. Physiological Factors Influencing Fruiting-Body Development

Most fungi do not form fruiting bodies continuously, but require special environmental conditions. Additionally, the vegetative mycelium has to acquire a certain stage of "competence" before differentiating fruiting bodies. The external factors needed to promote fruiting-body formation are mostly organism-specific, and depend on the ecological niche the fungus occupies. Most prominent among these are nutrients, light, temperature, aeration, pH, and the presence of a partner or host for symbiotic or pathogenic fungi, respectively. Endogenous factors that have been identified as relevant for fruiting-body formation often involve components of primary metabolism as well as pheromones or hormone-like substances (Dyer et al. 1992; Moore-Landecker 1992). Perception of these factors and initiation of an appropriate reaction require complex regulatory networks; in recent years, genetic and molecular biology methods have helped to start unraveling the molecular basis of fruiting-body development.

A. Environmental Factors

The influence of environmental factors on fruiting-body development is well-studied on the physiological level in economically important fungi, most of which are basidiomycetes (Kües and Liu 2000), and to some degree in several ascomycetes, which have been used to investigate the basic principles of fruiting-body development (Moore-Landecker 1992). In this section, a brief overview of some of these factors is given, focusing on cases where at least some genes involved in the perception of these factors have been identified.

1. Nutrients and Related Factors

The requirements for certain nutrients and other chemical substances for fruiting-body formation vary in different ascomycetes. In many species, fruiting bodies are formed preferentially at much lower nutrient concentrations than those promoting vegetative growth (Moore-Landecker 1992). One reason might be that the vegetative mycelium accumulates nutrients that can be used for the formation of fruiting bodies once a critical amount is reached. Thus, the mycelium would, in effect, nurture the developing fruiting bodies. Another reason could be that sexual development is initiated when the growth substrate is depleted of nutrients, and durable ascospores are produced that can lie dormant until more suitable conditions occur.

Some ascomycetes require specific factors, such as vitamins, for sexual development, but not for vegetative growth. *S. macrospora*, for example, needs biotin to complete the sexual cycle (Molowitz et al. 1976). Furthermore, it has been shown in *S. macrospora* that addition of arginine to the growth medium enhances fruiting-body formation (Molowitz et al. 1976). This finding, together with the observation that amino acid synthesis mutants of both *A. nidulans* and *S. macrospora* show defects in fruiting-body development, indicates an influence of amino acid metabolism in sexual development, an aspect discussed in more detail in Sect. II.B.1. In some ascomycetes, fruiting-body formation can be increased by addition of fatty acids to the growth medium. In *Ceratocystis ulmi* and *Nectria haematococca*, production of perithecia can be increased by exogenous linoleic acid, whereas in *N. crassa*, both oleate and linoleate enhance fruiting-body formation (Nukina et al. 1981; Marshall et al. 1982; Dyer et al. 1993; Goodrich-Tanrikulu et al. 1998). Mutants with defects in lipid metabolism often have developmental defects, too (see Sect. II.B.1).

Environmental factors that influence sexual development (and each other) are the pH of the growth medium, aeration (CO_2 pressure), osmotic pressure, and the presence and availability of mineral salts. In *N. crassa*, mutants in a gene encoding a subunit of the vacuolar H(+)-ATPase do not grow

in medium with a pH of 7.0 and above, and they are female-sterile, most likely due to insufficient cellular homeostasis (Bowman et al. 2000). It has long been known that in *A. nidulans*, increased partial pressure of carbon dioxide favors fruiting-body formation, whereas aeration, and thereby the removal of carbon dioxide, shifts the balance toward asexual sporulation (Champe et al. 1994).

In *A. nidulans*, high concentrations of potassium, sodium or magnesium ions inhibit sexual development and promote asexual sporulation. Regulation of this salt-dependent developmental balance requires the *lsdA* gene, which does not have any homology to previously characterized genes (Lee et al. 2001). Also involved in this regulation process is *veA*, a gene that integrates a number of signals and that is described more fully in Sect. II.A.2.

Another gene involved in salt-dependent developmental decisions between asexual and sexual development in *A. nidulans* is *phoA*, a gene for a cyclin-dependent kinase. Depending on both pH and the initial phosphorus concentration, *phoA* mutants can switch from asexual to sexual development, or do not show any form of spore differentiation (Bussink and Osmani 1998). Interacting with PHOA is the cyclin An-PHO80, which also is involved in regulating the balance between asexual and sexual differentiation. Effects of the *An-pho80* deletion also depend on phosphate concentration, but in contrast to *phoA* mutants, *An-pho80* mutants increase the production of conidia and do not form mature cleistothecia (Wu et al. 2004).

Many pathogenic or mycorrhizal fungi form fruiting bodies only in contact with their hosts or symbiont partners, which makes analysis of these differentiation processes difficult. For a taxol-producing *Pestalotiopsis microspora* isolate that lives as an endophyte of yew, it was found that a methylene chloride extract of yew needles induces the formation of perithecia (Metz et al. 2000). Most likely, one or more lipid-like compounds present in yew needles stimulate sexual differentiation in the fungus.

Lipid-derived factors produced by fungi themselves, as well as lipid metabolism in general have also been found to be involved in sexual development (see Sect. II.B.1).

For organisms which cannot easily be cultivated under laboratory conditions, but where RNA extraction is possible from field isolates, large-scale expression analysis techniques such as microarrays have great potential to help unraveling molecular mechanisms of development (Nowrousian et al. 2004). Lacourt and coworkers compared the expression of 171 genes in vegetative tissue and different stages of developing fruiting bodies of the mycorrhizal ascomycete *Tuber borchii*, using cDNA macroarrays. These investigations revealed that metabolism and cell wall synthesis are substantially altered during development (Lacourt et al. 2002), but how the plant partner influences fungal development at the molecular level remains to be elucidated.

2. Physical Factors

Most ascomycetes form fruiting bodies on the surface of their growth substrate to facilitate ascospore dispersal, the most notable exception being truffles and related fungi. This means that the fungus somehow has to organize the correct place and orientation of the fruiting bodies. Possible mechanisms for this process include the perception of gravity or of air/substrate interfaces. There has been some research into the effects of gravity on fungal sexual development that has established that at least some fungal fruiting bodies, or parts of fruiting bodies exhibit gravitropism, but the genetic and biochemical processes needed to perceive and respond to gravity remain enigmatic (see Corrochano and Galland, Chap. 13, this volume). Little more is known about the recognition of air/surface interfaces, or other means of spatial control of fruiting-body formation in ascomycetes. In basidiomycetes, a class of small secreted proteins called hydrophobins has been shown to be essential for breaching an air/water interface, and hydrophobins coat many fruiting-body surfaces (see Chap. 19, this volume). Hydrophobins have been identified in several ascomycetes, too, but whether they play a role in fruiting-body formation is not yet clear (see Sect. IV.B). Another effect that has been observed in several ascomycetes is the so-called edge effect – fruiting bodies are formed preferentially at the edges of a petri dish or other culture vessels. Investigation of this effect in *S. macrospora* has revealed that the determining parameter is not a recognition of edges or other surface structures, but rather an increased hyphal density, which can be due to mechanical obstacles and also to nutrient availability (Molowitz et al. 1976; Hock et al. 1978); similar results have been reached with other ascomycetes (Moore 1998).

Related to the "edge effect" might be the observation that fruiting bodies are often formed at

regular intervals; both effects require the fungus to sense hyphal or fruiting-body spacing, but the genetic basis behind this regulation remains to be elucidated.

Another possible signal for the production and orientation of fruiting bodies is light (see also Chap. 13, this volume). Light has been shown to influence fruiting-body formation in many ascomycetes from different phylogenetic groups. Influences of light range from complete light-dependence of fruiting-body formation to orientation of fruiting bodies or, in reverse, a preferential formation of fruiting bodies in the dark. Several genes involved in light perception have been identified in various fungi.

Early reports of light effects on fruiting-body formation have been for *Pyronema confluens* and *Pyronema domesticum*, where apothecium formation is completely light-dependent, and dark-grown mycelia are sterile (Claussen 1912; Moore-Landecker 1979). In other fungi, like *N. crassa*, fruiting bodies are formed in the dark and are placed on the surface of the growth substrate even without illumination, but perithecial necks are normally oriented toward the light, and in darkness point to various directions (Harding and Melles 1983). Furthermore, not only the orientation but also the position of the neck on the perithecium is light-dependent (Oda and Hasunuma 1997). Additionally, the number of protoperithecia that are formed is greatly increased upon blue-light illumination (Degli Innocenti and Russo 1983). All three effects were shown to depend on the *white collar* genes *wc-1* and *wc-2* (Harding and Melles 1983; Degli Innocenti and Russo 1984; Oda and Hasunuma 1997). WC-1 has been identified as a blue-light photoreceptor as well as a transcription factor, and it interacts with WC-2 to regulate the transcription of target genes in a light-dependent manner (Ballario et al. 1996; Linden and Macino 1997; Froehlich et al. 2002; He et al. 2002).

Another gene that plays a role in certain aspects of light-dependent morphogenesis is *ndk-1* (Ogura et al. 2001; Yoshida and Hasunuma 2004). *ndk-1* encodes a nucleoside diphosphate kinase, and is required for light-dependent neck positioning on the perithecia but not for orientation of the neck itself. Nucleoside diphosphate kinase is a conserved enzyme that is involved in signal transduction cascades in various organisms.

In *N. crassa*, NDK-1 is autophosphorylated after blue-light illumination, which makes it a likely candidate for a light signal transduction pathway component (Ogura et al. 2001).

Studies with double mutants have shown that *ndk-1* depends on the presence of functional *wc* genes (Yoshida and Hasunuma 2004). Both WC proteins are also part of the circadian clock of *N. crassa* (Loros and Dunlap 2001), and transcription of the pheromone precursor genes that are involved in sexual development of *N. crassa* (see Sect. B.2) is regulated by the endogenous clock (Loros et al. 1989; Bobrowicz et al. 2002). These findings indicate that in *N. crassa*, sexual development is not only light-regulated but the circadian clock may also contribute to the control of fruiting-body formation (see also The Mycota, Vol. III, Chap. 11).

The influence of light on developmental processes was also investigated extensively in *A. nidulans*. In this ascomycete, red light shifts the ratio of asexual to sexual reproduction structures (conidiophores vs. cleistothecia) toward asexual reproduction, whereas fruiting-body formation is favored in the dark. One gene that is necessary for maintaining this light-dependent balance is *veA* (Mooney and Yager 1990; Kim et al. 2002b). *veA*, which does not have homology to any genes with known function, is essential for fruiting-body formation; *veA* deletion mutants do not form any cleistothecia (Kim et al. 2002b). The *veA1* allele, a partially deleted form of *veA* that is present in many laboratory strains of *A. nidulans*, causes a less severe phenotype with a preference for conidiation, even without illumination (Kim et al. 2002b).

Another gene involved in controlling the light-dependent balance of asexual versus sexual reproduction of *Aspergillus* is *csnD*. This gene encodes a subunit of the COP9 signalosome, a conserved eukaryotic protein complex that regulates developmental processes by targeting proteins for ubiquitinylation and subsequent degradation by the 26S proteasome (Busch et al. 2003). In contrast to *veA* mutants, a *csnD* deletion mutant predominantly induces (but does not complete) the sexual cycle, irrespective of the light signal (Busch et al. 2003). However, both *veA* and *csnD* mutants have other, light-independent phenotypes, and may be part of signal transduction networks that integrate many signals controlling fruiting-body development (Kim et al. 2002b; Busch et al. 2003).

Another physical factor influencing fruiting-body development is temperature. For those ascomycetes in which this aspect has been investigated, it was found that the temperature range that controls fruiting-body formation is usually more

restricted than that controlling vegetative growth (Moore-Landecker 1992), but the genetic basis for this is not yet clear. *mod-E*, a heat-shock protein HSP90 homolog, was found to be involved in both sexual development and vegetative incompatibility in *Podospora anserina* (Loubradou et al. 1997). *mod-E* transcripts are accumulated after a shift from 26 to 37 °C, but effects of different temperatures on fruiting-body formation in the wild type versus *mod-E* mutants were not reported. Therefore, it remains to be determined whether *mod-E* or other (heat-shock) proteins are involved in temperature-dependence of fruiting-body development.

B. Endogenous Factors

The transition from vegetative growth to sexual development requires a physiologically "competent" mycelium. This competence often depends on nutrient availability, but the nutrients also have to be processed by the fungal metabolism; and genetic analyses have shown that fruiting-body formation requires metabolic reactions different from those of vegetative growth (see Sect. II.B.1). In several fungal species, pheromones or hormone-like substances are necessary for completion of the sexual cycle, as described in Sect. II.B.2 and in Chap. 11 (this volume).

1. Metabolic Processes

In several ascomycetes, it was found that mutations in genes for primary metabolism often interfere with sexual development under conditions where vegetative growth remains more or less normal. Examples for this are mutants blocked in amino acid biosynthesis pathways, and fatty acid biosynthesis mutants. Many effects of mutations leading to amino acid auxotrophy on fruiting-body morphogenesis have been investigated in *A. nidulans*. Deletion of the tryptophan synthase-encoding gene *trpB*, or the histidine biosynthesis gene *hisB* leads to loss of cleistothecia production on medium with low levels of tryptophan or histidine, respectively (Eckert et al. 1999, 2000; Busch et al. 2001). Both genes are regulated by the cross-pathway control system, a regulatory network that activates a variety of amino acid biosynthesis genes when the amounts of a single amino acid are low. Besides regulating amino acid biosynthesis, this cross-pathway network also comprises a control point for progression of sexual development (Hoffmann et al. 2000). This was demonstrated by investigating the functions in fruiting-body formation of two members of the cross-pathway network, *cpcA* and *cpcB*. *cpcA* encodes a transcriptional activator homologous to the yeast Gcn4p protein, which is the activating transcription factor for cross-pathway control (termed general control of amino acid biosynthesis) in *Saccharomyces cerevisiae* (Hoffmann et al. 2001b). CpcB is homologous to mammalian RACK1 (receptor for activated C-kinase 1), a scaffold protein involved in many cellular signaling processes (McCahill et al. 2002). *cpcA* and *cpcB* play antagonistic roles in cross-pathway control as well as in sexual development: *cpcA* activates amino acid biosynthesis gene transcription under conditions of amino acid deprivation, whereas *cpcB* represses the cross-pathway control network when amino acids are present. Overexpression of *cpcA* in the presence of amino acids leads to a block in sexual development, thereby mimicking a lack of amino acids, and the same effect can be reached by deletion of *cpcB* (Hoffmann et al. 2000). The connection between cross-pathway control and sexual development seems to be widespread in filamentous ascomycetes, as a mutant in the *N. crassa cpcB* homolog, *cpc-2*, is female-sterile (Müller et al. 1995). Also, a sterile mutant of *S. macrospora* was shown to have a defect in a gene for leucine biosynthesis (Kück 2005). This mutant, as well as the *A. nidulans* amino acid biosynthesis mutants mentioned above, grow normally on media with moderate amounts of the amino acid they are auxotrophic for, but if at all, fertility can be restored only by much higher amounts. These findings indicate that fungi are able to integrate nutrient availability and cellular metabolism, and react properly with respect to the initiation of energy-demanding processes such as fruiting-body formation.

Similar regulatory events can be proposed for fatty acid metabolism and fruiting-body development, although the evidence here is more spurious and signal transduction pathways have yet to be identified. Nevertheless, data from mutants in diverse genes involved in different aspects of fatty acid metabolism indicate that appropriate amounts and composition of fatty acids and their derivatives are essential for sexual development. *N. crassa* mutants of a fatty acid synthase subunit are sterile in homozygous crosses, and *A. nidulans* mutants of several desaturase genes show changes in

the balance between sexual and asexual development (Goodrich-Tanrikulu et al. 1999; Calvo et al. 2001; Wilson et al. 2004). In *A. nidulans*, several fatty acid-derived factors, so-called psi factors, are necessary for correct developmental decisions, and changes in fatty acid composition also influence psi factor composition, which could explain morphological defects in the mutants (see Chap. 11, this volume). It remains to be determined if similar fatty acid-derived specific developmental factors are present in other ascomycetes. Analyses of other mutants point to a more general requirement for fatty acids, probably as energy source for fruiting-body formation. In *S. macrospora*, the sterile mutant per5 was found to harbor a defect in the *acl1* gene that encodes a subunit of ATP citrate lyase (Nowrousian et al. 1999). This enzyme is involved in the production of cytosolic acetyl-CoA, which is used mainly for the synthesis of fatty acids and sterols, and the mutant can be partially rescued by addition of exogenous oleate, thereby indicating that it suffers from a lack of lipid biosynthesis products. The *acl1* gene is expressed mainly during vegetative growth prior to sexual development, which fits a model of the vegetative mycelium acquiring nutrients that are mobilized later during fruiting-body formation (Nowrousian et al. 1999, 2000). Another mutation with implications for fatty acid metabolism in fruiting-body development is *car1* of *Podospora anserina*. CAR1 is a peroxisomal protein necessary for peroxisome biogenesis, and interestingly, the *car1* mutant has defects in karyogamy, and therefore is sterile (Berteaux-Lecellier et al. 1995). In peroxisomes, mobilization of fatty acids by β-oxidation takes place, and the phenotype of the *car1* mutant might be due to a disturbed fatty acid metabolism.

Higher metabolic demands of fruiting-body formation versus vegetative growth or asexual sporulation can also be inferred by analyses of various complex I mutants of *N. crassa* (Videira and Duarte 2001). Complex I of the respiratory chain is a multi-subunit, proton-pumping NADH ubiquinone oxidoreductase that is found in most eukaryotic mitochondria. Mutants in several complex I subunits are sterile in homozygous crosses. This might be due to the subunits having other, non-respiratory functions, but the fact that mutants in subunits with different functions, such as complex assembly or reductase activity, display similar phenotypes with respect to sexual differentiation indicates that probably a lack of energy due to complex I malfunction is responsible for the developmental phenotype (Duarte and Videira 2000). This is consistent with an increased demand for energy and metabolites during fruiting-body formation.

Another aspect of increased respiration is the generation of reactive oxygen species (ROS), and thereby oxidative stress. It has long been known that ROS can have a deleterious effect on many cellular compounds, e.g., DNA and proteins, but only lately has it become acknowledged that the generation of ROS can be an actively regulated process, and that ROS can have diverse roles in cell physiology and signaling.

In *N. crassa*, it was shown recently that *sod-1*, which encodes a superoxide dismutase, is necessary for light-dependent positioning of perithecial necks (Yoshida and Hasunuma 2004). Superoxide dismutases catalyze the conversion of oxygen radicals that are generated during aerobic metabolism to hydrogen peroxide, thereby protecting the organism from damage by ROS. A possible explanation for the fact that *sod-1* is necessary for correct fruiting-body morphology is that SOD-1 is involved in generating a light-dependent ROS gradient that controls neck positioning.

In *Podospora anserina*, a mutant of the transcription factor GRISEA that is involved in cellular copper homeostasis is female-sterile (Osiewacz and Nuber 1996). Copper is an essential cofactor for several enzymes, among these some superoxide dismutases as well as cytochrome oxidase (COX), which is involved in the generation of ROS. Another mutant, in which the gene encoding a mitochondrial chaperone targeting copper to COX was deleted, showed delayed perithecial formation and reduced ascus production (Stumpferl et al. 2004). In contrast to the *grisea* mutant, this *PaCox17* mutant still had superoxide dismutase activity but respired via an alternative oxidase pathway, which might indicate that the balance between the generation and degradation of ROS is disturbed in both mutants, albeit not in the same way (Stumpferl et al. 2004). Further evidence for a participation of ROS in fruiting-body formation comes from investigation of *A. nidulans*. In this fungus, mutants of the NADPH oxidase gene, *noxA*, are sterile. NoxA generates superoxide, and is induced during sexual development of *A. nidulans*. At this time, superoxide can be detected in Hülle cells and cleistothecia (Lara-Ortíz et al. 2003). A similar expression pattern can be observed for the catalase-peroxidase gene *cpeA* (Scherer et al. 2002). Catalase-peroxidases are enzymes that convert ROS to harmless

compounds, thereby protecting the cell from oxidative damage. It is conceivable that *noxA* and *cpeA* act in concert to generate the correct amount of self-induced oxidative stress during fruiting-body formation. *noxA* expression is dependent on the MAP kinase SakA. *sakA* mutants show premature sexual development, and *noxA* is expressed in *sakA* mutants much earlier than in the wild type (Kawasaki et al. 2002; Lara-Ortíz et al. 2003).

Additional information on the involvement of *nox* genes in fruiting-body formation was recently gained from an investigation of *P. anserina*, where a mutant in the *noxA* ortholog *PaNox1* no longer differentiates mature fruiting bodies. Additionally, a mutant in a second member of the NADPH oxidase family, *PaNox2*, is blocked in ascospore germination, and both *PaNox1* and *PaNox2* are required for the controlled production of superoxide as well as peroxide during sexual development (Malagnac et al. 2004). These findings indicate that the generation of ROS is tightly regulated by a signal transduction network, and is an integral part of fruiting-body formation (see also Chap. 10, this volume).

2. Pheromones

In heterothallic ascomycetes, mating and subsequent fruiting-body development occur only after fusion of mycelial structures of opposite mating type. In *N. crassa*, diffusible pheromones have been suggested to be involved in the mating process, 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, thus suggesting that the mating-type locus regulates the pheromone production (Bistis 1981). Pheromone precursor genes encoding two different types of pheromones have been isolated from the heterothallic filamentous ascomycetes *Cryphonectria parasitica*, *Magnaporthe grisea*, *N. crassa* and *P. anserina*, as well as from the homothallic ascomycete *S. macrospora* (Zhang et al. 1998; Shen et al. 1999; Pöggeler 2000; Bobrowicz et al. 2002; Coppin et al. 2005). One of the precursor genes encodes a polypeptide containing multiple repeats of a putative pheromone sequence bordered by protease processing sites, and resembles the α-factor precursor gene of *S. cerevisiae* (Fig. 16.3). The other gene encodes a short polypeptide similar to the *S. cerevisiae* **a**-factor precursor. The short precursor has 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 carboxy methyl isoprenylated cysteine (Fig. 16.3). The two types of pheromone precursor genes are present in the same nucleus. In heterothallic ascomycetes, pro-

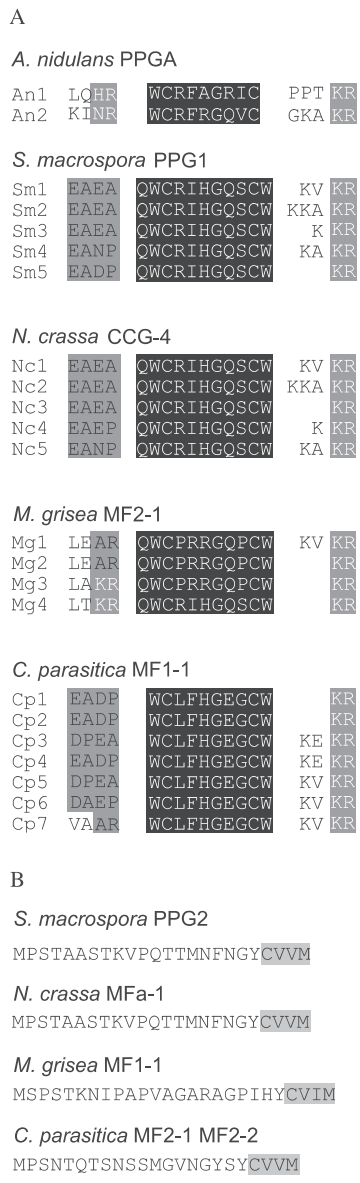

Fig. 16.3. **A**,**B** Pheromones of filamentous ascomycetes. **A** Sequences of predicted α-factor-like pheromones from filamentous ascomycetes. Repeats are shown in *white* and *boxed in black*, Kex2 processing sites (KR) in *white* and *boxed in gray*, STE13 processing sites in *black and boxed in gray*. **B** Sequences of predicted **a**-factor-like pheromones from filamentous ascomycetes. The prenylation signal motifs (CaaX) are *boxed in grey*

duction of either pheromone is directly controlled by transcription factors encoded by mating-type genes, and expression of pheromone genes seems to occur in a mating type-specific manner (see Debuchy and Turgeon, Chap. 15, this volume; Herskowitz 1989; Zhang et al. 1998; Shen et al. 1999; Bobrowicz et al. 2002; Coppin et al. 2005).

In contrast to all other hyphal ascomycetes, only one pheromone precursor gene, encoding a hydrophilic pheromone similar to the *S. cerevisiae* α-factor, was identified in the homothallic *Aspergillus nidulans* (Dyer et al. 2003).

In the heterothallic *N. crassa*, it was shown that pheromone precursor genes are highly expressed under conditions that favor sexual development (Bobrowicz et al. 2002). Interestingly, an elevated transcript level of the *N. crassa mfa-1* gene encoding the hydrophobic lipopeptide pheromone was observed in 7–9 day old perithecia (Kim et al. 2002a).

Furthermore, the expression of the *N. crassa* pheromone genes is regulated by the endogenous circadian clock in a time-of-day specific fashion. Both genes are repressed by RCO-1, a homolog of the *S. cerevisiae* transcriptional co-repressor Tup1p (Bobrowicz et al. 2002).

Recently, it was demonstrated that male and female fertility of heterothallic mycelial ascomycetes depends on interactions of pheromones with their specific receptors. When pheromone genes were deleted, spermatia were no longer able to fertilize the female partner, proving that pheromones are crucial for the fertility of male spermatia (Kim et al. 2002a; Turina et al. 2003; Coppin et al. 2005). In *P. anserina*, the function of pheromones is restricted to fertilization, while the *N. crassa* lipopeptide-pheromone gene *mfa-1* has also been shown to be involved in female sexual development, ascospore production, and vegetative growth of both mating types (Kim et al. 2002a; Coppin et al. 2005).

Similarly, in *C. parasitica*, deletion of only one of the two copies of CaaX-type pheromone genes (*Mf2-2*) was sufficient to prevent female fertility (Zhang et al. 1993). It was therefore speculated that *Mf2-2* of *C. parasitica* is required for a developmental phase after fertilization, and that the CaaX-type pheromone acts in a dosage-specific manner in post-fertilization events (Turina et al. 2003). Kim et al. (2002a) postulated an additional role for CaaX-type pheromones, in "conglutination" in perithecial development, or in the cementation of hyphae to stabilize the sclerotial structure of the maturing perithecium.

In contrast to the heterothallic mycelial ascomycetes *N. crassa*, *M. grisea* and *C. parasitica*, in the homothallic *S. macrospora* both pheromone precursor genes encoding structurally different pheromones are expressed in the same mycelium during the entire life cycle (Pöggeler 2000). It has recently been demonstrated that the disruption of the *S. macrospora ppg1* gene, encoding the α-factor-like peptide pheromone, prevents production of the peptide pheromone. However, this affects neither vegetative growth nor fruiting-body and ascospore development (Mayrhofer and Pöggeler 2005).

III. Signal Transduction Cascades

The molecular mechanisms underlying the development of fruiting bodies in ascomycetes are only poorly understood. However, there is increasing evidence that the external and internal stimuli are linked to genetically programmed cellular events. Usually, protein-mediated transduction of signals from the cell membrane to the nucleus is responsible for changes in gene expression. Components supposedly involved in signal transduction pathways can be subdivided into (1) receptors that percept the signal, (2) components that transmit the signal into the cell, and (3) nuclear transcription factors regulating gene expression. In the ascomycetous yeast *S. cerevisiae*, key components of the signaling cascade from the cell surface to the nucleus have been genetically characterized. In recent years, it has became evident that principles elucidated in yeast are applicable also to the more complex developmental programs of mycelial ascomycetes (Lengeler et al. 2000). However, analysis of the *N. crassa* genome demonstrated that filamentous ascomycetes encode classes of sensing molecules not found in *S. cerevisiae*, suggesting that alternative signaling cascades are involved in development processes of mycelial ascomycetes (Borkovich et al. 2004).

A. Perception of Environmental and Endogenous Signals

Central to many signaling pathways in eukaryotes is a cell surface receptor, which percepts an external chemical stimulus. Among the best characterized signaling systems are those mediated by guanine nucleotide binding (G)-protein-coupled seven-transmembrane-spanning receptors (GPCRs;

Neves et al. 2002). Typically, a ligand-bound GPCR activates or inhibits heterotrimeric G proteins, and transmits the signal to downstream effectors including adenylyl cyclases and protein kinases. Genome sequence analysis has shown that *N. crassa* possesses at least 10 predicted seven-transmembrane helix proteins that are potential G-PRCs (Galagan et al. 2003; Borkovich et al. 2004). Three of these were characterized at the molecular level. The *nop-1* gene encodes a seven-transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Analysis of *nop-1* deletion strains did not reveal obvious defects in light-regulated processes or fruiting-body development under normal laboratory conditions (Bieszke et al. 1999).

Two genes, designated *pre-1* and *pre-2*, encoding putative pheromone receptor genes similar to *S. cerevisiae* **a**-factor receptor (Ste3p) and α-factor receptor (Ste2p), respectively, have been identified in *N. crassa* and *S. macrospora* (Pöggeler and Kück 2001). Using a heterologous yeast system, it has been shown that the *S. macrospora* receptor PRE2 facilitates all aspects of the yeast pheromone response in *S. cerevisiae MAT***a** cells lacking the Ste2p receptor, when activated by the *S. macrospora* peptide pheromone. Therefore, one may conclude that the receptor encoded by the *pre2* gene functions as a GPCR in *S. macrospora*, too (Mayrhofer and Pöggeler 2005).

Northern and reverse transcription-polymerase chain reaction analyses indicate that in the heterothallic *N. crassa*, in contrast to pheromone precursor genes, expression of the receptor genes does not occur in a mating type-specific manner (Pöggeler and Kück 2001; Kim and Borkovich 2004). Recently, Kim and Borkovich (2004) demonstrated that deletion of the *N. crassa pre-1* gene does not affect vegetative growth or male fertility. However, protoperithecia from *Δpre1 mat A* mutants were shown to be female-sterile, because their trichogynes are unable to recognize and fuse with *mat a* cells. In the genome of the homothallic *A. nidulans*, nine genes (*gprA-gprI*) for potential seven-transmembrane spanning G-PRCs have been identified. Six of nine putative GPCRs have been disrupted and three genes, *gprA*, *gprB* and *gprD*, were found to play a central role in coordinating hyphal growth and sexual development (Han et al. 2004; Seo et al. 2004). Putative G-PRCs similar to the *S. cerevisiae* pheromone receptors Ste2p and Ste3p were shown to be encoded by *gprA* and *gprB*, respectively. Deletion of *gprA* or *gprB* resulted in the production of a few small cleistothecia carrying a reduced number of ascospores. Under homothallic conditions, an *A. nidulans* double-receptor-knockout strain *ΔgprA/ΔgprB* was completely abolished in fruiting-body and ascospore formation. Interestingly, outcrossing of *A. nidulans* receptor-mutant strains (*ΔgprA/ΔgprB*×*ΔgprA/ΔgprB*) resulted in fruiting-body and ascospore formation at wild-type level, suggesting that in *A. nidulans* the pheromone receptors GprA/B are specifically required for self-fertilization, and not for sexual development per se (Seo et al. 2004). By contrast, deletion of the GPRD-encoding *gprD* gene causes extremely restricted hyphal growth, delayed conidial germination, and uncontrolled activation of sexual development. Since elimination of sexual development rescues both growth and developmental abnormalities in *ΔgprD* strains, it was suggested that the primary role of GprD is to negatively regulate sexual development (Han et al. 2004). Moreover, it was demonstrated that deletion of pheromone receptor genes *gprA* and or *gprB* suppressed growth defects caused by the deletion of the *gprD* gene. This result implies that pheromone receptors GprA and GprB function downstream of GprD-mediated negative control of sexual development (Seo et al. 2004).

As has been described in the section above, fruiting-body development in filamentous ascomycetes is influenced by a variety of environmental stimuli and endogenous factors. In *N. crassa*, known elements in light sensing involved in fruiting-body development include the white collar complex, which acts as a blue-light photoreceptor as well as a transcription factor to regulate transcription of target genes in a light-dependent manner (see Sect. II.A.2, and Corrochano and Galland, Chap. 13, this volume). In addition to these two proteins, numerous other putative light-sensing genes have been identified in the genomic sequence of *N. crassa*, but their role in fruiting-body development has still to be elucidated (Borkovich et al. 2004).

Other environmental signals influencing fruiting-body development, such as osmolarity, nutrient levels, oxygen levels, and cellular redox status, might be sensed by two-component signal transduction pathways.

Two-component regulatory systems are composed of an autophosphorylating sensor histidine kinase and a response regulator. In contrast to yeasts, filamentous ascomycetes encode an extensive family of two-component signaling proteins

(Catlett et al. 2003). Eleven genes encoding putative histidine kinases have been identified in the *N. crassa* genome. So far, only two of them, *nik-1*/*os-1* and *nik-2*, have been characterized, but seem not to be involved in fruiting-body development. Whereas NIK-1 is an osmosensing histidine kinase that plays an important role in the regulation of cell wall assembly and cell responses to changes in external osmolarity, *nik-2* deletion mutants exhibit no obvious phenotypes (Alex et al. 1996; Schumacher et al. 1997; Borkovich et al. 2004). Similarly, two histidine kinases analyzed in *A. nidulans* were either shown to be involved in the formation of asexual spores, or disruption yielded no obvious clues to their function (Virginia et al. 2000; Furukawa et al. 2002). The involvement of the other two-component systems in ascocarp development awaits testing.

B. Signal Transduction Pathways

In response to extracellular stimuli, two cytoplasmic signaling branches defined by cAMP-dependent protein kinases (PKA) and mitogen-activated protein kinases regulate gene expression that finally leads to ascocarp formation (Lengeler et al. 2000). Upstream of these two signaling cascades, either heterotrimeric G proteins or ras and ras-like proteins act to relay extracellular ligand-stimulated signals to the cytoplasm. In addition to these four classes of proteins, several highly conserved proteins of unknown function have been identified by complementation of fungal developmental mutants (Fig. 16.4). These proteins are supposedly part of signal transduction pathways that lead to ascocarp formation (Table 16.1).

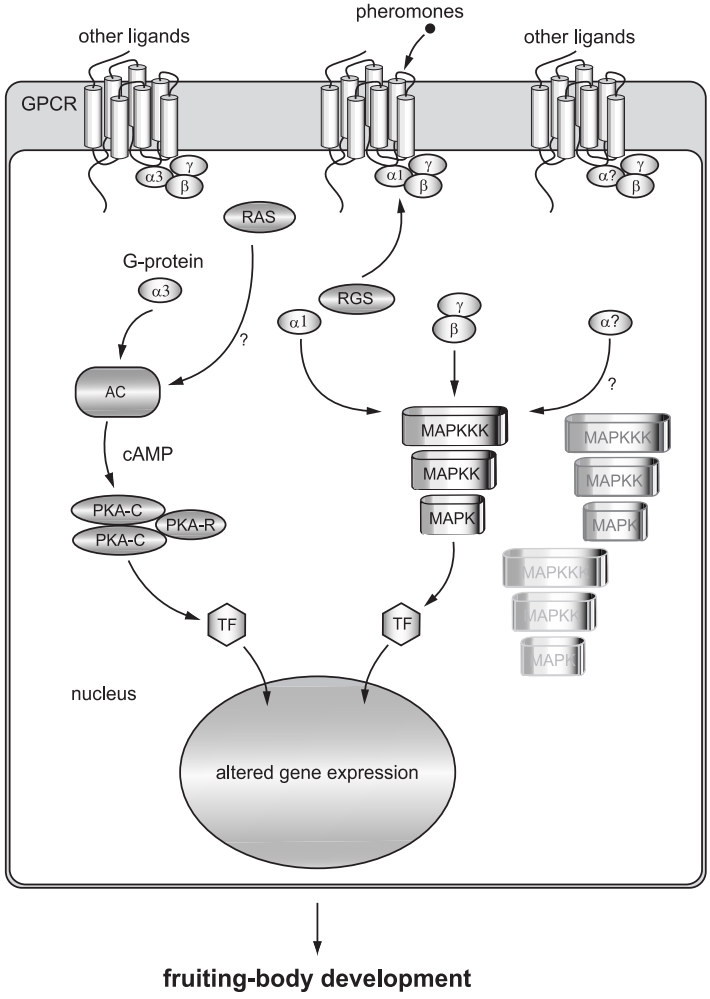

Fig. 16.4. Signal transduction pathways leading to fruiting-body development in filamentous ascomycetes

1. Heterotrimeric G Proteins

Upon activation of G-PRC receptors, heterotrimeric G proteins catalyze the exchange of GDP for GTP on the G protein α subunit, which leads to its dissociation from the βγ subunits. Either Gα, or Gβγ, or both are then free to activate downstream effectors (Dohlman 2002). In *N. crassa* and *A. nidulans* as well as other filamentous ascomycetes, it was demonstrated that subunits of G proteins are important for hyphal growth, conidiation, and fruiting-body development (Table 16.1). The genome of *N. crassa* contains three genes (*gna-1*, *gna-2* and *gna-3*) encoding Gα subunits, a single gene encoding the Gβ (*gnb-1*) subunit, and a single gene encoding the Gγ (*gng-1*) subunit (Borkovich et al. 2004). During the sexual cycle, GNA-1 is necessary for female fertility, whereas GNA-3, which is also important for asexual sporulation, mediates ascospore maturation (Ivey et al. 1996; Yang and Borkovich 1999; Kays et al. 2000). Loss of *gna-2* has no obvious effect on *N. crassa* growth and development; however, the double mutant *Δgna-1/Δgna-2* was shown to have more pronounced defects in female fertility than was the case for *Δgna-1* strains. This suggests that *gna-1* and *gna-2* have overlapping functions in sexual development (Baasiri et al. 1997; Kays and Borkovich 2004). *Δgnb-1* and *Δgng-1* strains of *N. crassa* are able to function as males during crosses with wild-type strains. When used as female parents, however, protoperithecia do not develop normally after fertilization, and they produce only small perithecia with aberrant fertilized reproductive structures (Yang et al. 2002; Krystofova and Borkovich 2005).

In the closely related pyrenomycete *Podospora anserina*, the *mod-D* gene encoding a Gα subunit and cloned as a suppressor of nonallelic heterokaryon incompatibility was shown to be involved in protoperithecial development (Loubradou et al. 1999).

Similar to *N. crassa*, the rice pathogen *Magnaporthe grisea* encodes three Gα subunits. Female fertility is mediated by MAG-B, whereas the other two subunits MAG-A and MAG-C are required for the production of mature asci (Liu and Dean 1997; Fang and Dean 2000). Targeted disruption of two Gα subunit genes in the chestnut blight fungus *Cryphonectria parasitica* revealed roles for the subunit CPG-1 in fungal reproduction, virulence, and vegetative growth. Disruption of the second Gα subunit gene, *cpg-2*, was found to enhance perithecial development (Gao and Nuss 1996). Mutation of the Gα subunit gene *cga-1* of the maize pathogen *Cochliobolus heterostrophus* leads to defects in several developmental pathways. Conidia from *cga-1* mutants germinate as abnormal, straight-growing germ tubes that form few appressoria, and the mutants are female-sterile (Horwitz et al. 1999; Degani et al. 2004). In *A. nidulans*, the Gα subunit FadA, along with the Gβ subunit SfdA, was shown to be an active participant in signaling pathways that govern critical decisions in the ascocarp development. *A. nidulans* strains carrying a deletion of the *fadA* gene fail to form cleistothecia. By contrast, strains carrying a dominant-negative mutation in the *fadA* ($fadA^{G203R}$) gene display an increase in Hülle cell formation but no cleistothecia formation. A similar phenotype was observed when the *sfaD* gene was deleted (Rosén et al. 1999). In addition to FadA, FlbA, a member of the regulator of G protein signalling (RGS) proteins that function as GTPase activating proteins, was shown to be involved in cleistothecia formation in *A. nidulans* (Han et al. 2001).

2. RAS and RAS-Like Proteins

RAS and RAS-like proteins are small GTP-binding proteins, which reside on the inner surface of the plasma membrane. They are able to transduce signals to the cytoplasmic signaling cascades, and control a variety of essential cellular processes (Vojtek and Der 1998).

Small GTP-binding proteins are generally grouped into five subfamilies named after their prototypical member: RAS, RHO, RAN, RAB, and ARF. The RAS superfamily of GTP-binding proteins comprises over 100 members, which act as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state (Takai et al. 2001).

The pyrenomycete *N. crassa* encodes two RAS proteins and three RAS-like proteins (Borkovich et al. 2004). To date, an involvement in ascocarp development has been demonstrated only for the RAS-like *N. crassa* protein KREV-1, a homolog of the mammalian RAP protein, and the *S. cerevisiae* Rsp1p protein. Disruption of the *N. crassa krev-1* gene by RIP was not correlated with growth abnormalities, but mutants overexpressing a constitutively active KREV-1 were shown to have defects in the sexual cycle. Mutations that are thought to lock the KREV-1 protein into the active GTP-bound form completely inhibit the development of protoperithecia into perithecia, whereas a dominant

negative mutation of KREV-1 resulted in some enlargement of protoperithecia (Ito et al. 1997).

In *A. nidulans*, mutations in the *rasA* gene cause aberrations in conidial germination and asexual development, but the role of RAS- and RAS-like proteins in cleistothecium formation has not yet been determined (Som and Kolaparthi 1994; Osherov and May 2000; Fillinger et al. 2002).

3. cAMP Activated Protein Kinases PKA

Activated RAS, RAS-like proteins, Gα or Gβγ subunits can regulate downstream effectors such as adenylyl cyclase and mitogen-activated protein kinase (MAPK) cascades (see Sect. III.B.4). Adenylyl cyclase is a membrane-bound enzyme that produces cyclic AMP (cAMP) from ATP. cAMP is a ubiquitous secondary messenger in prokaryotic and eukaryotic cells. In filamentous ascomycetes, cAMP signaling is involved in such diverse cellular processes as stress response, metabolism, pathogenicity, and sexual development (Kronstad et al. 1998; Lengeler et al. 2000). A well-characterized intracellular target of cAMP is the regulatory subunit of protein kinase A (PKA). PKA is a tetrameric enzyme that is composed of two regulatory subunits and two catalytic subunits. Binding of cAMP to the regulatory subunits releases the catalytic subunits. The latter phosporylate target proteins involved in cAMP-regulated processes (Dickman and Yarden 1999; Taylor et al. 2004).

In the plant pathogen *M. grisea*, it was shown that the sexual cycle is dependent on cAMP. *M. grisea* mutants lacking the adenylyl cyclase gene *mac-1* are female-sterile, and mate only when exogenous cAMP is supplied (Choi and Dean 1997; Adachi and Hamer 1998). By contrast, the *N. crassa cr-1* mutant, lacking adenylyl cyclase activity and cAMP, is able to function as male and female partner in sexual crosses, but exhibits delayed perithecial and ascospore production, compared to the wild type (Perkins et al. 1982; Ivey et al. 2002).

Female sterility of the *cr-1* mutant was observed only in a *Δgna-1* background. Unlike the *Δgna-1* mutant, the *Δgna-1*/*Δcr-1* double mutant does not form protoperithecia, suggesting that cAMP may be required for protoperithecial development. A recent study explored the contribution of all three Gα subunits on the cAMP level. The effects of mutating *gna-1* and *gna-3* were shown to be additive with respect to the adenylyl cyclase activity, whereas loss of *gna-2* did not appreciably affect adenylyl cyclase activity (Kays and Borkovich 2004).

The involvement of catalytic and regulatory subunits of PKA has so far been demonstrated only with respect to the asexual development, hyphal growth, and pathogenicity of *N. crassa*, *A. nidulans* and *M. grisea*, respectively (Mitchell and Dean 1995; Bruno et al. 1996; Fillinger et al. 2002). However, effects of PKA on fruiting-body formation have not yet been analyzed. Due to the presence of genes in the *N. crassa* genome encoding G-PRCs similar to slime mold cAMP receptors, it was recently suggested that cAMP may also serve as an environmental signal and G-PRC ligand in *N. crassa* (Borkovich et al. 2004).

4. Mitogen-Activated Protein Kinase (MAPK) Pathways

Mitogen-activated protein kinase (MAPK) pathways regulate eukaryotic gene expression in response to extracellular stimuli. The basic assembly of MAPK pathways is a three-component module conserved from yeast to humans.

The MAPK module includes three kinases that establish a sequential activation pathway comprising a MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK (Widmann et al. 1999). MAPK is activated by phosphorylation on conserved tyrosine and threonine residues by MAKK. In turn, MAPKK is activated by phosphorylation on conserved serine and threonine residues by MAPKKK. MAPKKK become activated by phosphorylation in response to various extracellular stimuli. The activated MAPK can translocate to the nucleus where it phosphorylates, and thus activates transcription factors (Dickman and Yarden 1999).

In the filamentous ascomycete *N. crassa*, three MAPK modules have been identified by genome sequence analysis (Borkovich et al. 2004). These correspond to those for pheromone response/filamentation, osmosensing/stress and cell integrity pathways in the yeasts *S. pombe* and *S. cerevisiae*. Three different MAPKs and two different MAPKKKs have been shown to be involved in fruiting-body development in different mycelial ascomycetes (Table 16.1). Mutation of the corresponding genes always leads to multiple phenotypic defects, including defects in ascocarp formation. Deletion of the *N. crassa mak-2* gene encoding a Fus3p MAPK was shown to result in loss of hyphal fusion and female sterility (Pandey et al. 2004; Li et al. 2005).

The MAPK Hog1p plays an essential role in osmotic stress signaling and osmoadaptation in the

yeast *S. cerevisiae* (Hohmann 2002). In *A. nidulans*, the Hog1p homolog SakA/HogA was demonstrated to be involved not only in stress signal transduction but also in sexual development. *A. nidulans ΔsakA* mutants display premature sexual development, and produce two times more cleistothecia at least 24 h earlier than wild-type strains (Kawasaki et al. 2002). In the plant pathogens *M. grisea* and *Fusarium graminearum*, it was demonstrated that homologs of the *S. cerevisiae* Slt2p MAPK are required for plant infection, and also for female fertility (Xu et al. 1998; Hou et al. 2002).

The MAPKKK NRC-1 of *N. crassa* functions to repress the onset of conidiation and is required for female fertility, because a *N. crassa nrc-1* mutant is unable to make protoperithecia (Kothe and Free 1998). It was recently demonstrated by Pandey et al. (2004) that an *nrc-1* mutant shares many of the same phenotypic traits as the *mak-2* mutant, and is also a hyphal fusion mutant, implying that NRC-1 acts upstream of MAK-2 during fruiting-body development. Similarly to the *N. crassa* NRC-1, the *A. nidulans* SteC regulates conidiophore development and is essential for cleistothecial development (Wei et al. 2003).

5. Other Putative Signaling Proteins

In addition to the components of conventional signal transduction pathways, forward genetic screens in filamentous ascomycetes identified several conserved proteins that may play key roles in fruiting-body development, but have so far not been shown to be involved in signal transduction processes in other eukaryotes. During a screen for suppressors of vegetative incompatibility in *N. crassa*, Xiang et al. (2002) isolated a deletion mutant that displayed pleiotropic defects including suppression of vegetative incompatibility, altered conidiation pattern, lack of hyphal fusion, and female sterility. A single gene termed *ham-2* was shown to complement hyphal fusion and female fertility, but not vegetative incompatibility and condiation pattern. The *ham-2* gene encodes a putative transmembrane protein that is highly conserved, but of unknown function among eukaryotes. Since the *ham-2* mutant forms only few protoperithecial-like structures, it was speculated that a HAM-2-dependent hyphal fusion process may be required for the formation of female reproductive structures (Xiang et al. 2002). In addition, the *S. cerevisiae* homolog of HAM-2, Far11p, was shown to be transcriptionally induced by exposure to pheromone, and to participate in the signaling pathway leading to pheromone-mediated G1 arrest (Kemp and Sprague 2003).

Similarly, in the related pyrenomycete *Podospora anserina*, there is evidence for a link between genes involved in incompatibility and perithecial development. A screen for suppressors of vegetative incompatibility led to the isolation of the *P. anserina mod-A* gene, which is not only responsible for growth arrest in the self-incompatible strains, but also involved in the control of the development of female organs. The MODA-encoded polypeptide is rich in proline residues, which are clustered in a domain containing a motif that displays similarity to SH3-binding motifs (Barreau et al. 1998).

In the homothallic pyrenomycete *Sordaria macrospora*, the sterile mutant pro11 carries a defect in the *pro11* gene encoding a multimodular WD40-repeat protein. PRO11 shows significant homology to several vertebrate WD40 proteins, such as striatin or zinedin, which seem to be involved in Ca^{2+}-dependent signaling in cells of the central nervous system, and supposedly function as scaffolding proteins linking signaling and eukaryotic endocytosis. Most importantly, a cDNA encoding the mouse striatin caused a functional substitution of the *S. macrospora* wild-type gene with a restoration of fertility in the *pro11* mutant, suggesting that an evolutionary conserved cellular process in eukaryotic cell differentiation may regulate fruiting-body formation (Pöggeler and Kück 2004).

C. Transcription Factors

From the preceding section, it is evident that diverse signal transduction pathways interpret environmental or intrinsic signals. Eventually, signal transduction initiates morphogenesis by activating transcription factors that in turn activate or repress cell- or tissue-specific expression of morphogenetic genes. Most transcription factors involved in ascocarp formation have been identified in *A. nidulans* (Table 16.1). Some of these are thought to be direct targets of signaling pathways.

In *A. nidulans*, SteA, a homolog of the *S. cerevisiae* homeodomain transcription factor Ste12p, was isolated and shown to be required for cleistothecia formation. In the budding yeast, Ste12p plays a key role in coupling signal transduction

through MAP kinase modules to cell-specific or morphogenesis-specific gene expression required for mating and pseudohyphal filamentous growth. An *A. nidulans ΔsteA* strain is sterile and differentiates neither ascogenous hyphae nor cleistothecia. However, the development of sexual cycle-specific Hülle cells and asexual conidiation is unaffected (Vallim et al. 2000). A similar phenotype has been observed in *N. crassa*. *pp-1* mutants of *N. crassa* fail to develop protoperithecia. In addition, ascospores carrying null mutations of the *pp-1* gene are nonviable (Li et al. 2005).

In a forward genetic screen of *A. nidulans*, Han et al. (2001) isolated the *nsdD* (*n*ever in *s*exual *d*evelopment) gene encoding a GATA-type transcription factor. Deletion of *nsdD* resulted in loss of cleistothecia or Hülle cell formation, even under conditions that preferentially promote sexual development, indicating that NsdD is necessary for ascocarp formation. Transcription factors StuA, MedA, and DopA are also required during sexual reproduction in *A. nidulans*. Whereas *ΔstuA* and *ΔdopA* strains fail to differentiate Hülle cells and cleistothecia, *ΔmedA* strains form extensive masses of unorganized Hülle cells, but fail to differentiate cleistothecia.

In contrast to *steA* and *nsdD*, *stuA*, *medA* as well as *dopA* are developmental modifier genes that are also involved in asexual development of *A. nidulans* (Busby et al. 1996; Dutton et al. 1997; Wu and Miller 1997; Pascon Castiglioni and Miller 2000). The *N. crassa* homolog of the *A. nidulans stuA* gene, *asm-1*, has also been shown to be involved in sexual development, since deletion of *asm-1* destroys the ability to make protoperithecia, but does not affect male-specific functions (Aramayo et al. 1996).

So far, information about the sexual regulatory network in *A. nidulans* is limited. However, Vallim et al. (2000) reported that *steA* lies upstream of *medA* in the same regulatory pathway, and that SteA directly or indirectly represses *medA* transcription (see Fischer and Kües, Chap. 14, this volume). Upregulation of *nsdD* resulted in the production of barren cleistothecia in the *ΔgprA*/*ΔgprB* double mutant. This result suggests that NsdD can partially rescue the developmental defects caused by the deletion of GPCRs, and that GprA/B-mediated signaling may activate other genes necessary for the maturation of cleistothecia (Seo et al. 2004). In turn, *nsdD* expression was shown to be under control of FadA-mediated signaling (Han et al. 2001). As summarized in Table 16.1, other transcription factors of different classes from hyphal ascomycetes have been identified that regulate fruiting-body formation. Amongst these is the C_6 zinc finger transcription factor PRO1, isolated in a forward genetic screen from the homothallic pyrenomycete *Sordaria macrospora*. The developmental mutant pro1 forms only protoperithecia, and is unable to perform the transition into mature perithecia (Masloff et al. 1999). Functional analysis of the PRO1 transcription factor revealed that the $Zn(II)_2Cys_6$ binuclear cluster is a prerequisite for the developmental function of the protein. Fertility of the *S. macrospora pro1* mutant can be restored by the *pro1* homolog from *N. crassa* (Masloff et al. 2002). Recently, it was shown that the putative $Zn(II)_2Cys_6$ transcription factor RosA from *A. nidulans*, sharing 38% sequence similarity to the *S. macrospora* PRO1, is a negative regulator of sexual development in *A. nidulans* (Vienken et al. 2004).

Besides the transcription factors described above, mating type-encoded transcription factors are also involved in fruiting-body development of filamentous ascomycetes (reviewed by Debuchy and Turgeon, Chap. 15, this volume). Mating proteins, as master regulatory transcription factors, control pathways of cell speciation as well as sexual morphogenesis in heterothallic and homothallic ascomycetes. They are not only necessary for fertilization, but are also required for subsequent development of the fertilized pre-fruiting bodies (Coppin et al. 1997; Pöggeler 2001).

IV. Structural Components Involved in Fruiting-Body Development

Fruiting-body formation involves the differentiation of many morphologically distinct cell types. Besides the cells that participate directly in karyogamy and meiosis, many more specialized cell types are formed that comprise the mature fruiting body. Of the 28 recognized cell types of *N. crassa*, 15 occur only during fruiting-body formation (Bistis et al. 2003). As the cell wall is the main form-giving structure of the fungal cell, it can be expected that enzymes involved in cell wall biogenesis and metabolism are required and coordinately regulated during sexual development. In addition, genes for cytoskeleton structure and organization have in some cases been shown to be specifically involved in sexual development, and these and the cell wall-related genes will be discussed in this section.

A. The Cytoskeleton in Fruiting-Body Development

Genes encoding components or regulators of the cytoskeleton have been shown to contribute to sexual development in *A. nidulans* and *P. anserina*. *A. nidulans* has two genes encoding α-tubulin, *tubA* and *tubB*. Whereas *tubA* is necessary for mitosis and nuclear migration, *tubB* is essential for ascospore formation (Kirk and Morris 1991). In *P. anserina*, it was shown that an ami1 mutant is male-sterile and displays delayed fruiting-body formation (Bouhouche et al. 2004). *ami1* encodes a homolog of the *A. nidulans apsA* gene, which is a protein necessary for nuclear positioning, most likely by regulating components of the dynein pathway. Thus, the cytoskeleton-related genes involved in sexual development have so far turned out to be important for nuclear migration and cell cycle events.

B. The Cell Wall in Fruiting-Body Development

The function of fruiting bodies is the protection and discharge of the ascospores, and this is achieved by developing morphologically distinct structures that are often characterized by cells with rigid and heavily pigmented cell walls. Thus, it has long been proposed that enzymes involved in cell wall biogenesis and metabolism are essential for sexual development in ascomycetes. Analyses have concentrated on several classes of genes considered to have a role in development, namely, genes involved in pigmentation, chitin synthases, lectins, hydrophobins, and genes involved in carbohydrate metabolism of the cell wall. Some of the results are described below (see also Chaps. 4 and 5, this volume).

1. Chitin

One of the characteristic components of the fungal cell wall is chitin, and central enzymes in chitin biosynthesis are chitin synthases. Fungi usually possess several chitin synthase genes, e.g., the genome of *N. crassa* contains seven chitin synthase genes (Borkovich et al. 2004). Chitin synthases that might be specifically required for fruiting-body formation have not been identified with certainty. However, chitin synthase genes that are expressed preferentially in fruiting-body tissue have been found in *A. nidulans* and *T. borchii*. In *A. nidulans*, the three chitin synthase genes *chsA*, *chsB* and *chsC* have been investigated. *chsA* was expressed solely during asexual sporulation, and *chsB* during all stages of growth and development, whereas *chsC* was expressed during early vegetative growth as well as sexual development. In the latter case, *chsC* expression was restricted to cleistothecia and ascospores (Lee et al. 2004). In *T. borchii*, expression of three genes for chitin synthases was analyzed in vegetative hyphae and fruiting bodies. All three were constitutively expressed in vegetative mycelium, but two of them were additionally expressed in fruiting bodies. One of these was found in sporogenic tissue, and the other in the vegetative tissue of the fruiting body (Balestrini et al. 2000). These examples indicate that, most likely, chitin synthases have specific but overlapping functions during different stages of fungal development.

2. Carbohydrates

After chitin, carbohydrates are another important constituent of the fungal cell wall, one main component being β-1,3-glucan (Walser et al. 2003). Apart from being structural elements, it has been proposed that carbohydrates are stored during vegetative growth to be mobilized as a carbon source for sexual development. One of these potential storage carbohydrates is α-1,3-glucan. It was shown to accumulate during vegetative growth of *A. nidulans*, and is degraded at the onset of sexual development (Zonneveld 1972). This finding correlates well with the fact that the gene for α-1,3-glucanase, *mutA*, is expressed during sexual development and degrades α-1,3-glucan to yield glucose.

Interestingly, MUTA is mainly found in Hülle cells whereas α-1,3-glucan is located mostly in the walls of cells other than Hülle cells. This might indicate that Hülle cells produce glucanase, which then degrades glucan in the cell walls of storage hyphae to yield carbohydrates that can be absorbed by the still intact Hülle cells (Zonneveld 1972; Wei et al. 2001).

However, *mutA* mutants are still able to form fruiting bodies, which indicates that other forms of carbohydrates can be used during fruiting-body development (Wei et al. 2001). One of these might be rhamnogalacturonan, as it has been shown that a putative rhamnogalacturonase encoded by the *asd-1* gene is essential for ascospore formation in *N. crassa* (Nelson and Metzenberg 1992; Nelson et al. 1997b). It is not yet clear whether rhamnogalacturonan inhibits sexual development and is degraded by ASD-1, or if the rhamnogalacturonan degradation products are needed for development.

The fact that fruiting-body formation requires high amounts of energy in the form of carbohydrates initially stored in vegetative hyphae, and subsequently released mostly in the extracellular space, would imply the existence of effective transport systems for nutrient uptake into the cells of the developing fruiting body. One such transporter for hexoses that is localized in ascogenous hyphae was described in *A. nidulans* (Wei et al. 2004). The gene for this transporter, *hxtA*, is not essential for sexual development, but as there are at least 17 putative hexose transporter genes in the *A. nidulans* genome, this might be due to the existence of functionally redundant proteins (Wei et al. 2004).

3. Pigments

Other common components of fungal cell walls are pigments, e.g., brown or black melanins. Melanins stabilize the cell wall and offer protection against UV light-induced DNA damage, but also have additional functions. For example, they are necessary for appressorial penetration of plant tissue in several fungal plant pathogens, and for pathogenicity of several human pathogens (Howard and Valent 1996; Perpetua et al. 1996; Jacobson 2000). Two biochemical pathways have been recognized for fungal melanin production, the DHN (dihydroxynaphtalene)- and the DOPA (dihydroxyphenylalanine)-melanin biosynthesis pathways (Langfelder et al. 2003). Both pathways contain laccases for the first (DOPA) or last (DHN) biosynthetic step, respectively. The DHN pathway involves a polyketide synthase in its initial step, but laccases and polyketide synthases also participate in a variety of biosynthetic pathways not necessarily involved in pigment formation. Laccase and polyketide synthase genes have been identified in several fungi, and have been implied in fruiting-body pigmentation of ascomycetes, but so far evidence for a genetic control of melanin biosynthesis in black or darkly pigmented fruiting bodies is missing.

It was shown for *A. nidulans* that laccase activity is located in young cleistothecia and Hülle cells, and that a sterile mutant had reduced laccase activity, but the corresponding laccase gene(s) have not yet been identified (Kurtz and Champe 1981; Hermann et al. 1983). In the plant pathogen *Nectria haematococca*, a polyketide synthase, PKSN, was shown to be essential for the synthesis of the red perithecial pigment by complementation of a mutant with white perithecia (Graziani et al. 2004). The mutant, however, is fertile. Therefore, the lack of perithecial pigmentation does not preclude ascospore maturation (Babai-Ahary et al. 1982), although it might be interesting to determine performance of the mutant strain under natural growth conditions.

4. Cell Wall Proteins

In addition to carbohydrates and pigment molecules, proteins comprise a sizeable part of the fungal cell wall. Two classes of proteins, hydrophobins and lectins, have been characterized extensively in higher basidiomycetes where they are implied in mushroom formation (see also Chap. 19, this volume). Hydrophobins can self-assemble at water/air interfaces, and form highly insoluble amphipathic films. These films can attach to the hydrophilic cellular surfaces, thereby orienting the hydrophobic side to the outside, which allows the fungus to break a water/air interface and grow into the air (Wösten et al. 1999). Lectins are carbohydrate-binding, mostly extracellular proteins that occur in virtually all classes of organisms. They have been implicated in the interaction of fungi with other organisms as well as fruiting-body formation in basidiomycetes (Walser et al. 2003). Genes encoding hydrophobins and lectins have been found in ascomycetes, too, but whether any of these play a role in sexual development is not yet clear.

Hydrophobins from *N. crassa*, *A. nidulans* and *M. grisea* have been shown to be involved in the formation of the water-repellent coat of conidiospores, but not in fruiting-body development (Stringer et al. 1991; Bell-Pedersen et al. 1992; Stringer and Timberlake 1995; Talbot et al. 1996).

As fungi can contain several members of the hydrophobin family in their genomes (Segers et al. 1999; Fuchs et al. 2004), addressing the question whether any of these are involved in fruiting-body morphogenesis might be a complex task involving the generation of mutants in more than one hydrophobin gene. Similarly to hydrophobins, lectins are thought to be involved in fruiting-body formation, but a requirement for lectins has yet to be shown in any fungus. The only described mutant in a lectin-encoding gene of an ascomycete is the aol mutant of *Arthrobotrys oligospora* (Balogh et al. 2003), but as no perfect stage of *A. oligospora* is known, the question of an involvement of AOL in sexual development cannot be addressed.

5. Are Multiple Genes with Overlapping Functions Involved in Cell Wall Metabolism?

The information about the molecular and genetic basis of cell wall metabolism during fruiting-body formation in ascomycetes is rather limited. A recurring theme are mutants in "candidate genes" that surprisingly do not have any discernible phenotype (see Sect. IV.B.2–4). Especially with the availability of whole genome sequences, it has become increasingly clear that one of the reasons for these findings might be the existence of large gene families most likely with partly overlapping functions. This does seem to be the case for most of the genes involved in the biosynthesis of cell wall components. A possible explanation for this might be the fact that the cell wall is a highly important structure for fungi – it is form-giving as well as protecting – but makes assessments of single components somewhat tedious. However, analysis of whole genome data, especially cross-species comparisons as well as large-scale expression studies, could help to identify candidates for future gene inactivation projects.

V. Conclusions

Fruiting-body formation in filamentous ascomycetes is a highly complex differentiation process that in some cases produces up to 15 different cell types. This complexity is further enhanced by morphogenic signals, such as light, temperature, and nutrients as well as species-specific cell communication factors such as pheromones and other signaling molecules. The many parameters determining this process may explain why several genes encoding G proteins, receptors, pheromones, and transcription factors have been identified as being involved in this developmental process. Mainly two different signal transduction pathways, MAPK cascades, and a cAMP-PKA cascade, function coordinately to regulate sexual cell differentiation processes by activating or repressing numerous transcription factors of different classes. Most probably, these regulate the transcription of genes encoding enzymes involved in cell wall biogenesis and metabolism, and genes for cytoskeleton structure and organization. Classical genetic studies have shown that ascocarp development involves a series of developmentally regulated genes. Therefore, it seems not surprising that currently no "main stream" signal transduction pathway is obvious that predominantly directs fruiting-body development in ascomycetes.

In summary, it has become evident that multicellular ascocarp development requires precise integration of a number of fundamental biological processes. The sexual pathway in ascomycetes provides a valuable experimental system for studying the mechanism and regulation of developmental processes that are usually more complex in animal and plant development. The analysis of fungal genomes is a starting point for further understanding developmental processes in mycelial fungi and other multicellular eukaryotes. Post-genomic analysis, including transcriptome and proteome assessments, will hopefully decipher components directing multicellular differentiation processes in ascomycetes, and finally as a long-term objective will help to understand eukaryotic differentiation processes at the molecular level.

Acknowledgements. We thank Dr. S. Masloff and Dr. S. Risch for microscopic images. The work of the authors is funded by the 'Deutsche Forschungsgemeinschaft' (DFG).

References

Adachi K, Hamer JE (1998) Divergent cAMP signaling pathways regulate growth and pathogenesis in the rice blast fungus *Magnaporthe grisea*. Plant Cell 10:1361–1374

Adams TH, Wieser JK, Yu J-H (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Alex LA, Borkovich KA, Simon MI (1996) Hyphal development in *Neurospora crassa*: involvement of a two-component histidine kinase. Proc Natl Acad Sci USA 93:3416–3421

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory mycology. Wiley, New York

Almeida T, Duarte M, Melo AM, Videira A (1999) The 24-kDa iron-sulphur subunit of complex I is required for enzyme activity. Eur J Biochem 265:86–93

Aramayo R, Peleg Y, Addison R, Metzenberg R (1996) *Asm-1*$^{+}$, a *Neurospora crassa* gene related to transcriptional regulators of fungal development. Genetics 144:991–1003

Arnaise S, Zickler D, Poisier C, Debuchy R (2001) *pah1*: a homeobox gene involved in hyphal morphology and microconidiogenesis in the filamentous ascomycete *Podospora anserina*. Mol Microbiol 39:54–64

Baasiri RA, Lu X, Rowley PS, Turner GE, Borkovich KA (1997) Overlapping functions for two G protein α subunits in *Neurospora crassa*. Genetics 147:137–145

Babai-Ahary A, Daboussi-Bareyre MJ, Parisot D (1982) Isolation and genetic analysis of self-sterility and perithecial pigmentation mutants in a homothallic isolate of *Nectria haematococca*. Can J Bot 60:79–84

Bailey LA, Ebbole DJ (1998) The *fluffy* gene of *Neurospora crassa* encodes a Gal4p-type C6 zinc cluster protein required for conidial development. Genetics 148:1813–1820

Balestrini R, Mainieri D, Soragni E, Garnero L, Rollino S, Viotti A, Ottonello S, Bonfante P (2000) Differential expression of chitin synthase III and IV mRNAs in ascomata of *Tuber borchii* Vittad. Fungal Genet Biol 31:291–232

Ballario P, Vittorioso P, Magrelli A, Talora C, Cabibbo A, Macino G (1996) White collar-1, a central regulator of blue-light responses in *Neurospora*, is a zinc finger protein. EMBO J 15:1650–1657

Balogh J, Tunlid A, Rosen S (2003) Deletion of a lectin gene does not affect the phenotype of the nematode-trapping fungus *Arthrobotrys oligospora*. Fungal Genet Biol 39:128–135

Barr ME (2001) Ascomycota. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota, vol 7A. Systematics and evolution, part A. Springer, Berlin Heidelberg New York, pp 161–177

Barreau C, Iskandar M, Loubradou G, Levallois V, Begueret J (1998) The mod-A suppressor of nonallelic heterokaryon incompatibility in *Podospora anserina* encodes a proline-rich polypeptide involved in female organ formation. Genetics 149:915–926

Bell-Pedersen D, Dunlap JC, Loros JJ (1992) The *Neurospora* circadian clock-controlled gene, *ccg-2*, is allelic to *eas* and encodes a fungal hydrophobin required for formation of the conidial rodlet layer. Genes Dev 6:2382–2394

Benjamin CR (1955) Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47:669–687

Berbee ML, Carmean DA, Winka K (2000) Ribosomal DNA and resolution of branching order among the Ascomycota: how many nucleotides are enough? Mol Phylogenet Evol 17:337–344

Berteaux-Lecellier V, Picard M, Thompson-Coffe C, Zickler D, Panvier-Adoutte A, Simonet JM (1995) A non-mammalian homolog of the PAF1 gene (Zellweger syndrome) discovered as a gene involved in caryogamy in the fungus *Podospora anserina*. Cell 81:1043–1051

Berteaux-Lecellier V, Zickler D, Debuchy R, Panvier-Adoutte A, Thompson-Coffe C, Picard M (1998) A homologue of the yeast SHE4 gene is essential for the transition between the syncytial and cellular stages during sexual reproduction of the fungus *Podospora anserina*. EMBO J 17:1248–1258

Bieszke JA, Braun EL, Bean LE, Kang S, Natvig DO, Borkovich KA (1999) The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Proc Natl Acad Sci USA 96:8034–8039

Bistis GN (1981) Chemotrophic interactions between trichogynes and conidia of the opposite mating type in *Neurospora crassa*. Mycologia 73:959–975

Bistis GN (1983) Evidence for diffusible mating-type specific trichogyne attractants in *Neurospora crassa*. Exp Mycol 7:292–295

Bistis GN, Perkins DD, Read ND (2003) Different cell types in *Neurospora crassa*. Fungal Genet Biol 50:17–19

Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ (2002) The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804

Borghouts C, Osiewacz HD (1998) GRISEA, a copper-modulated transcription factor from *Podospora anserina* involved in senescence and morphogenesis, is an ortholog of MAC1 in *Saccharomyces cerevisiae*. Mol Gen Genet 260:492–502

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Bouhouche K, Zickler D, Debuchy R, Arnaise S (2004) Altering a gene involved in nuclear distribution increases the repeat-induced point mutation process in the fungus *Podospora anserina*. Genetics 167:151–159

Bowman EJ, Kendle R, Bowman BJ (2000) Disruption of *vma-1*, the gene encoding the catalytic subunit of the vacuolar H(+)-ATPase, causes severe morphological changes in *Neurospora crassa*. J Biol Chem 275:167–176

Braus GH, Krappmann S, Eckert SE (2002) Sexual development in ascomycetes – fruit body formation of *Aspergillus nidulans*. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 215–244

Bruggeman J, Debets AJ, Wijngaarden PJ, deVisser JA, Hoekstra RF (2003) Sex slows down the accumulation of deleterious mutations in the homothallic fungus *Aspergillus nidulans*. Genetics 164:479–485

Bruggeman J, Debets AJ, Hoekstra RF (2004) Selection arena in *Aspergillus nidulans*. Fungal Genet Biol 41:181–188

Bruno KS, Aramayo R, Minke PF, Metzenberg RL, Plamann M (1996) Loss of growth polarity and mislocalization of septa in a *Neurospora* mutant altered in the regulatory subunit of cAMP-dependent protein kinase. EMBO J 15:5572–5582

Busby TM, Miller KY, Miller BL (1996) Suppression and enhancement of the *Aspergillus nidulans medusa* mutation by altered dosage of the *bristle* and *stunted* genes. Genetics 143:155–163

Busch S, Hoffmann B, Valerius O, Starke K, Düvel K, Braus GH (2001) Regulation of the *Aspergillus nidulans hisB* gene by histidine starvation. Curr Genet 38:314–322

Busch S, Eckert SE, Krappmann S, Braus GH (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 49:717–730

Bussink HJ, Osmani SA (1998) A cyclin-dependent kinase family member (PHOA) is required to link developmental fate to environmental conditions in *Aspergillus nidulans*. EMBO J 17:3990–4003

Calvo AM, Gardner HW, Keller NP (2001) Genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans*. J Biol Chem 276:25766–25774

Catlett NL, Yoder OC, Turgeon BG (2003) Whole-genome analysis of two-component signal transduction genes in fungal pathogens. Eukaryot Cell 2:1151–1161

Champe SP, Nagle DL, Yager LN (1994) Sexual sporulation. In: Martinelli SD, Kinghorn JR (eds) *Aspergillus*: 50 years on. Elsevier, Amsterdam, pp 429–454

Choi W, Dean RA (1997) The adenylate cyclase gene MAC1 of *Magnaporthe grisea* controls appressorium formation and other aspects of growth and development. Plant Cell 9:1973–1983

Claussen P (1912) Zur Entwicklungsgeschichte der Ascomyceten. *Pyronema confluens*. Zeitschr Bot 4:1–63

Contamine V, Zickler D, Picard M (2004) The *Podospora rmp1* gene implicated in nucleus-mitochondria crosstalk encodes an essential protein whose subcellular location is developmentally regulated. Genetics 166:135–150

Coppin E (2002) The *fle1* gene encoding a C_2H_2 zinc finger protein co-ordinates male and female sexual differentiation in *Podospora anserina*. Mol Microbiol 43:1255–1268

Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61:411–428

Coppin E, de Renty C, Debuchy R (2005) The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Eukaryot Cell 4:407–420

Davis R, Perkins DD (2002) *Neurospora*: a model of model microbes. Nat Rev Genet 3:7–13

Degani O, Maor R, Hadar R, Sharon A, Horwitz BA (2004) Host physiology and pathogenic variation of *Cochliobolus heterostrophus* strains with mutations in the G protein alpha subunit, CGA1. Appl Environ Microbiol 70:5005–5009

Degli Innocenti F, Russo VEA (1983) Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem Photobiol 37:49–51

Degli Innocenti F, Russo VEA (1984) Isolation of new white collar mutants of *Neurospora crassa* and studies on their behavior in the blue light-induced formation of protoperithecia. J Bacteriol 159:757–761

Dickman MB, Yarden O (1999) Serine/threonine protein kinases and phosphatases in filamentous fungi. Fungal Genet Biol 26:99–117

Dohlman HG (2002) G proteins and pheromone signaling. Annu Rev Physiol 64:129–152

Duarte M, Videira A (2000) Respiratory chain complex I is essential for sexual development in *Neurospora* and binding of iron sulfur clusters are required for enzyme assembly. Genetics 156:607–615

Duarte M, Mota N, Pinto L, Videira A (1998) Inactivation of the gene coding for the 30.4-kDa subunit of respiratory chain NADH dehydrogenase: is the enzyme essential for *Neurospora*? Mol Gen Genet 257:368–375

Dutton JR, Johns S, Miller BL (1997) StuAp is a sequence-specific transcription factor that regulates developmental complexity in *Aspergillus nidulans.* EMBO J 16:5710–5721

Dyer PS, Ingram DS, Johnstone K (1992) The control of sexual morphogenesis in the Ascomycotina. Biol Rev 67:421–458

Dyer PS, Ingram DS, Johnstone K (1993) Evidence for the involvement of linoleic acid and other endogenous lipid factors in perithecial development of *Nectria haematococca* mating population VI. Mycol Res 97:485–496

Dyer PS, Paoletti M, Archer DB (2003) Genomics reveals sexual secrets of *Aspergillus*. Microbiology 149:2301–2303

Eckert SE, Hoffmann B, Wanke C, Braus GH (1999) Sexual development of *Aspergillus nidulans* in tryptophan auxotrophic strains. Arch Microbiol 172:157–166

Eckert SE, Kübler E, Hoffmann B, Braus GH (2000) The tryptophan synthase-encoding *trpB* gene of *Aspergillus nidulans* is regulated by the cross-pathway control system. Mol Gen Genet 263:867–876

Ellis TT, Reynolds DR, Alexopoulus CJ (1973) Hülle cell development in *Emericella nidulans*. Mycologia 65:1028–1035

Esser K (1982) Cryptogams. Cambridge University Press, Cambridge

Esser K, Straub J (1958) Genetische Untersuchungen an *Sordaria macrospora* Auersw.: Kompensation und Induktion bei genbedingten Entwicklungsdefekten. Z Vererbungslehre 89:729–746

Fang EG, Dean RA (2000) Site-directed mutagenesis of the *magB* gene affects growth and development in *Magnaporthe grisea*. Mol Plant Microbe Interact 13:1214–1227

Fecke W, Sled VD, Ohnishi T, Weiss H (1994) Disruption of the gene encoding the NADH-binding subunit of NADH: ubiquinone oxidoreductase in *Neurospora crassa*. Formation of a partially assembled enzyme without FMN and the iron-sulphur cluster N-3. Eur J Biochem 220:551–558

Feng B, Haas H, Marzluf GA (2000) ASD4, a new GATA factor of *Neurospora crassa*, displays sequence-specific DNA binding and functions in ascus and ascospore development. Biochemistry 39:11065–11073

Fillinger S, Chaveroche MK, Shimizu K, Keller N, d'Enfert C (2002) cAMP and ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 44:1001–1016

Fischer R (2002) Conidiation in *Aspergillus nidulans*. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 59–86

Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. Science 297:815–819

Fuchs U, Czymmek KJ, Sweigard JA (2004) Five hydrophobin genes in *Fusarium verticillioides* include two required for microconidial chain formation. Fungal Genet Biol 41:852–864

Furukawa K, Katsuno Y, Urao T, Yabe T, Yamada-Okabe T, Yamada-Okabe H, Yamagata Y, Abe K, Nakajima T (2002) Isolation and functional analysis of a gene, *tcsB*, encoding a transmembrane hybrid-type histidine kinase from *Aspergillus nidulans*. Appl Environ Microbiol 68:5304–5310

Gagny B, Silar P (1998) Identification of the genes encoding the cytosolic translation release factors from *Podospora anserina* and analysis of their role during the life cycle. Genetics 149:1763–1775

Galagan JE, Selker EU (2004) RIP: the evolutionary cost of genome defense. Trends Genet 20:417–423

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S et al. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868

Gao S, Nuss DL (1996) Distinct roles for two G protein α subunits in fungal virulence, morphology, and reproduction revealed by targeted gene disruption. Proc Natl Acad Sci USA 93:14122–14127

Goodrich-Tanrikulu M, Howe K, Stafford A, Nelson MA (1998) Changes in fatty acid composition of *Neurospora crassa* accompany sexual development and ascospore germination. Microbiology 144:1713–1720

Goodrich-Tanrikulu M, Jacobson DJ, Stafford AE, Lin JT, McKeon TA (1999) Characterization of *Neurospora crassa* mutants isolated following repeat-induced

point mutation of the beta subunit of fatty acid synthase. Curr Genet 36:147–152
Graia F, Berteaux-Lecellier V, Zickler D, Picard M (2000) *ami1*, an orthologue of the *Aspergillus nidulans apsA* gene, is involved in nuclear migration events throughout the life cycle of *Podospora anserina*. Genetics 155:633–646
Graziani S, Vasnier C, Daboussi MJ (2004) Novel polyketide synthase from *Nectria haematococca*. Appl Environ Microbiol 70:2984–2988
Han DM, Han YK, Lee YH, Jahng KY, Jahng SH, Chae KS (1990) Inhibitory conditions of asexual development and their application for the screening of mutants defective in sexual development. Korean J Mycol 18:225–232
Han DM, Han YK, Kim JH, Jahng KY, Chung YS, Chae KS (1994) Isolation and characterization of NSD mutants in *Aspergillus nidulans*. Korean J Mycol 22:1–7
Han KY, Cheong SS, Hoe HS, Han DM (1998) Characterization of several NSD mutants of *Aspergillus nidulans* that never undergo sexual development. Korea J Genet 20:257–264
Han KH, Han KY, Yu JH, Chae KS, Jahng KY, Han DM (2001) The *nsdD* gene encodes a putative GATA-type transcription factor necessary for sexual development of *Aspergillus nidulans*. Mol Microbiol 41:299–309
Han KH, Seo JA, Yu JH (2004) A putative G protein-coupled receptor negatively controls sexual development in *Aspergillus nidulans*. Mol Microbiol 51:1333–1345
Harding RW, Melles S (1983) Genetic analysis of phototropism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. Plant Physiol 72:996–1000
He Q, Cheng P, Yang Y, Wang L, Gardner K, Liu Y (2002) White collar-1, a DNA binding transcription factor and a light sensor. Science 297:840–843
Hermann TE, Kurtz MB, Champe SP (1983) Laccase localized in Hülle cells and cleistothecial primordia of *Aspergillus nidulans*. J Bacteriol 154:955–964
Herskowitz I (1989) A regulatory hierarchy for cell specialization in yeast. Nature 342:749–757
Hock B, Bahn M, Walk RA, Nitschke U (1978) The control of fruiting body formation in the ascomycete *Sordaria macrospora* Auersw. by regulation of hyphal development. Planta 141:93–103
Hoffmann B, Wanke C, Lapaglia SK, Braus GH (2000) c-Jun and RACK1 homologues regulate a control point for sexual development in *Aspergillus nidulans*. Mol Microbiol 37:28–41
Hoffmann B, Eckert SE, Krappmann S, Braus GH (2001a) Sexual diploids of *Aspergillus nidulans* do not form by random fusion of nuclei in the heterokaryon. Genetics 157:141–147
Hoffmann B, Valerius O, Anderman M, Braus GH (2001b) Transcriptional autoregulation and inhibition of mRNA translation of amino acid regulator gene *cpcA* of filamentous fungus *Aspergillus nidulans*. Mol Biol Cell 12:2846–2857
Hohmann S (2002) Osmotic stress signaling and osmoadaptation in yeasts. Microbiol Mol Biol Rev 66:300–372
Horwitz BA, Sharon A, Lu SW, Ritter V, Sandrock TM, Yoder OC, Turgeon BG (1999) A G protein alpha subunit from *Cochliobolus heterostrophus* involved in mating and appressorium formation. Fungal Genet Biol 26:19–32
Hou Z, Xue C, Peng Y, Katan T, Kistler HC, Xu JR (2002) A mitogen-activated protein kinase gene (MGV1) in *Fusarium graminearum* is required for female fertility, heterokaryon formation, and plant infection. Mol Plant Microbe Interact 15:1119–1127
Howard RJ, Valent B (1996) Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. Annu Rev Microbiol 50:491–512
Ito S, Matsui Y, Toh-e A, Harashima T, Inoue H (1997) Isolation and characterization of the *krev-1* gene, a novel member of *ras* superfamily in *Neurospora crassa*: involvement in sexual cycle progression. Mol Gen Genet 255:429–437
Ivey FD, Hodge PN, Turner GE, Borkovich KA (1996) The $G\alpha_i$ homologue *gna-1* controls multiple differentiation pathways in *Neurospora crassa*. Mol Biol Cell 7:1283–1297
Ivey FD, Kays AM, Borkovich KA (2002) Shared and independent roles for a $G\alpha_i$ protein and adenylyl cyclase in regulating development and stress responses in *Neurospora crassa*. Eukaryot Cell 1:634–642
Jacobson ES (2000) Pathogenic roles for fungal melanins. Clin Microbiol Rev 13:708–717
Johnson TE (1978) Isolation and characterization of perithecial developmental mutants in *Neurospora*. Genetics 88:27–47
Kawasaki L, Sanchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45:1153–1163
Kays AM, Borkovich KA (2004) Severe impairment of growth and differentiation in a *Neurospora crassa* mutant lacking all heterotrimeric Gα proteins. Genetics 166:1229–1240
Kays AM, Rowley PS, Baasiri RA, Borkovich KA (2000) Regulation of conidiation and adenylyl cyclase levels by the Gα protein GNA-3 in *Neurospora crassa*. Mol Cell Biol 20:7693–7705
Kemp HA, Sprague GFJ (2003) Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast *Saccharomyces cerevisiae*. Mol Cell Biol 23:1750–1763
Kicka S, Silar P (2004) PaASK1, a mitogen-activated protein kinase kinase kinase that controls cell degeneration and cell differentiation in *Podospora anserina*. Genetics 166:1241–1252
Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52:1781–1798
Kim H, Metzenberg RL, Nelson MA (2002a) Multiple functions of *mfa-1*, a putative pheromone precursor gene of *Neurospora crassa*. Eukaryot Cell 1:987–999
Kim H, Kyu-Yong H, Kim KJ, Han DM, Jahng KY, Chae KS (2002b) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80
Kirk KE, Morris NR (1991) The *tubB* alpha-tubulin gene is essential for sexual development in *Aspergillus nidulans*. Genes Dev 5:2014–2023
Kirk PM, Cannon PF, David JC, Staplers JA (2001) Ainsworth and Bisby's dictionary of the fungi. CAB International, Wallingford, Oxon

Kothe GO, Free SJ (1998) The isolation and characterization of *nrc-1* and *nrc-2*, two genes encoding protein kinases that control growth and development in *Neurospora crassa*. Genetics 149:117–130

Kronstad J, Staben C (1997) Mating type in filamentous fungi. Annu Rev Genet 31:245–276

Kronstad J, de Maria AD, Funnell D, Laidlaw RD, Lee N, de Sa MM, Ramesh M (1998) Signaling via cAMP in fungi: interconnections with mitogen-activated protein kinase pathways. Arch Microbiol 170:395–404

Krystofova S, Borkovich KA (2005) The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a Gβγ dimer required for normal female fertility, asexual development, and Gα protein levels in *Neurospora crassa*. Eukaryot Cell 4:365–378

Kück U (2005) A *Sordaria macrospora* mutant, lacking the *leu1* gene, shows a developmental arrest during fruiting body formation. Mol Gen Genomics DOI:10.1007/s00438-005-0021-8

Kües U, Liu Y (2000) Fruiting body production in basidiomycetes. Appl Microbiol Biotechnol 54:141–152

Kurtz MB, Champe SP (1981) Dominant spore color mutants of *Aspergillus nidulans* defective in germination and sexual development. J Bacteriol 148:629–638

Kwon KJ, Raper KB (1967) Sexuality and cultural characteristics of *Aspergillus heterothallis*. Am J Bot 54:36–48

Lacourt I, Duplessis S, Abbà S, Bonfante P, Martin F (2002) Isolation and characterization of differentially expressed genes in the mycelium and fruit body of *Tuber borchii*. Appl Env Microbiol 68:4574–4582

Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38:143–158

Lara-Ortíz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol Microbiol 50:1241–1255

Lee DW, Kim S, Kim SJ, Han DM, Jahng KY, Chae KS (2001) The *lsdA* gene is necessary for sexual development inhibition by a salt in *Aspergillus nidulans*. Curr Genet 39:237–243

Lee JI, Choi JH, Park BC, Park YH, Lee MY, Park HM, Maeng PJ (2004) Differential expression of the chitin synthase genes of *Aspergillus nidulans*, *chsA*, *chsB*, and *chsC*, in response to developmental status and environmental factors. Fungal Genet Biol 41:635–646

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen WC, Wang P, Pan X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Leslie JF, Raju NB (1985) Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. Genetics 111:759–777

Lev S, Sharon A, Hadar R, Ma H, Horwitz BA (1998) A mitogen-activated protein kinase of the corn leaf pathogen *Cochliobolus heterostrophus* is involved in conidiation, appressorium formation, and pathogenicity: diverse roles for mitogen-activated protein kinase homologs in foliar pathogens. Proc Natl Acad Sci USA 96:13542–13547

Li D, Bobrowicz P, Wilkinson HH, Ebbole DJ (2005) A MAP kinase pathway essential for mating and contributing to vegetative growth in *Neurospora crassa*. Genetics (in press)

Linden H, Macino G (1997) White collar 2, a partner in blue-light signal transduction, controlling expression of light-regulated genes in *Neurospora crassa*. EMBO J 16:98–109

Lindenmuth R, Wirtz N, Lumbsch HT (2001) Phylogeneic analysis of nuclear and mitochondrial rDNA sequences supports the view that loculoascomycetes (Ascomycota) are not monophyletic. Mycol Res 105:1176–1181

Liu S, Dean RA (1997) G protein α-subunit genes control growth, development, and pathogenicity of *Magnaporthe grisea*. Mol Plant Microbe Interact 10:1075–1086

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16:1799–1808

Loros JJ, Dunlap JC (2001) Genetic and molecular analysis of circadian rhythms in *Neurospora*. Annu Rev Physiol 63:757–794

Loros JJ, Denome SA, Dunlap JC (1989) Molecular cloning of genes under the control of the circadian clock in *Neurospora*. Science 243:385–388

Loubradou G, Bégueret J, Turcq B (1997) A mutation in an *HSP90* gene affects the sexual cycle and suppresses vegetative incompatibility in the fungus *Podospora anserina*. Genetics 147:581–588

Loubradou G, Bégueret J, Turcq B (1999) MOD-D, a Gα subunit of the fungus *Podospora anserina*, is involved in both regulation of development and vegetative incompatibility. Genetics 152:519–528

Lumbsch HT (2000) Phylogeny of filamentous ascomycetes. Naturwissenschaften 87:335–342

Lumbsch HT, Lindemuth R, Schmitt I (2000) Evolution of filamentous ascomycetes interferred from LSU rRNA sequence data. Plant Biol 2:525–529

Malagnac F, Lalucque H, Lepere G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserina*. Fungal Genet Biol 41:982–997

Marshall MR, Hindal DF, MacDonald WL (1982) Production of perithecia in culture by *Ceratocystis ulmi*. Mycologia 74:376–381

Masloff S, Pöggeler S, Kück U (1999) The *pro1*$^+$ gene from *Sordaria macrospora* encodes a C_6 zinc finger transcription factor required for fruiting body development. Genetics 152:191–199

Masloff S, Jacobsen S, Pöggeler S, Kück U (2002) Functional analysis of the C_6 zinc finger gene *pro1* involved in fungal sexual development. Fungal Genet Biol 36:107–116

Mayrhofer S, Pöggeler S (2005) Functional characterization of an α-factor-like *Sordaria macrospora* peptide pheromone and analysis of its interaction with its cognate receptor in *Saccharomyces cerevisiae*. Eukaryot Cell 4:661–672

McCahill A, Warwicker J, Bolger GB, Houslay MD, Yarwood SJ (2002) The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. Mol Pharmacol 62:1261–1273

Metz AM, Haddad A, Worapong J, Long DM, Ford EJ, Hess WM, Strobel GA (2000) Induction of the sexual stage of *Pestalotiopsis microspora*, a taxol-producing fungus. Microbiology 146:2079–2089

Mitchell TK, Dean RA (1995) The cAMP-dependent protein kinase catalytic subunit is required for appressorium formation and pathogenesis by the rice blast pathogen *Magnaporthe grisea*. Plant Cell 7:1869–1878

Molowitz R, Bahn M, Hock B (1976) The control of fruiting body formation in the ascomycete *Sordaria macrospora* Auersw. by arginine and biotin: a two-factor analysis. Planta 128:143–148

Mooney JL, Yager LN (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge

Moore-Landecker E (1979) Effect of light regimens and intensities on morphogenesis of the discomycete *Pyronema domesticum*. Mycologia 71:699–712

Moore-Landecker E (1992) Physiology and biochemistry of ascocarp induction and development. Mycol Res 96:705–716

Müller F, Krüger D, Sattlegger E, Hoffmann B, Ballario P, Kanaan M, Barthelmeß IB (1995) The *cpc-2* gene of *Neurospora crassa* encodes a protein entirely composed of WD-repeat segments that is involved in general amino acid control and female fertility. Mol Gen Genet 248:162–173

Nelson MA, Metzenberg RL (1992) Sexual development genes of *Neurospora crassa*. Genetics 132:149–162

Nelson MA, Kang S, Braun EL, Crawford ME, Dolan PL, Leonard PM, Mitchell J, Armijo AM, Bean L, Blueyes E et al. (1997a) Expressed sequences from conidial, mycelial, and sexual stages of *Neurospora crassa*. Fungal Genet Biol 21:348–363

Nelson MA, Merino ST, Metzenberg RL (1997b) A putative rhamnogalacturonase required for sexual development of *Neurospora crassa*. Genetics 146:531–540

Neves SR, Ram PT, Iyengar R (2002) G protein pathways. Science 296:1636–1639

Nowrousian M, Masloff S, Pöggeler S, Kück U (1999) Cell differentiation during sexual development of the fungus *Sordaria macrospora* requires ATP citrate lyase activity. Mol Cell Biol 19:450–460

Nowrousian M, Kück U, Loser K, Weltring KM (2000) The fungal *acl1* and *acl2* genes encode two polypeptides with homology to the N- and C-terminal parts of the animal ATP citrate lyase polypeptide. Curr Genet 37:189–193

Nowrousian M, Dunlap JC, Nelson MA (2004) Functional genomics in fungi. In: Kück U (ed) The Mycota, vol II. Springer, Berlin Heidelberg New York, pp 115–128

Nukina M, Sassa T, Ikeda M, Takahashi K, Toyota S (1981) Linoleic acid enhances perithecial production in *Neurospora crassa*. Agric Biol Chem 45:2371–2373

Oda K, Hasunuma K (1997) Genetic analysis of signal transduction through light-induced protein phosphorylation in *Neurospora crassa* perithecia. Mol Gen Genet 256:593–601

Ogura Y, Yoshida Y, Yabe N, Hasunuma K (2001) A point mutation in nucleoside diphosphate kinase results in a deficient light response for perithecial polarity in *Neurospora crassa*. J Biol Chem 276:21228–21234

Osherov N, May G (2000) Conidial germination in *Aspergillus nidulans* requires RAS signaling and protein synthesis. Genetics 155:647–656

Osiewacz HD, Nuber U (1996) GRISEA, a putative copper-activated transcription factor from *Podospora anserina* involved in differentiation and senescence. Mol Gen Genet 252:115–124

Pandey A, Roca MG, Read ND, Glass NL (2004) Role of a mitogen-activated protein kinase pathway during conidial germination and hyphal fusion in *Neurospora crassa*. Eukaryot Cell 3:348–358

Pascon Castiglioni R, Miller BL (2000) Morphogenesis in *Aspergillus nidulans* requires Dopey (DopA), a member of a novel family of leucine zipper-like proteins conserved from yeast to humans. Mol Microbiol 36:1250–1264

Perkins DD, Davis R (2000) *Neursopora* at the millenium. Fungal Genet Biol 31:153–167

Perkins DD, Radford A, Newmeyer D, Bjorkman M (1982) Chromosomal loci of *Neurospora crassa*. Microbiol Rev 46:426–570

Perpetua NS, Kubo Y, Yasuda N, Takano Y, Furusawa I (1996) Cloning and characterization of a melanin biosynthetic THR1 reductase gene essential for appressorial penetration of *Colletotrichum lagenarium*. Mol Plant Microbe Interact 9:323–329

Pöggeler S (2000) Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. Curr Genet 37:403–411

Pöggeler S (2001) Mating-type genes for classical strain improvements of ascomycetes. Appl Microbiol Biotechnol 56:589–601

Pöggeler S, Kück U (2001) Identification of transcriptionally expressed pheromone receptor genes in filamentous ascomycetes. Gene 280:9–17

Pöggeler S, Kück U (2004) A WD40 repeat protein regulates fungal cell differentiation and can functionally be replaced by the mammalian homologue striatin. Eukaryot Cell 3:232–240

Pöggeler S, Nowrousian M, Jacobsen S, Kück U (1997) An efficient procedure to isolate fungal genes from an indexed cosmid library. J Microbiol Methods 29:49–61

Pontecorvo G (1953) The genetics of *Aspergillus nidulans*. In: Demerec M (ed) Advances in genetics. Academic Press, New York, pp 141–238

Raju BB (1992) Genetic control of the sexual cycle in *Neurospora*. Mycol Res 96:241–262

Randall TA, Metzenberg RL (1998) The mating type locus of *Neurospora crassa*: identification of an adjacent gene and characterization of transcripts surrounding the idiomorphs. Mol Gen Genet 259:615–621

Rosén S, Yu JH, Adams TH (1999) The *Aspergillus nidulans sfaD* gene encodes a G protein β-subunit that is required for normal growth and repression of sporulation. EMBO J 18:5592–5600

Scherer M, Wei H, Liese R, Fischer R (2002) *Aspergillus nidulans* catalase-peroxidase gene (*cpeA*) is transcriptionally induced during sexual development through the transcription factor StuA. Eukaryot Cell 1:725–735

Schumacher MM, Enderlin CS, Selitrennikoff CP (1997) The *osmotic-1* locus of *Neurospora crassa* encodes a putative histidine kinase similar to osmosensors of bacteria and yeast. Curr Microbiol 34:340–347

Segers GC, Hamada W, Oliver RP, Spanu PD (1999) Isolation and characterisation of five different hydrophobin-encoding cDNAs from the fungal tomato pathogen *Cladosporium fulvum*. Mol Gen Genet 261:644–652

Seo JA, Han KH, Yu JH (2004) The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol Microbiol 53:1611–1623

Shen WC, Bobrowicz P, Ebbole DJ (1999) Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet Biol 27:253–263

Silar P, Lalucque H, Haedens V, Zickler D, Picard M (2001) eEF1A controls ascospore differentiation through elevated accuracy, but controls longevity and fruiting body formation through another mechanism in *Podospora anserina*. Genetics 158:1477–1489

Som T, Kolaparthi VS (1994) Developmental decisions in *Aspergillus nidulans* are modulated by Ras activity. Mol Cell Biol 14:5333–5348

Stringer MA, Timberlake WE (1995) *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall. Mol Microbiol 16:33–44

Stringer MA, Dean RA, Sewall TC, Timberlake WE (1991) Rodletless, a new *Aspergillus* developmental mutant induced by direct gene inactivation. Genes Dev 5:1161–1171

Stumpferl SW, Stephan O, Osiewacz HD (2004) Impact of a disruption of a pathway delivering copper to mitochondria on *Podospora anserina* metabolism and life span. Eukaryot Cell 3:200–211

Swart K, van Heemst D, Slakhorst M, Debets F, Heyting C (2001) Isolation and characterization of sexual sporulation mutants of *Aspergillus nidulans*. Fungal Genet Biol 33:25–35

Takai Y, Sasaki T, Matozaki T (2001) Small GTP-binding proteins. Physiol Rev 81:153–208

Talbot NJ, Kershaw MJ, Wakley G, de Vries O, Wessels JG, Hamer JE (1996) *MPG1* encodes a fungal hydrophobin involved in surface interactions during infection-related development of *Magnaporthe grisea*. Plant Cell 8:985–999

Taylor SS, Yang J, Wu J, Haste NM, Radzio-Andzelm E, Anand G (2004) PKA: a portrait of protein kinase dynamics. Biochim Biophys Acta 11:259–269

Tsitsigiannis DI, Zarnowski R, Keller NP (2004) The lipid body protein, PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Turina M, Prodi A, Alfen NK (2003) Role of the *Mf1-1* pheromone precursor gene of the filamentous ascomycete *Cryphonectria parasitica*. Fungal Genet Biol 40:242–251

Vallim MA, Miller KA, Miller BL (2000) *Aspergillus* SteA (Sterile12-like) is a homeodomain-C_2/H_2-Zn^{2+} finger transcription factor required for sexual reproduction. Mol Microbiol 36:290–301

Van Heemst D, Swart K, Holub EF, van Dijk R, Offenberg HH, Goosen T, van den Broek HW, Heyting C (1997) Cloning, sequencing, disruption and phenotypic analysis of uvsC, an *Aspergillus nidulans* homologue of yeast RAD51. Mol Gen Genet 254:654–664

Van Heemst D, James F, Pöggeler S, Berteaux-Lecellier V, Zickler D (1999) Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programs. Cell 98:261–271

Videira A, Duarte M (2001) On complex I and other NADH:ubiquinone reductases of *Neurospora crassa* mitochondria. J Bioenerg Biomembr 33:197–203

Vienken K, Scherer M, Fischer R (2004) The $Zn(II)_2Cys_6$ putative *Aspergillus nidulans* transcription factor repressor of sexual development inhibits sexual development under low-carbon conditions and in submersed culture. Genetics 169:619–630

Virginia M, Appleyard CL, McPheat WL, Stark MJ (2000) A novel 'two-component' protein containing histidine kinase and response regulator domains required for sporulation in *Aspergillus nidulans*. Curr Genet 37:364–372

Vojtek AB, Der CJ (1998) Increasing complexity of the Ras signaling pathway. J Biol Chem 273:19925–19928

Walser PJ, Velagapudi R, Aebi M, Kües U (2003) Extracellular matrix proteins in mushroom development. Recent Res Dev Microbiol 7:381–415

Walz M, Kück U (1995) Transformation of *Sordaria macrospora* to hygromycin B resistance: characterization of transformants by electrophoretic karyotyping and tetrad analysis. Curr Genet 29:88–95

Wei H, Scherer M, Singh A, Liese R, Fischer R (2001) *Aspergillus nidulans* α-1,3 glucanase (mutanase), *mutA*, is expressed during sexual development and mobilizes mutan. Fungal Genet Biol 34:217–227

Wei H, Requena N, Fischer R (2003) The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol Microbiol 47:1577–1588

Wei H, Vienken K, Weber R, Bunting S, Requena N, Fischer R (2004) A putative high affinity hexose transporter, *hxtA*, of *Aspergillus nidulans* is induced in vegetative hyphae upon starvation and in ascogenous hyphae during cleistothecium formation. Fungal Genet Biol 41:148–156

Widmann C, Gibson S, Jarpe MB, Johnson GL (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 79:143–180

Wilson RA, Chang PK, Dobrzyn A, Ntambi JM, Zarnowski R, Keller NP (2004) Two Δ9-stearic acid desaturases are required for *Aspergillus nidulans* growth and development. Fungal Genet Biol 41:501–509

Wösten HA, van Wetter MA, Lugones LG, van der Mei HC, Busscher HJ, Wessels JG (1999) How a fungus escapes the water to grow into the air. Curr Biol 9:85–88

Wu J, Miller BL (1997) *Aspergillus* asexual reproduction and sexual reproduction are differentially affected by transcriptional and translational mechanisms regulating *stunted* gene expression. Mol Cell Biol 17:6191–6120

Wu D, Dou X, Hashmi SB, Osmani SA (2004) The Pho80-like cyclin of *Aspergillus nidulans* regulates development independently of its role in phosphate acquisition. J Biol Chem 279:37693–37703

Xiang Q, Rasmussen C, Glass NL (2002) The *ham-2* locus, encoding a putative transmembrane protein, is required for hyphal fusion in *Neurospora crassa*. Genetics 160:169–180

Xu JR, Staiger CJ, Hamer JE (1998) Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA 95:12713–12718

Yamashiro CT, Ebbole DJ, Lee BU, Brown RE, Bourland C, Madi L, Yanofsky C (1996) Characterization of *rco-1* of *Neurospora crassa*, a pleiotropic gene affecting growth and development that encodes a homolog of Tup1 of *Saccharomyces cerevisiae*. Mol Cell Biol 16:6218–6228

Yang Q, Borkovich KA (1999) Mutational activation of a $G\alpha_i$ causes uncontrolled proliferation of aerial hyphae and increased sensitivity to heat and oxidative stress in *Neurospora crassa*. Genetics 151:107–117

Yang Q, Poole SI, Borkovich KA (2002) A G-protein β-subunit required for sexual and vegetative development and maintenance of normal Gα protein levels in *Neurospora crassa*. Eukaryot Cell 1:378–390

Yatzkan E, Yarden O (1999) The B regulatory subunit of protein phosphatase 2A is required for completion of macroconidiation and other developmental processes in *Neurospora crassa*. Mol Microbiol 31:197–209

Yoshida Y, Hasunuma K (2004) Reactive oxygen species affect photomorphogenesis in *Neurospora crassa*. J Biol Chem 279:6986–6993

Zhang L, Churchill ACL, Kazmierczak P, Kim DH, van Alfen NK (1993) Hypovirulence-associated traits induced by a mycovirus of *Cryphonectria parasitica* are mimicked by targeted inactivation of a host gene. Mol Cell Biol 13:7782–7792

Zhang L, Baasiri RA, van Alfen NK (1998) Viral repression of the fungal pheromone precursor gene expression. Mol Cell Biol 18:953–959

Zonneveld BJM (1972) Morphogenesis in *Aspergillus nidulans*. The significance of α-1,3-glucan of the cell wall and α-1,3-glucanase for cleistothecium development. Biochim Biophys Acta 273:174–187