

Development in *Aspergillus*

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Abstract: The genus *Aspergillus* represents a diverse group of fungi that are among the most abundant fungi in the world. Germination of a spore can lead to a vegetative mycelium that colonizes a substrate. The hyphae within the mycelium are highly heterogeneous with respect to gene expression, growth, and secretion. Aspergilli can reproduce both asexually and sexually. To this end, conidiophores and ascocarps are produced that form conidia and ascospores, respectively. This review describes the molecular mechanisms underlying growth and development of *Aspergillus*.

Key words: *Aspergillus*, fungi, asexual reproduction, sexual reproduction, development, conidium, conidiophore, vegetative mycelium, heterogeneity, ascocarp, ascospore, fruiting body.

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INTRODUCTION

Aspergillus is an anamorph genus, which comprises between 260 (Geiser *et al.* 2007, Samson & Varga 2009) and 837 species (Hawksworth 2011). These species are classified in approximately ten different teleomorph genera (Geiser 2009). For instance, *A. nidulans* is part of the teleomorph genus *Emericella*, while *A. fumigatus* and *A. flavus* belong to the genera *Neosartorya* and *Petromyces*, respectively. This shows that *Aspergillus* is a diverse group of fungi. Indeed, comparison of the genomic sequences of *A. nidulans* and *A. fumigatus* (Galagan *et al.* 2005) showed that these fungi are as related to each other as fish to humans. These animals separated about 450 million years ago but diversification in the genus *Aspergillus* is assumed to be restricted to about 200 million years (Galagan *et al.* 2005). The large differences in genomic sequence have been proposed to be caused by an accelerated evolutionary rate (Cai *et al.* 2006).

Aspergillus species are among the most abundant fungi worldwide. They are not very selective with respect to abiotic growth conditions (Table 1). For instance, they can grow over a wide range of temperature (6–55 °C) and at relatively low humidity. In fact, *A. penicilloides* is among the most xerophilic fungi (Williams & Hallsworth 2009). Moreover, *Aspergillus* species feed on a large variety of substrates including animal faeces and human tissue. Nonetheless, they are predominantly found on complex plant polymers (Bennett 2010) and are considered to be common food spoilage fungi. The success of *Aspergillus* is also explained by their effective dispersal. Spores of this genus are among the most dominant fungal structures in the air, dispersing themselves both short and long distances (Bennett 2010). Aspergilli are not only known because of their saprobic life style. *Aspergillus niger* has been reported to be a pathogen of *Zingiber officinale* plants

(Pawar *et al.* 2008). Moreover, a wide variety of aspergilli are opportunistic pathogens of animals and humans. They do not infect healthy individuals but do invade individuals with a compromised immune system (Pitt 1994, Brakhage 2005). Aspergilli (*i.e.* *A. fumigatus*, and to a lesser extent species such *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*) cause invasive aspergillosis (involving several organ systems, particularly pulmonary disease), non-invasive pulmonary aspergilloma, and allergic bronchopulmonary aspergillosis (Denning 1998, Stevens *et al.* 2000).

Aspergillus spp secrete a wide variety of enzymes that degrade polymers within the substrate into molecules that can be taken up to serve as nutrients. For instance, amylases are secreted to degrade starch, xylanases to degrade xylan and pectinases to degrade pectin within plant material. Similarly, elastase is secreted in the human lung to degrade elastin. The capacity to secrete large amounts of proteins (and other metabolites such as organic acids) in combination with established fermentation technology and molecular biology make aspergilli such as *A. niger*, *A. oryzae*, *A. awamori*, *A. sojae*, and *A. terreus* attractive cell factories for the production of homologous and heterologous proteins (Meyer *et al.* 2011). The potential of these fungi is exemplified by strains of *A. niger* that produce more than 30 grams per liter of glucoamylase (Finkelstein *et al.* 1989). Of concern, *Aspergillus spp* can form mycotoxins that are toxic for animals and humans. *Aspergillus flavus* produces aflatoxin, which is one of the most carcinogenic natural molecules (Varga *et al.* 2011). In addition, different aspergilli, including *A. westerdijkiae*, can form ochratoxin on food products such as coffee and grapes (Leong *et al.* 2007).

This review describes the current understanding of development of aspergilli. Germination of spores, formation of a differentiated vegetative mycelium, and formation of asexual and sexual spores are discussed. Table 2 summarises the role of genes in these

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Table 1. Conditions for vegetative growth of selected *Aspergilli*.

Species	Optimum Temp (°C)	Temp range (°C)	Optimum pH	pH range	Minimal Water activity	Optimum Water activity	Minimum Relative humidity (%)	Optimum Relative humidity (%)	References
<i>A. niger</i>	35–37	6–47	6.0	1.5–9.8	0.77	0.97	88–89	96–98	(Astoreca <i>et al.</i> 2007, Ayerst 1969, Leong <i>et al.</i> 2006, Mehra & Jaitly 1995, Panasenکو 1967, Pitt 1981)
<i>A. oryzae</i>	30–37	7–47	6.0–7.5	4–8		0.99			(Chipeta <i>et al.</i> 2008, Gibson <i>et al.</i> 1994, Nasser <i>et al.</i> 2002, Panasenکو 1967)
<i>A. fumigatus</i>	37	10–55	5.5–6.5	3.0–8.0	0.82	0.97	85	98–99	(Al-Doory 1984, Ayerst 1969, Ogundero 1981, Panasenکو 1967, Singh & Sandhu 1982)
<i>A. clavatus</i>	20–25	5–42			0.88		88	98	(Panasenکو 1967)
<i>A. terreus</i>	37	15–42	5.0		0.78				(Al-Doory 1984, Mehra & Jaitly 1995, Singh & Sandhu 1982)
<i>N. fischeri</i>	26–45	11–52				0.98			(Beuchat 1986, Nielsen <i>et al.</i> 1988, Samson <i>et al.</i> 2000, Valik & Pieckova 2001)
<i>A. nidulans</i>	35–37	6–51	7.0	2–12	0.78		80	95	(Agnihotri 1964, Al-Doory 1984, Lacey 1980, Panasenکو 1967)

processes. *Aspergillus nidulans*, *A. fumigatus*, *A. oryzae*, and *A. niger* have been chosen as the lead organisms for this review. The effect of light on the formation of asexual and sexual spores will serve as an example how environmental factors can influence development. The process of meiosis is beyond the scope of this review (for a review see Pöggeler *et al.* 2006), and the relation between primary and secondary metabolism will not be discussed as well. For this we refer to Yu & Keller (2005) and Pöggeler *et al.* (2006). For the effect of other environmental factors than light we refer to Clutterbuck 1977, Skromne *et al.* 1995, Penalva & Arst 2004, and Etxebeste *et al.* 2010b.

VEGETATIVE GROWTH

In nature, aspergilli grow within and on solid substrates. A colony can result from a single sexual or asexual spore but it may also arise after conidia and/or germlings that are in close vicinity to each other have fused. It has been described that fusion in *A. oryzae*, *A. sojae* and *A. tamarii* most often occurs between conidia (> 80%), while fusions between conidia and germlings and fusion of germlings are much less frequent (Ishitani & Sakaguchi 1956). Fusion is mediated by fusion bridges that are formed by conidia or germ tubes. They may be similar to the conidial anastomosis tubes that are formed by *Colletotrichum* and *Neurospora* (Roca *et al.* 2003, Roca *et al.* 2005a, Roca *et al.* 2005b). These anastomosis tubes are morphologically and physiologically distinct from germ tubes. They are typically short, thin, and unbranched. Fusion of conidia and germlings has been described to occur within *Aspergillus* strains, between *Aspergillus* strains, between different aspergilli and even between *Aspergillus* and *Penicillium* species (Ishitani & Sakaguchi 1956). However, fusion between strains and between species often results in heterokaryon incompatibility. For instance, heterokaryon incompatibility is a widespread phenomenon among *A. niger* strains. The underlying mechanism is, however, not known (van Diepingen *et al.* 2009). Fusion of hyphae was reported to be rare when germlings of *A. oryzae*, *A. sojae* and *A. tamarii* had

formed hyphae (Ishitani & Sakaguchi 1956). Whether this also holds for other aspergilli is not known. At least, fusion of hyphae has been shown to occur in other ascomycetes (for references see Ishitani & Sakaguchi 1956).

Colonies can reach a diameter in the (sub-)millimeter (micro-colonies) to centimeter (macro-colonies) scale depending on the size and the composition of the substrate. For instance, micro-colonies are formed on a wheat kernel, whereas macro-colonies can be formed within the lobes of a lung. In the laboratory, aspergilli are routinely grown on agar media or in liquid media. On agar medium, aspergilli form radial symmetrical macro-colonies. The mycelium of *A. nidulans* (Lee & Adams 1994a) and *A. niger* extend their diameter with approximately 0.25 mm per h in excess of nutrients and at a temperature of 37 °C and 30 °C, respectively. Colonies can also be grown between porous polycarbonate membranes on an agar medium (Levin *et al.* 2007a, Levin *et al.* 2007b, Masai *et al.* 2006, Wösten *et al.* 1991). Scanning electron microscopy shows that the periphery of a 7 d old sandwiched colony of *A. niger* consists of a single layer of hyphae (Fig. 1A, D). A few millimeters behind the periphery this layer becomes thicker and comprises of up to six layers of hyphae growing on top of each other. Notably, three distinct layers are observed another two millimeters towards the centre (Fig. 1B, E). The upper and lower layer consist of up to five hyphae on top of each other, while the intermediate layer comprises a loose network of thin and thick hyphae, and some non-sporulating conidiophores. Three distinct layers are also observed in the innermost centre of the colony (Fig. 1C, F). In this case, the upper and lower layers consist of up to twenty and six layers of hyphae, respectively. The intermediate layer comprises a dense network of both thin and thick hyphae, and a relatively high number of non-sporulating conidiophores. An *A. niger* colony grows in a similar way when a 0.45 mm thin agarose layer is present in between the polycarbonate membranes.

Mycelium can grow dispersed, as clumps or as micro-colonies, also known as pellets, during submerged growth in liquid medium. Clumps are aggregated hyphae that are considered to be an intermediate state between pelleted and dispersed growth. The

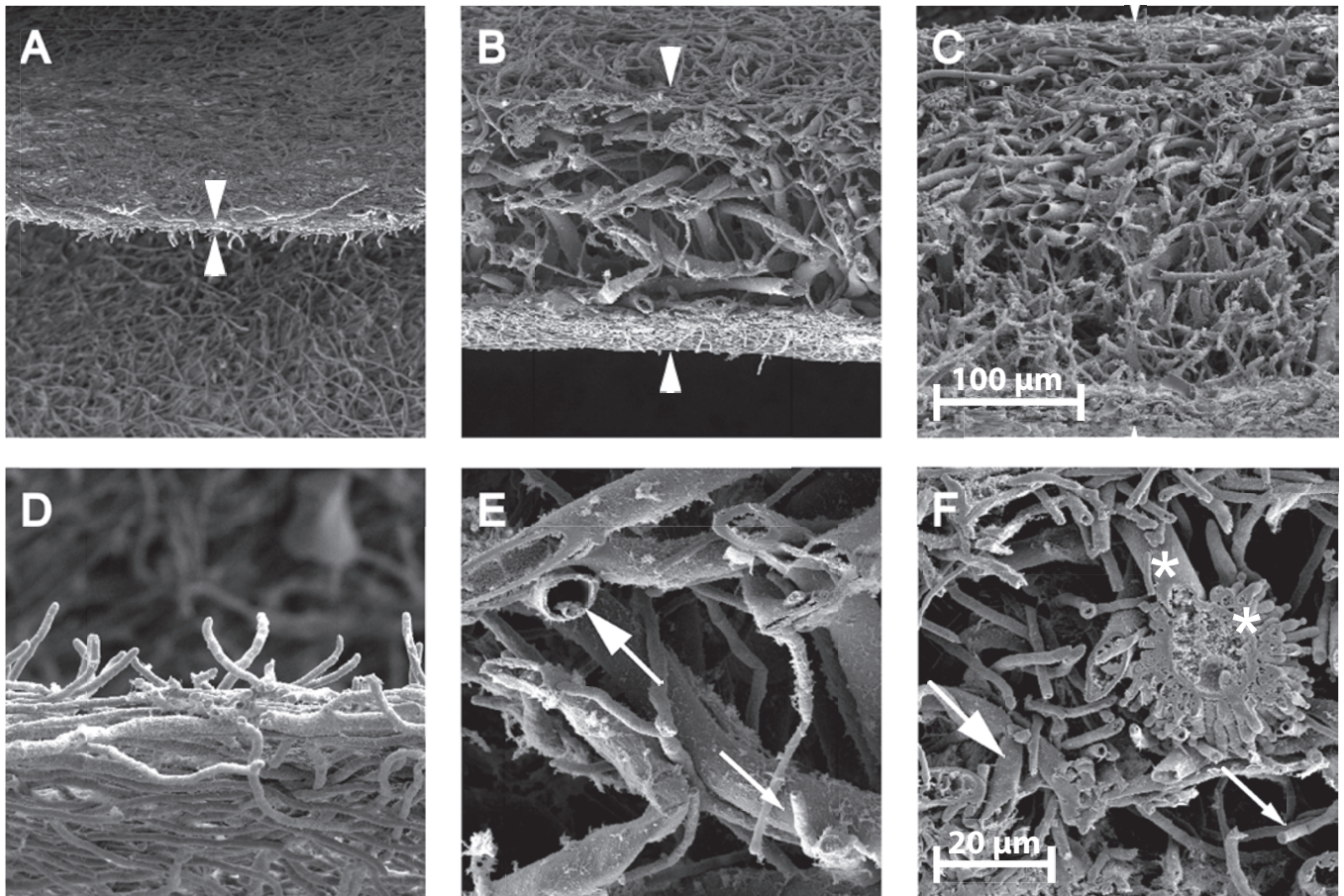


Fig. 1. Scanning electron microscopy of cross sections of a 7 d old sandwiched *A. niger* colony. Cross sections were made at the periphery (A, D), four millimeter behind the periphery (B, E) and at the innermost center (C, F). The thickness of the colony is indicated by the distance between the white triangles. Panels D–F represent higher magnifications of A–C, respectively. Thin and thick arrows point at thin and thick hyphae, respectively. In H asterisks mark a non-sporulating conidiophore. Bars in panel C, for A–C, and F, for D–F, represent 100 and 20 μm .

morphology of the mycelium has an enormous impact on the production of enzymes and primary or secondary metabolites. For instance, micro-colonies are required for the production of citric acid by *A. niger* (Vecht-Lifshitz *et al.* 1990). It is not clear how morphology exactly affects productivity. It has been proposed that this is due to the effect of the fungal morphology on the viscosity of the medium (Bhargava *et al.* 2003). Viscosity correlates with the extent of dispersed growth; large micro-colonies thus result in a low viscosity. The center of large pellets may experience oxygen starvation and other nutrients may also become limiting in this part of the mycelium. These conditions may also impact productivity of the pellets.

Pellet formation is caused by coagulation of the conidia in the culture. Parameters that affect coagulation of *A. niger* and *A. oryzae* conidia are initial pH, agitation, and medium composition (Metz & Kossen 1977, Carlsen *et al.* 1996). For instance, the chelating agents EDTA and ferrocyanide lead to small and compact pellets, whereas anionic polymers like carboxypolyethylene and polyacrylate give rise to small but loose pellets. Pellet formation can also be manipulated by changing the surface composition of spores. Formation of micro-colonies was affected in strains of *A. nidulans* in which either or both *dewa* and *rodA* were inactivated (Dynesen & Nielsen 2003). The effect was strongest when both these hydrophobin genes were inactivated, which was accompanied by a huge drop in surface hydrophobicity of the conidia (see below). Pellet formation in *A. niger* was also affected by inactivation of one of the pigmentation genes (van Veluw *et al.* 2013). Conidia were no longer hydrophobic in the case of the Δolva strain but the other deletion strains affected in pigmentation (*i.e.* the

ΔfwnA and ΔbrnA strains) were still hydrophobic. Taken together, surface hydrophobicity of conidia plays a role in pellet morphology but other factors are important as well.

Research in the last two decades has shown that the mycelium of *Aspergillus* is heterogeneous with respect to gene expression, growth, and secretion. Genome wide expression analysis has shown that the RNA composition of central and peripheral zones of colonies of *A. niger* (Levin *et al.* 2007a) and *A. oryzae* (Masai *et al.* 2006) is different. In the case of 7 d old colonies of *A. niger*, 25 % of the active genes show a two-fold or more difference in RNA accumulation between the innermost and outermost zone of the mycelium (Levin *et al.* 2007a). For instance, RNA levels of the glucoamylase gene *glaA* are 3-fold higher at the periphery of maltose-grown colonies when compared to the center. Similarly, accumulation of transcripts of the ferulic acid esterase gene *faeA* is 5-fold higher at the periphery of xylose grown colonies. Notably, 9 % of the genes that are active in a 7 d old colony are expressed in only one of five concentric zones. For instance, genes related to nitrate metabolism are specifically expressed in the outer zone of the colony, whereas mRNA of the hydrophobin *hfbD* is almost exclusively found in a central zone. Half the variation in RNA profiles is explained by differences in the composition of the medium underlying each zone of the colony, whereas the other half of the variation is caused by medium-independent mechanisms (Levin *et al.* 2007a). These findings imply that differentiation occurs within the vegetative mycelium of *Aspergillus*.

The heterogeneity of the mycelium of *A. niger* is also indicated by the fact that distinct zones of the colony grow and secrete

Table 2. Overview of *Aspergillus* genes involved in the different developmental stages. Functions of genes refer to *A. nidulans*, unless otherwise indicated.

Name	Description	Developmental stage	Function	Page number
<i>abaA</i>	ATTS Transcription factor	Asexual development	Regulation of sterigmata formation during conidiophore development	9, 10, 12, 14, 19, 24
<i>abr1</i>	Vermelone dehydratase	Spore protection	Melanin biosynthesis in <i>A. fumigatus</i>	21, 24
<i>abr2</i>	Laccase (with homology to <i>yA</i>)	Spore protection	Melanin biosynthesis in <i>A. fumigatus</i>	21
<i>alb1</i>	Polyketide synthase	Spore protection	Melanin biosynthesis in <i>A. fumigatus</i>	21
<i>arp1</i>	Scytalone dehydratase	Spore protection	Melanin biosynthesis in <i>A. fumigatus</i>	21
<i>arp2</i>	Hydroxynaphthalene (HN) reductase	Spore protection	Melanin biosynthesis in <i>A. fumigatus</i>	21
<i>ayg1</i>	Polyketide carbon backbone modification	Spore protection	Melanin biosynthesis in <i>A. fumigatus</i>	21
<i>brlA</i>	C ₂ H ₂ zinc finger transcription factor	Asexual development	Regulation of stalk development	9–14, 17–19, 21, 24
<i>brnA</i>	Multicopper oxidase	Spore protection	Melanin biosynthesis in <i>A. niger</i>	3, 9, 21
<i>chiB</i>	Class V endochitinase B	Vegetative growth	Autolysis	10
<i>chsA</i>	Chitin synthase	Asexual development	Septum formation in conidiophores	10
<i>chsC</i>	Chitin synthase	Asexual development	Septum formation in conidiophores	10
<i>cryA</i>	Cryptochrome/photolyase	Blue light response	Inhibition of sexual development in the light	19–21
<i>cyaA</i>	Adenylate cyclase	Germination	GanB mediated germination	24, 25
<i>dewA</i>	Hydrophobin	Asexual development	Coating of conidia	3, 9, 14
<i>fadA</i>	G α -subunit heterotrimeric G-protein complex	Vegetative growth Sexual development	Inhibition sexual and asexual development Homothallic cleistothecia and Hülle cell formation	10–13, 16
<i>flbA</i>	Regulator of G-protein signaling	Asexual development Sexual development	Inhibition of vegetative growth enabling asexual development Homothallic cleistothecia formation	10–13, 16
<i>flbB</i>	bZIP-type transcription factor	Asexual development	Regulation of conidiophore formation	7, 12, 13
<i>flbC</i>	C ₂ H ₂ zinc finger transcription factor	Asexual development Sexual development Germination	Regulation of conidiophore formation Repression sexual development Germination	12, 13, 16, 17, 24
<i>flbD</i>	c-Myb transcription factor	Asexual development	Regulation of conidiophore formation	12–13
<i>flbE</i>	Protein involved in conidiophore formation	Asexual development Sexual development	Regulation of conidiophore formation Repression sexual development	12, 13, 16, 17
<i>fluG</i>	Homology to bacterial glutamine syntetase	Asexual development Germination	Production of extracellular signaling molecule involved in conidiophore development Germination	8, 9, 11–13, 24
<i>fphA</i>	Phytochrome	Red Light response	Stimulation asexual development and repression of sexual development in the light	19–21
<i>fwnA</i>	Polyketide synthase	Spore protection	Melanin biosynthesis	3, 9, 21
<i>ganA</i>	G α -subunit of heterotrimeric G-protein complex of <i>A. nidulans</i> and <i>A. oryzae</i>	Not known		12, 16
<i>ganB</i>	G α -subunit of heterotrimeric G-protein complex of <i>A. nidulans</i> and <i>A. oryzae</i>	Vegetative growth Germination	Repression asexual development cAMP dependent Germination	11, 12, 16, 23, 24
<i>gaoC</i>	G α -subunit of heterotrimeric G-protein complex of <i>A. oryzae</i>	Not known		12
<i>gpaA</i>	G α -subunit of heterotrimeric G-protein complex of <i>A. fumigatus</i>	Vegetative growth	Promotion vegetative growth	12
<i>gpaB</i>	G α -subunit of heterotrimeric G-protein complex of <i>A. fumigatus</i>	Asexual development	Regulation asexual development	12
<i>gpgA</i>	G γ -subunit of heterotrimeric G-protein complex	Vegetative growth Sexual development Germination	Stimulation vegetative growth Regulation of cleistothecia formation Trehalose degradation during germination	11, 12, 15, 16, 23, 24
<i>gprA</i>	G-protein receptor (GPCR)	Sexual development	Homothallic cleistothecia formation	15–17
<i>gprB</i>	G-protein receptor (GPCR)	Sexual development	Homothallic cleistothecia formation	15–16

Table 2. (Continued).

Name	Description	Developmental stage	Function	Page number
<i>gprD</i>	G-protein receptor (GPCR)	Vegetative growth	Repression sexual development	15
<i>kapA</i>	α -importin	Light response	Protein import into nucleus in the dark	18, 20
<i>laeA</i>	Transcription factor	Light response	Regulation of asexual development, sexual cleistothecia and Hülle cell formation	19, 20
<i>lreA</i>	White collar blue light receptor	Blue light response	Stimulation sexual development in the light, repression asexual development in the light	19, 20
<i>lreB</i>	White collar blue light receptor	Blue light response	Stimulation sexual development in the light, repression asexual development in the light	19, 20
<i>MAT1-1</i>	α -homeodomain transcription factor	Sexual development	Regulation of sexual reproduction	15
<i>MAT1-2</i>	High mobility group domain (HMG)-transcription factor	Sexual development	Regulation of sexual reproduction	15
<i>medA</i>	Temporal regulation conidiophore formation	Asexual development Sexual development	Regulation of conidiophore development Regulation of cleistothecia and Hülle cell formation	9, 10, 16, 17, 24
<i>mpkB</i>	Mitogen activated protein kinase (MAPK)	Sexual development	Signalling in cleistothecia and Hülle cell formation	14, 16, 17
<i>nosA</i>	Zn(II) ₂ Cys ₆ transcription factor	Sexual development	Regulation of cleistothecia formation (primordium maturation)	16, 17, 19
<i>nsdC</i>	Zinc finger transcription factor	Vegetative growth Sexual development	Repressing asexual development Regulation of cleistothecia formation	14, 16, 17
<i>nsdD</i>	GATA-like transcription factor	Asexual development Sexual development	Repressing asexual development Regulation of cleistothecia and Hülle cell formation	14, 16–18, 21
<i>olvA</i>	Homologue of <i>aygA</i>	Spore protection	Melanin biosynthesis in <i>A. niger</i>	3, 9, 21
<i>phnA</i>	Phosducin like protein	Vegetative growth	Positive regulation G $\beta\gamma$ stimulating vegetative growth	11
<i>pkaA</i>	Protein kinase	Vegetative growth Germination	Stimulation vegetative growth Signalling involved in germination	11, 12, 24
<i>pkaB</i>	Protein kinase activity	Vegetative growth Asexual development Germination	Potential backup for <i>pkaA</i> Germination spores	11, 12, 24
<i>ppoA</i>	Fatty acid oxygenase	Balance Sexual and Asexual development	Production oleic and linoleic acid derived oxylipins (ψ IB α) regulating asexual and sexual development	17–19
<i>ppoB</i>	Fatty acid oxygenase	Balance Sexual and Asexual development	Production oleic and linoleic acid derived oxylipins (ψ IB β) regulating asexual and sexual development	17–19
<i>ppoC</i>	Fatty acid oxygenase	Balance Sexual and Asexual development	Production oleic and linoleic acid derived oxylipins (ψ IB β) regulating asexual and sexual development	17–19
<i>pptA</i>	Polyketide synthase	Spore protection	Melanin biosynthesis in <i>A. niger</i>	21
<i>rasA</i>	GTPase of the RAS superfamily	Asexual development Germination	Polarised growth during germination	23, 24
<i>rgsA</i>	Regulator of G-protein signaling	Asexual development	Enhancing intrinsic activity GanB (G- α subunit) Regulation <i>brlA</i>	11, 12, 23, 24
<i>rodA</i>	Hydrophobin	Asexual development	Formation rodlet layer during conidiophore development	3, 9, 14
<i>rodB</i>	Hydrophobin	Asexual development	Formation rodlet layer during Conidiophore development	14
<i>rolA</i>	RodA-like Hydrophobin	Asexual development	Cutinase recruitment during conidiophore formation in <i>A. oryzae</i>	14
<i>rosA</i>	Zn(II) ₂ Cys ₆ transcription factor	Sexual development	Cleistothecia formation (VeA dependent)	16, 17, 21
<i>schA</i>	Ser/Thr protein kinase	Germination	Signalling leading to germination	24
<i>sfaD</i>	G β -subunit of heterotrimeric G-protein complex	Vegetative growth Sexual development Germination	Regulation vegetative growth Regulation cleistothecia formation Trehalose degradation during germination	11, 12, 15, 16, 23, 24
<i>sfgA</i>	Gal4-type Zn(II) ₂ Cys ₆ type transcription factor	Vegetative growth	Repression asexual development	12, 13

Table 2. (Continued).

Name	Description	Developmental stage	Function	Page number
<i>steA</i>	STE-like transcription factor	Sexual development	Repression Cleistothecia formation, and regulating Hülle cell formation	14, 16, 17, 19, 24
<i>steC</i>	C ₂ H ₂ zinc finger transcription factor MAPKKK	Asexual development	Mitogen activated protein kinase kinase kinase (MAPKKK)	13, 14, 16
		Sexual development	Cleistothecia formation	
		Sexual development	Heterokaryon formation	
<i>stuA</i>	APSES domain transcription factor	Asexual development	Spatial regulation conidiophore formation	9, 10, 16, 17, 24
		Sexual development	Cleistothecia and Hülle cell formation	
		Germination	Germination	
<i>tpsA</i>	α-α-Trehalose-6-phosphate synthase	Spore protection	Trehalose biosynthesis	22, 23
<i>tpsB</i>	α-α-Trehalose-6-phosphate synthase	Spore protection	Trehalose biosynthesis	22
<i>treB</i>	Neutral trehalase B	Germination	Degradation intracellular trehalose during germination	22, 23
<i>veA</i>	Velvet-protein with nuclear localisation signal	Light response	Regulation sexual development (inhibition asexual development)	10, 16–21, 23
		Asexual and Sexual development		
<i>veB</i>	Velvet-like protein	Light response	Regulation asexual/sexual development	10, 18–20, 22
		Asexual and Sexual development		
		Germination	Regulation trehalose synthetic genes during germination	
<i>vosA</i>	Velvet-like transcription factor	Light response	Repression conidiophore formation in the dark	9, 10, 12, 13, 19, 22, 24
		Asexual and Sexual development		
		Germination	Regulation trehalose synthesis genes during germination	
<i>wetA</i>	Synthesis cell wall layers	Asexual development	Regulation conidiophore maturation and formation	9, 10, 12, 14, 24
<i>yA</i>	Conidial laccase	Asexual development	Production dark green pigment in <i>A. nidulans</i>	9

proteins (Levin *et al.* 2007a, Levin *et al.* 2007b, Masai *et al.* 2006, Wösten *et al.* 1991). Proteins are formed throughout the *A. niger* mycelium (Levin *et al.* 2007a, Levin *et al.* 2007b, Wösten *et al.* 1991) (Fig. 2) but they are mainly secreted at the periphery. Growth is observed in this outer zone but also in the innermost centre (Fig. 2). Spatial growth and protein production is not affected when 6 d old colonies are transferred to fresh medium for 16 h. However, after transfer protein secretion is not only observed at the periphery of the colony but also in central parts of the mycelium (Fig. 2). These data show that non-growing zones of the mycelium abundantly secrete proteins upon transfer to fresh medium (Levin *et al.* 2007a). This is a remarkable finding considering the fact that protein secretion is generally assumed to take place in growing hyphae only (Moukha *et al.* 1993, Wessels 1989, Wessels 1990, Wösten *et al.* 1991).

The finding that 7 d old macro-colonies are heterogeneous with respect to RNA accumulation, growth and protein secretion raised the question whether heterogeneity is also observed between and within micro-colonies. Indeed, micro-colonies within liquid shaken cultures are heterogeneous with respect to size and gene expression (de Bekker *et al.* 2011b). A population of small and a population of large micro-colonies can be distinguished by flow cytometry in cultures of *A. niger* that consist of pellets with a maximum diameter of 1 mm. These populations differ 90 µm in diameter. Similarly, two populations of micro-colonies were distinguished when expression of the glucoamylase gene *glaA* and the ferulic acid esterase gene *faeA* were monitored. Notably, the population of lowly expressing micro-colonies is larger than the population of small pellets. This

indicates that size of micro-colonies is not the only determinant for expression of genes encoding secreted proteins (de Bekker *et al.* 2011b). It is not yet clear how heterogeneous gene expression is between zones of micro-colonies. At least, the total amount of RNA per hypha is about 50 times higher at the periphery of 1 mm wide micro-colonies when compared to the center (de Bekker *et al.* 2011b).

Heterogeneous gene expression is not only observed between micro-colonies or between zones of micro- or macro-colonies of *Aspergillus*; it is also observed between hyphae in a particular zone. It has been described that only part of the hyphae at the periphery of macro-colonies of *A. niger* secrete glucoamylase (Wösten *et al.* 1991). This observation is explained by heterogeneous expression of the glucoamylase gene *glaA* within this zone (Vinck *et al.* 2005). In fact, two populations of hyphae can be distinguished at the outer zone of the colony; those highly and those lowly expressing *glaA*. The hyphae highly expressing *glaA* also highly express other genes encoding secreted proteins (Vinck *et al.* 2011). Moreover, these hyphae highly express the glyceraldehyde-3-phosphate dehydrogenase gene *gpdA* and show a high 18S rRNA content. Thus, two populations of hyphae are present at the periphery of a colony; those that are lowly and those that are highly metabolically active. From the fact that the lowly active hyphae have a growth rate similar to that of the highly active hyphae it has been concluded that a “low” activity of hyphae is sufficient to support hyphal growth. However, a “high” metabolism would be needed to support secretion of large amounts of proteins (Vinck *et al.* 2011). Recently, it has been described that transcriptionally and translationally

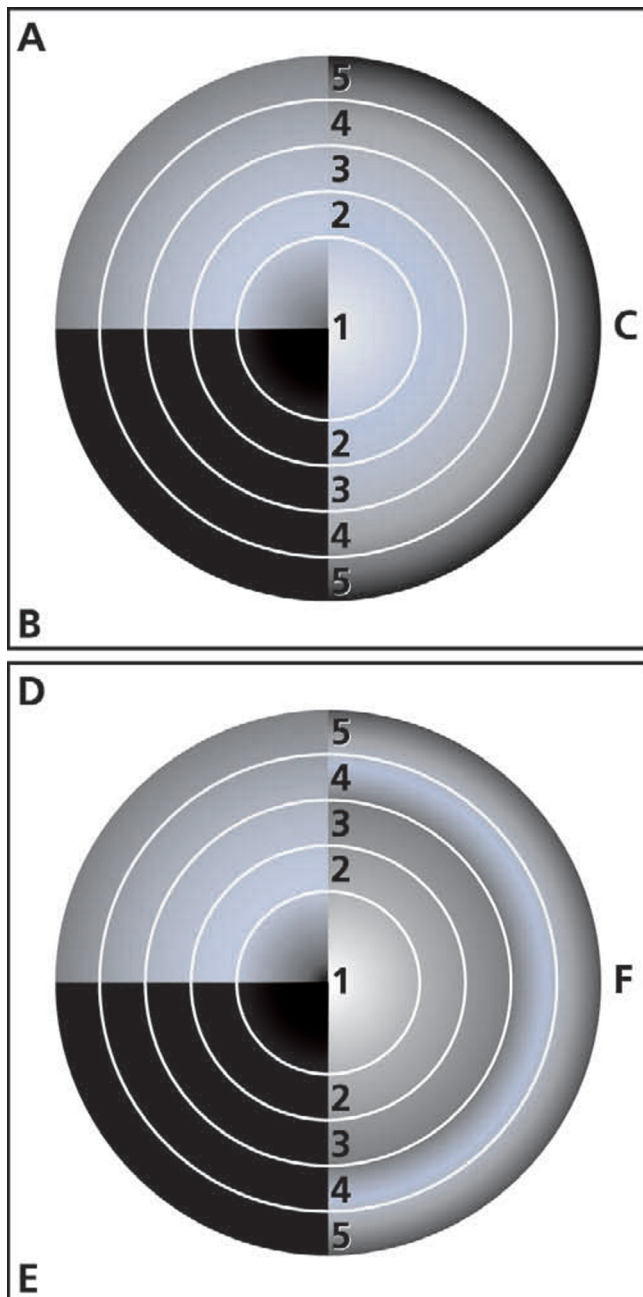


Fig. 2. Growth (A, D), protein synthesis (B, E) and protein secretion (C, F) in a 7 d old xylose grown sandwiched colony of *A. niger* before (A–C) and after transfer (D–F) to fresh medium. (Adapted from Levin *et al.* 2007).

highly active and lowly active hyphae also occur at the periphery of micro-colonies. However, the existence of distinct populations of these types of hyphae seems to be less robust when compared to macro-colonies grown on solid medium (van Veluw *et al.* 2013). Possibly, signalling between hyphae is involved in maintaining or enhancing heterogeneity. Gradients of signalling molecules cannot be formed between hyphae in liquid shaken cultures, which may explain why heterogeneity is less evident in these cultures.

Single hypha transcriptome analysis indicates that heterogeneity between neighboring hyphae goes beyond two types of hyphae. Individual hyphae each have their own composition of RNA (de Bekker *et al.* 2011a). So far, we can only guess why hyphae are heterogeneous at the colony periphery. The leading hyphae explore the substrate and they may be exposed to rapid changes in the environment. A heterogeneous hyphal population may contribute to the survival under such conditions. Notably, the transcription factor FlbB, which is involved in asexual development

(see below), has been shown to accumulate at 60 % of the tips of newly formed branches (Etxebeste *et al.* 2009). This is another example of heterogeneity within the *Aspergillus* mycelium.

Heterogeneity within the mycelium is surprising considering the fact that the cytoplasm of a fungal mycelium is assumed to be continuous. This is based on the fact that the septa within and between hyphae are porous allowing streaming of water, (in)organic compounds, proteins and even organelles (Jennings 1984, Jennings 1987, Bleichrodt *et al.* 2013). Heterogeneity between hyphae would require a certain immobility of molecules. This could be caused by the fact that many proteins are part of large complexes that are immobilised at membranes (Gavin *et al.* 2006). For instance, the yeast GPD homologs were found to be part of 17 protein complexes (Gavin *et al.* 2006). One of such protein complexes includes two transmembrane proteins that may well decrease the streaming rate by temporally immobilising the complex at the membrane. In agreement, the streaming rate of a fusion between GFP and GpdA was lower in *A. niger* than that of GFP itself (Bleichrodt *et al.* 2013). Closure of septa is another mechanism to maintain differences in composition between hyphae. Septa of vegetative hyphae of *A. oryzae* and *A. niger* (Bleichrodt, 2012) and the basidiomycete *Schizophyllum commune* (van Peer *et al.* 2009a, van Peer *et al.* 2009b) can be in a closed or open state. The incidence of closed septa in *A. niger* and *S. commune* depends on the environmental conditions and is reversible.

ASEXUAL DEVELOPMENT

After a period of vegetative growth, air-exposed colonies of *A. nidulans* and *A. niger* form two types of aerial hyphae (Fig. 3). One type is quite similar to vegetative hyphae of these aspergilli and has a diameter of about 2–3 μm . The second type of aerial hyphae has a diameter of about 4–5 and 6–7 μm in the case of *A. nidulans* and *A. niger*, respectively. These so-called stalks can differentiate into conidiophores (Fig. 3). The conidiophore stalk of *A. nidulans* extends about 100 μm into the air and is formed from a specialised foot-cell within the substrate mycelium (Adams *et al.* 1998). When the stalk has reached its maximum height, the tip swells and forms a vesicle with a diameter of 10 μm . In biserate species like *A. nidulans* and *A. niger*, the vesicle surface buds resulting in a layer of primary sterigmata termed metulae. The metulae in turn bud twice. This results in a second layer of sterigmata called phialides. The phialides give rise to chains of mainly uninucleate conidia. As a result, more than 10.000 conidia can be produced per conidiophore. *Aspergillus oryzae* can be both uniserate and biserate. In the case of uniserate species, spore producing phialides are positioned directly at the surface of the conidiophore vesicles.

The 2–3 μm wide aerial hyphae of *A. nidulans* and *A. niger* are formed about 8 h after inoculation of spores on complete medium. Although timing of this type of aerial hyphae seems to be medium-independent, the density of aerial hyphae is lower in the case of minimal medium. The first stalks of *A. nidulans* and *A. niger* are formed 10 h after spore inoculation on complete medium and growth at 37 $^{\circ}\text{C}$ and 30 $^{\circ}\text{C}$, respectively. In both cases, conidiophores are formed 20 h post-inoculation. Formation of aerial hyphae in both aspergilli starts in the centre of the colony and moves outwards ending a few millimeters from the edge of the mycelium. This observation implies that the competence of hyphae to form aerial hyphae is acquired faster when a colony gets older (Adams *et al.* 1998). The process of aerial growth has been

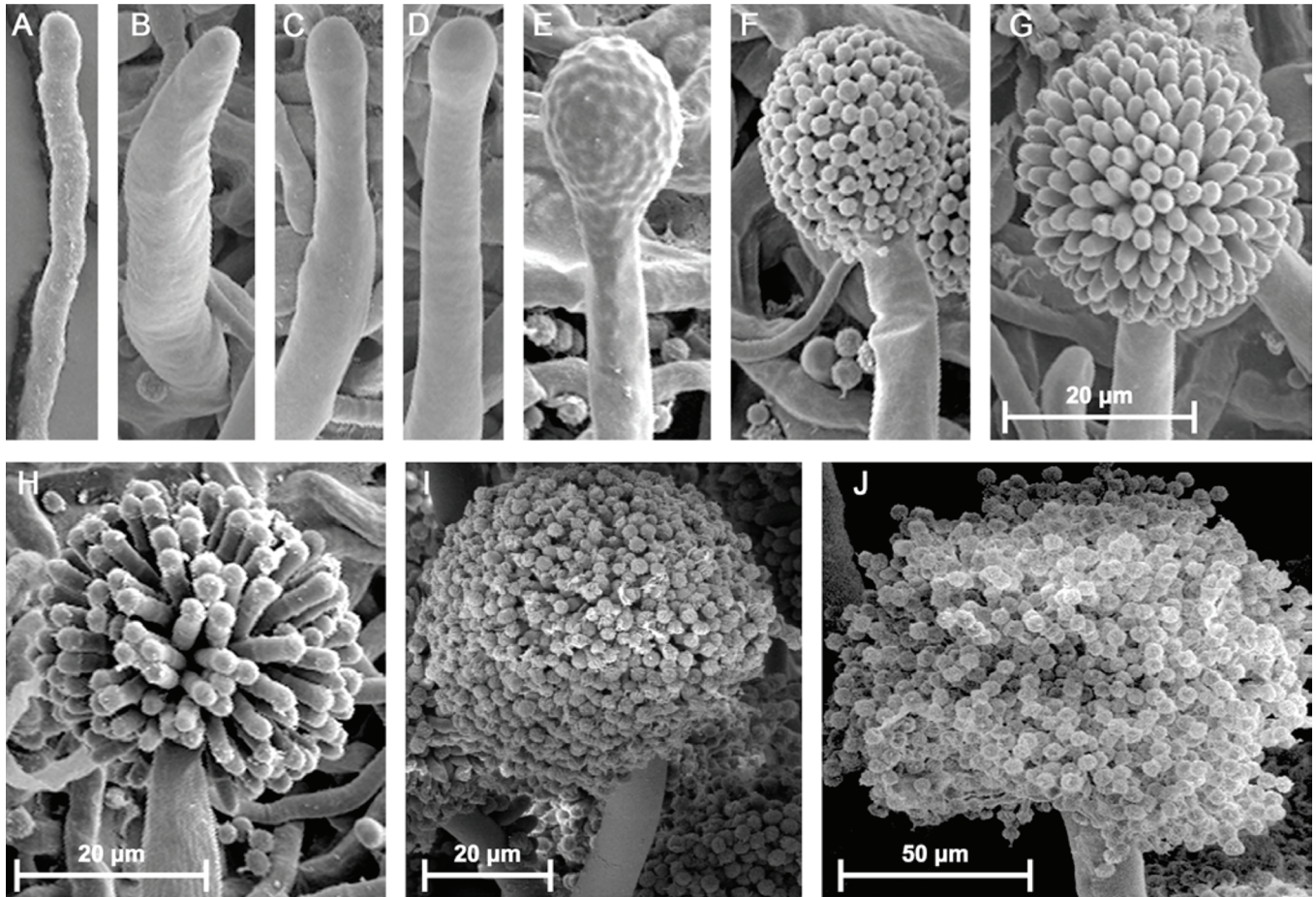


Fig. 3. Development of *A. niger* monitored by scanning electron microscopy. The vegetative mycelium forms two types of aerial hyphae. One type is similar to vegetative hyphae (A), while the other type is 2–3 times thicker (B). The tips of the latter aerial hyphae may swell to form a vesicle (C,D). Buds are formed on the vesicle (E) that develop into metulae (F, G). Phialides are formed on top of the metulae (H), which give rise to chains of conidia (I, J). The bar in G also holds for A–F.

proposed to involve signaling of the cell density of the vegetative mycelium (Lee & Adams 1994b, Wösten *et al.* 1999a, Wösten & Willey 2000). The signaling molecule would induce hydrophobin genes. These genes encode proteins that lower the water surface tension to enable hyphae to breach the interface to grow into the air (Wösten *et al.* 1999b, Wösten 2001). Which hydrophobin is secreted into the aqueous environment in *Aspergillus* cultures with the aim to lower the water surface tension is not yet known.

Aspergillus nidulans can also form conidia in submerged cultures (Adams *et al.* 1998). In this case, conidiation is induced when the culture gets stressed or when nutrients are limited (*e.g.* limitation of the carbon and the nitrogen source). On the other hand, formation of conidiophores in air-exposed colonies is assumed to be induced by an internal signal that activates a genetic program of sporulation (see below) (Adams *et al.* 1998). In both cases, competence to sporulate is acquired in a time-dependent way (Skromne *et al.* 1995). Like *A. nidulans*, *A. niger* can also form conidiophores in submerged conditions. However, these conidiophores do not form spore chains (Fig. 1F).

Aspergillus nidulans strains in which the *fluG* (*fluffy*) gene is inactivated (*i.e.* a $\Delta fluG$ strain) do form aerial hyphae but conidiophores are not being formed in excess of nutrients (Lee & Adams 1994b). During nutrient deprivation, however, some conidiophores are being formed on a solid medium. Similarly, submerged cultures of the $\Delta fluG$ strain start to sporulate in the absence of a carbon source (Lee & Adams 1996). These data indicate that FluG is involved in a developmental program of sporulation but not in the stress-related sporulation pathway.

Formation of conidiophores in the $\Delta fluG$ strain can be rescued by growing the mutant next to a wild-type strain. Complementation is also observed when the strains are physically separated by a dialysis membrane with a size exclusion of 6–8 kDa. This indicates that FluG is involved in the production of a low-molecular weight extracellular signaling molecule that is involved in the formation of conidiophores. A similar phenomenon is observed in *Penicillium* species (Roncal & Ugalde 2003). In this case, an extracellular molecule called conidiogenone induces conidiation. Conidiogenone is a diterpene that accumulates during vegetative growth. At a certain point, a certain threshold level is exceeded and conidiation is initiated (Roncal & Ugalde 2003).

Regulation of asexual development

Formation of conidiophores has been well studied in *A. nidulans*. Experimental evidence has shown that mechanisms underlying asexual development in *A. fumigatus* and *A. oryzae* are similar but not identical in *A. nidulans* (see below). So far, formation of conidiophores and conidia has not been studied in *A. niger*. However, its genomic sequence predicts that mechanisms of asexual development are also similar, if not identical, to that in *A. nidulans* (Pel *et al.* 2007). About 1300 genes have been found to be up-regulated in whole colonies of *A. nidulans* during asexual reproduction (Timberlake 1980). Recently, RNA was isolated from the vegetative mycelium and from aerial structures (aerial hyphae, conidiophores, and spores) of 7 d old colonies of *A. niger*. Microarray analysis showed that 34 genes are found in the top 100 of

most highly expressed genes of both the vegetative mycelium and the aerial structures (Bleichrodt *et al.* 2013). These genes include histones, ribosomal proteins, and a hydrophobin homologous to *dewA*. Of the 8 predicted hydrophobin genes (Pel *et al.* 2007, Jensen *et al.* 2010), 6 are within the top 100 of most highly expressed genes in the aerial structures. This top 100 also includes the pigmentation genes *fwnA*, *olvA* and *brnA* (for these genes see Dormancy and Germination). Seven genes encoding carbohydrate degrading enzymes are in the top 100 of highest expressed genes in the vegetative mycelium. One of these genes is the glucoamylase gene *glaA* (Bleichrodt *et al.* 2013).

Regulation by *fluG*, *brlA*, *abaA*, *wetA*, *medA*, *stuA*, and *vosA*

FluG is believed to be at the start of the developmental program leading to asexual sporulation in *A. nidulans*. Indeed, overexpression of *fluG* in vegetative hyphae is sufficient to cause sporulation under conditions that normally suppress conidia formation (Lee & Adams 1996). The *fluG* transcripts are present in relatively constant levels during late vegetative growth and conidiation. Notably, a 4-fold higher *fluG* expression level is found in germinating spores during their isotropic growth (3 h after inoculation) when compared to polar growing germlings (5 h after inoculation) (Breakspear & Momany 2007). This suggests that *fluG* is not only involved in conidiophore formation but also in germination.

FluG activates the *brlA* (*bristle*) gene. A $\Delta brlA$ strain of *A. nidulans* forms stalks that do not stop their growth after they have reached a length of 100 μm . These stalks can reach a length 20–30 times longer than those of the wild-type, which results in the characteristic bristle phenotype (Adams *et al.* 1988). Moreover, isotropic growth is not initiated at the apex of the stalks of the $\Delta brlA$ strain. As a result, conidiophore vesicles are not being formed. Conidiophore development becomes independent from *fluG* by placing *brlA* under control of an inducible promoter (Adams *et al.* 1988). Similar results have been obtained in *A. oryzae* (Ogawa *et al.* 2010, Yamada *et al.* 1999). Inactivation of *brlA* in *A. oryzae* results in the inability to form conidiophores. In contrast, fully developed conidiophores are formed in submerged culture when the *brlA* gene is expressed under the control of the *amyB* promoter. *BrlA* is also essential for conidiophore formation in *A. fumigatus* (Mah & Yu 2006). However, in contrast to *A. nidulans* (Adams *et al.* 1988) and *A. oryzae* (Ogawa *et al.* 2010), the *A. fumigatus* gene seems to function earlier in conidiophore development. This is based on the fact that conidiophore development is completely abolished in a $\Delta brlA$ strain of *A. fumigatus*. The appearance of the colonies of this strain is more similar to that of the fluffy mutants of *A. nidulans* (see below) (Mah & Yu 2006). In addition, the *A. fumigatus* gene seems to function independent from *fluG*. At least, a $\Delta fluG$ strain of *A. fumigatus* still sporulates in air-exposed cultures. Possibly, *A. fumigatus* has more than one *brlA* activating mechanism (Mah & Yu 2006). The *brlA* gene of *A. fumigatus* has also been shown to be involved in suppressing ribosomal protein genes during nitrogen stress (Twumasi-Boateng *et al.* 2009). This finding conforms to the general starvation response in fungi, which involves both down-regulation of ribosomal protein biogenesis and induction of sporulation (Bahn *et al.* 2007, de la Sema *et al.* 1999, Gasch *et al.* 2000, Li *et al.* 1999, Mogensen *et al.* 2006, Warner 1999). However, down-regulation of ribosomal protein encoding genes is not impaired during carbon stress in *A. fumigatus* (Twumasi-Boateng *et al.* 2009). Nevertheless, these findings suggest that *brlA* of *A. fumigatus* is not only a regulator of formation of conidiophores

but also influences the vegetative mycelium by affecting its protein synthesising capacity.

Transcription of *brlA* in *A. nidulans* results in two transcripts that are called *brlA α* and *brlA β* . Both transcripts are essential for proper conidiophore development (Prade & Timberlake 1993) and are controlled at the transcriptional (*brlA α* and *brlA β*) and translation level (*brlA β*) (Han & Adams 2001). Transcript *brlA β* contains a short upstream ORF (μORF) and a downstream ORF that encodes the same polypeptide as *BrlA α* but with an N-terminal extension of 23 aa (Prade & Timberlake 1993). Both polypeptides contain two C_2H_2 zinc finger DNA binding motifs. The *brlA α* and *brlA β* transcripts have different functions during asexual development. As mentioned above, inactivation of *brlA* results in indefinitely elongating stalks. In contrast, aberrant primary conidiophores develop in the $\Delta brlA\beta$ strain that can form secondary conidiophores (*i.e.* a conidiophore that develops from another conidiophore). Asexual development proceeds further in the $\Delta brlA\alpha$ strain but conidia are not produced (Fischer & Kües 2006). So far, it is not known whether transcription of *brlA* of *A. oryzae* and *A. fumigatus* also results in two transcripts.

BrlA activates a central regulatory pathway controlling temporal and spatial expression of conidiation specific genes (Boylan *et al.* 1987, Mirabito *et al.* 1989). This cascade is complex and involves, amongst others, the regulatory genes *abaA*, *wetA*, *stuA*, *medA*, and *vosA* (Fig. 4). Gene *abaA* (*abacus*) is a regulatory gene that is activated in *A. nidulans* by *BrlA* during sterigmata differentiation (Boylan *et al.* 1987, Breakspear & Momany 2007). A $\Delta abaA$ strain forms metulae that bud apically resulting in chains of cells with metula-like, rather than phialide-like, properties. In other words, phialides are not produced and therefore conidia are not formed (Boylan *et al.* 1987, Clutterbuck 1969, Sewall *et al.* 1990). The interaction of *AbaA* with *brlA* is complex (Fig. 4A). Gene *abaA* is activated by *BrlA* and, in turn, *AbaA* stimulates formation of *brlA α* transcripts but represses *brlA β* accumulation (Adams *et al.* 1998, Andrianopoulos & Timberlake 1994, Han & Adams 2001, Sewall *et al.* 1990). This is caused by *AbaA* binding to a responsive element in the *brlA β* locus (Han & Adams 2001). The net result of *abaA* inactivation is that *brlA* is over-activated (Aguirre 1993). The positive feedback loop of *brlA* itself is likely to be independent of *AbaA* because the over-expression of *brlA β* activates expression of *brlA α* in an *abaA* mutant (Han & Adams 2001) (Fig. 4A). Taken together, both *BrlA* and *AbaA* control transcript levels of *brlA α* and *brlA β* . *AbaA* regulates several other genes including *abaA* itself, *medA*, *wetA* (Fig. 4A), *vosA* (Fig. 4B), and the structural genes *yA* and *rodA* (for their functions see below) (Andrianopoulos & Timberlake 1994). Recently *abaA* was identified in *A. oryzae* (Ogawa *et al.* 2010) and *A. fumigatus* (Tao & Yu 2011). The role of *abaA* in *A. oryzae* is similar to that in *A. nidulans*. In the case of *A. fumigatus* *abaA* also delays autolysis and cell death.

During the late phase of conidiation, *wetA* (*wet white*) is activated by *abaA* (Fig. 4A). Normal conidiophores are formed by *wetA* mutants. However, the conidia do not form pigments, are not water repellent, and go in autolysis (Marshall & Timberlake 1991, Sewall *et al.* 1990). Gene *wetA* activates a set of genes in phialides and spores (*e.g.* *wA*), which are involved in making the conidial wall impermeable and mature (Marshall & Timberlake 1991). In addition, *WetA* seems to activate itself (Adams *et al.* 1998, Boylan *et al.* 1987, Marshall & Timberlake 1991, Ni & Yu 2007) and represses *abaA* and *brlA* (Tao & Yu 2011) (Fig. 4A). Gene *wetA* of *A. oryzae* (Ogawa *et al.* 2010) has a role similar to that in *A. nidulans*. In the case of *A. fumigatus* *wetA* seems to have an additional role (Tao & Yu 2011). It would also function in germ tube formation and reduced hyphal branching.

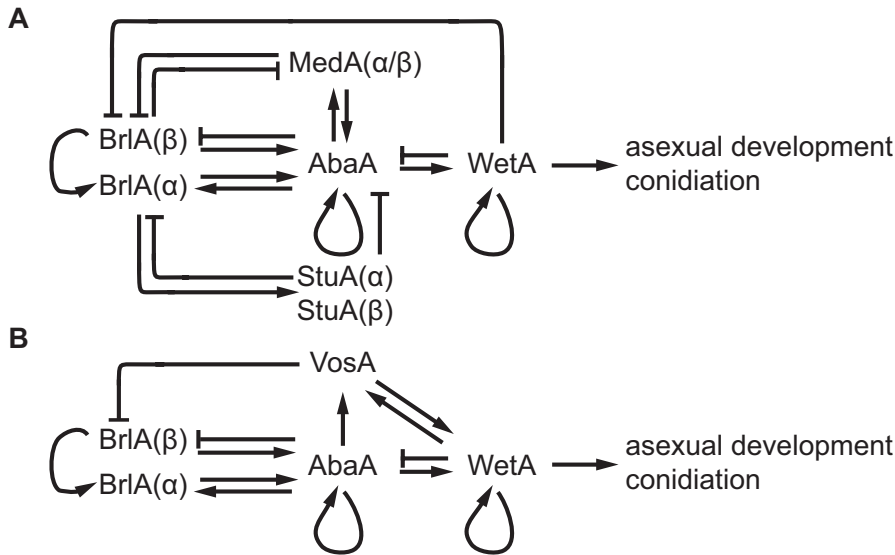


Fig. 4. The central regulatory network consisting of BrIA, AbaA and WetA initiates asexual development in *A. nidulans*. StuA and MedA (**A**) and VosA (**B**) are regulators of *brlA*, *abaA*, and *wetA*.

VosA (*viability of spore*) is a putative transcription factor of the velvet family (Tao & Yu 2011). This family, which is conserved in filamentous fungi, also includes VeA and VelB of *A. nidulans* (see below). Inactivation of *vosA* results in uncontrolled activation of asexual development, whereas its over-expression blocks sporulation. This may be the result of the observed inhibition of *brlA* by VosA (Tao & Yu 2011) (Fig. 4B). It should be noted that *vosA* is lowly expressed in the vegetative mycelium. Yet, these expression levels may be sufficient to control *brlA*. Gene *vosA* is particularly expressed during the formation of conidia and sexual ascospores, where it plays a role in resistance to stress conditions (see below).

The *stuA* and *medA* genes are classified as developmental modifiers. Their encoded proteins affect *brlA* and *abaA* expression (Fig. 4A). Mutations in *stuA* (*stunted*) of *A. nidulans* results in shortened aerial hyphae, shortened conidiophores and the absence of metulae and phialides. Conidiophores that are formed have reduced vesicles with abnormal numbers of nuclei. Only a few conidia can directly bud from the conidiophore vesicle. Thus, the morphology of the conidiophores is aberrant in *stuA* mutants, but neither temporal development nor conidiophore density is affected (Wu & Miller 1997). Gene *stuA* has a similar role in asexual development in *A. fumigatus* (Sheppard *et al.* 2005). *StuA* is a helix-loop-helix transcription factor with two transcription start sites. This leads to *stuA α* and *stuA β* transcripts, of which the former is most important for correct development (Aguirre 1993, Miller *et al.* 1991, Miller *et al.* 1992). Expression of *stuA* depends on *brlA*. As a result, transcript levels of *stuA* are increased 20-fold in conidiating cultures (Breakspear & Momany 2007, Busby *et al.* 1996, Miller *et al.* 1992). In turn, *StuA* directly or indirectly represses and spatially restricts *brlA* and *abaA* expression (Fig. 4A). With this ability *stuA* is involved in proper spatial distribution of AbaA and BrIA (Miller *et al.* 1992, Wu & Miller 1997). The *StuA* protein also stimulates *stuA* expression. This seems to be an indirect effect because its responsive elements are absent in the promoter (Wu & Miller 1997).

The *medA* (*medusa*) gene is conserved in filamentous fungi. Like other regulators, *medA* is transcribed at two initiation sites. While *stuA* of *A. nidulans* is required for proper spatial expression of *abaA* and *brlA*, *medA* is involved in proper temporal expression of these genes (Adams *et al.* 1998, Busby *et al.* 1996). Accumulation of both *brlA* transcripts is observed earlier in development in a $\Delta medA$ strain. Moreover, the mutant strain shows higher levels of *brlA β* , but not *brlA α* , transcripts. As a result, the ratio of *brlA α* and *brlA β* transcripts

is lowered. Gene *medA* thus acts as a repressor of *brlA* expression. In contrast, it is an activator of *abaA* expression. This is concluded from the observation that *abaA* transcription levels are reduced or even absent in the *medA* mutant (Busby *et al.* 1996, Miller *et al.* 1992). The molecular basis of MedA function is still unclear. A $\Delta medA$ strain forms repeated layers of sterigmata and frequent reinitiated secondary conidiophores (Clutterbuck 1969, Sewall *et al.* 1990). This phenotype resembles that of a strain of *A. nidulans* in which the chitin synthase genes *chsA* and *chsC* genes have been inactivated (Ichinomiya *et al.* 2005). In the latter strain, *abaA* expression is reduced. This indicates that *chsA* and *chsC* regulate expression of *abaA*, most probably in an indirect way. The $\Delta chsA\Delta chsC$ mutant shows a defective septum formation (Ichinomiya *et al.* 2005). Therefore, it was proposed that MedA is involved in septum formation on conidiophore structures. Taken together, conidiophore morphogenesis requires a finely tuned balance of at least BrIA, AbaA, MedA, and *StuA* (Busby *et al.* 1996), and possibly VosA and other velvet complex genes (Boylan *et al.* 1987, Ni & Yu 2007).

Trimeric G-protein signaling

Trimeric G-protein signaling is involved in the decision to grow vegetatively or to start asexual development. Gene *flbA* (*fluffy low brlA expression*) encodes an RGS domain protein, which negatively regulates vegetative growth signaling (Fig. 5). It does so by stimulating the intrinsic GTPase activity of the G α subunit FadA (*fluffy autolytic dominant*) of a heterotrimeric G-protein. As a result, the G α subunit is converted into the inactive GDP bound state (D'Souza *et al.* 2001, Yu *et al.* 1996, Yu *et al.* 1999) (Fig. 5). Overexpression of *flbA* in vegetative cells inhibits hyphal growth and stimulates conidiophore development even under conditions that normally prevent sporulation (Lee & Adams 1994a, Lee & Adams 1996). On the other hand, a mutation in *flbA* results in reduced *brlA* expression and a fluffy phenotype (hence the name *fluffy low brlA expression*). The $\Delta flbA$ strain does not form conidiophores. Instead, the mycelium proliferates uncontrolled and masses of undifferentiated aerial hyphae are formed. Both the submerged and aerial hyphae autolyse when colonies mature (Lee & Adams 1994a, Wieser *et al.* 1994). The autolytic phenotype of the *flbA* mutant can be partially overcome by deleting a class V endochitinase B (*chiB*). However, reduced cell viability cannot be restored in this way (Shin *et al.* 2009). Inactivation of *fadA* ($\Delta fadA$ or dominant-interfering *fadA* mutant) can also counteract the autolytic

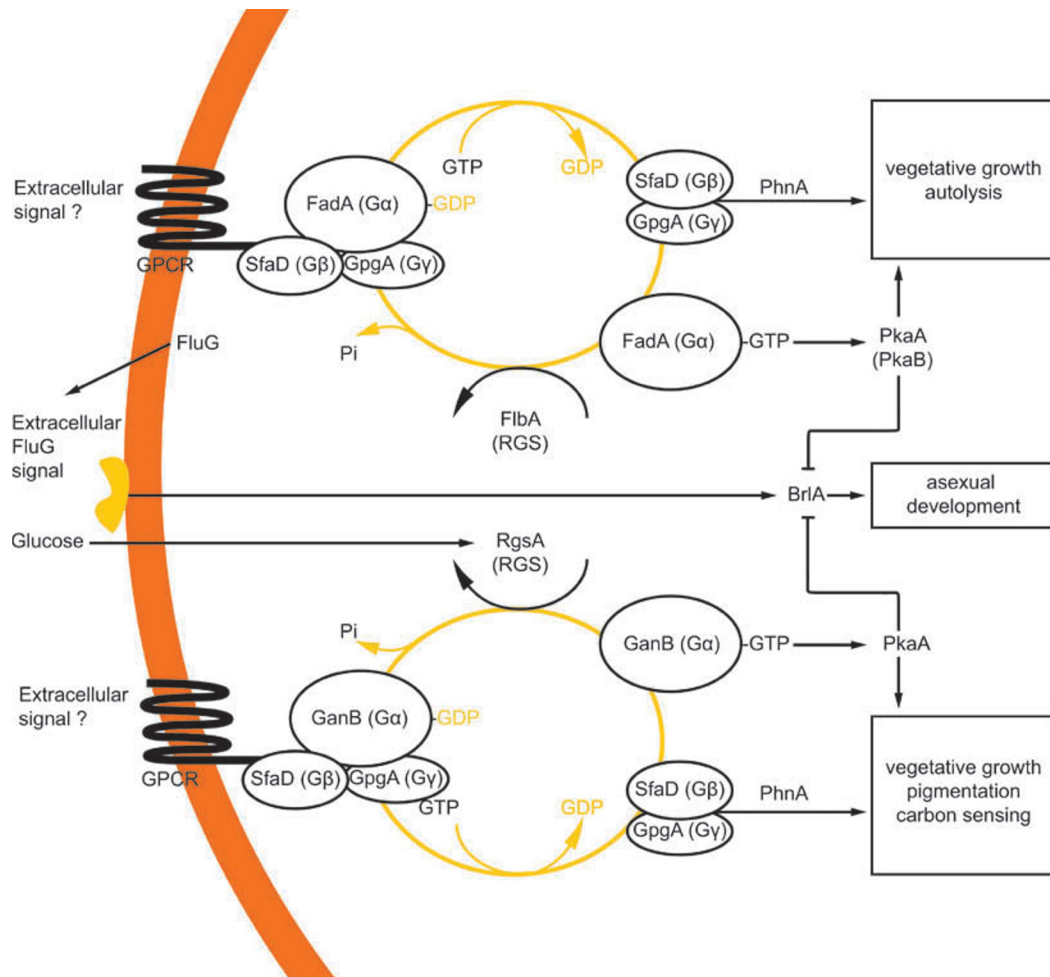


Fig. 5. Signaling cascades resulting in vegetative growth or asexual reproduction in *A. nidulans*. Signalling involves FluG (see Figure 6) and independently, two heterotrimeric G-protein complexes, both consisting of SfaD and GpgA (the G β γ subunits) and the G α subunits FadA and GanB, respectively. GTP-bound FadA and GanB stimulate vegetative growth via the cAMP PkaA pathway and repress asexual reproduction via *brlA*. The RGS proteins FlbA and RgsA hydrolyze the GTP bound to FadA and GanB, respectively, thereby repressing vegetative growth and promoting asexual development. The SfaD-GpgA dimer also stimulates vegetative growth. This is regulated by PhnA. (Adapted from Yu 2006).

phenotype of the *flbA* mutant. This is in agreement with the function of FlbA to convert FadA into the inactive GDP bound state (Fig. 5). A constitutively active *fadA* mutant, *fadA*^{G42R}, results in autolytic mutants similar to the *flbA* mutant (Hicks *et al.* 1997, Yu *et al.* 1996). The constitutively active *fadA*^{G42R} mutant phenotype cannot be suppressed by overexpression of *flbA* (Yu *et al.* 1999).

In its inactive GDP-bound form, FadA of *A. nidulans* forms a heterotrimeric G-protein with the β - and γ -subunits encoded by *sfaD* and *gpgA*, respectively (Rosén *et al.* 1999, Seo *et al.* 2005, Yu *et al.* 1996, Yu *et al.* 1999). When FadA becomes GTP bound, this α -subunit dissociates from SfaD and GpgA (Fig. 5). Deletion of *sfaD* (Rosén *et al.* 1999) or *gpgA* (Seo *et al.* 2005) suppress the phenotype of the *flbA* mutant. Moreover, in a wild-type background reduced vegetative growth is observed in these deletion strains. Inactivation of *sfaD* (Rosén *et al.* 1999) but not *gpgA* (Seo *et al.* 2005) also causes hyper-sporulation. The Δ *sfaD* Δ *gpgA* double mutant shows a phenotype identical to those of the Δ *sfaD* mutant (Seo *et al.* 2005). This shows that Δ *sfaD* is epistatic to Δ *gpgA* and that SfaD can induce inappropriate conidiation even in the absence of GpgA. Notably, constitutive activation of *fadA* in the absence of *sfaD* is sufficient to cause proliferation of undifferentiated hyphae (Seo *et al.* 2005, Wieser *et al.* 1997). Taken together, FadA and SfaD-GpgA have overlapping functions in stimulating vegetative growth (Rosén *et al.* 1999, Seo *et al.* 2005). The phosphodiesterase like protein A (PhnA) seems to be involved in positively regulating G β γ

signaling in *A. nidulans*. Deletion of *phnA* results in a phenotype similar to that of a Δ *sfaD* strain (Seo & Yu 2006). This would agree with the role of phosphodiesterase like proteins to act as chaperones for G β γ assembly (Yu 2006). Finally, deletions in *sfaD*, *fadA* or *gpgA* do not suppress conidiation defects in a *fluG* mutant. Therefore, the FadA/SfaD/GpgA vegetative growth-signaling cascade seems to be distinct from that of the FluG pathway (Seo *et al.* 2005).

GTP-bound-FadA promotes vegetative growth and inhibits asexual and sexual development by activating a cAMP-PKA signaling cascade (Shimizu & Keller 2001) (Fig. 5). The cAMP dependent protein kinase A catalytic subunit (PKA) encoded by *pkaA* has a major role in the stimulation of vegetative growth and the repression of conidiation (Lafon *et al.* 2005, Lafon *et al.* 2006, Seo *et al.* 2005, Yu & Keller 2005). Inactivation of *pkaA* results in hyper-sporulation and reduced radial growth (Shimizu & Keller 2001). On the other hand, overexpression of *pkaA* leads to decreased sporulation accompanied by a fluffy-like appearance. Deletion of the other *pka* gene in the genome of *A. nidulans*, *pkaB*, causes no apparent phenotype (Ni *et al.* 2005). However, over-expression of *pkaB* reduces conidiation and increases vegetative growth on solid medium. Moreover, it complements the reduced radial growth of the Δ *pkaA* strain. Apparently, PkaB functions as a backup for PkaA (Ni *et al.* 2005, Seo *et al.* 2003).

The FlbA/FadA/SfaD/GpgA pathway seems to be conserved in *A. nidulans*, *A. oryzae* and *A. fumigatus* (Mah & Yu 2006, Ogawa *et*

al. 2010, Yu 2006). Like in *A. nidulans*, the SfaD-GpgA complex is involved in stimulating vegetative growth in *A. fumigatus* (Shin *et al.* 2009). However, there are some differences in the case of the other components. Deletion of *flbA* in *A. nidulans* (Wieser *et al.* 1994), *A. oryzae* (Ogawa *et al.* 2010) or *A. fumigatus* (Mah & Yu 2006) results in low *brlA* expression, and reduced conidiation. In contrast to *A. nidulans* and *A. oryzae*, the autolysis phenotype is missing in *A. fumigatus*. Moreover, in both *A. oryzae* and *A. fumigatus* hyphal proliferation is reduced in the $\Delta flbA$ strain, blocking formation of aerial hyphae in the case of *A. oryzae* (Ogawa *et al.* 2010). Like in *A. nidulans*, FadA of *A. oryzae* (Ogawa *et al.* 2010) and *A. fumigatus* (Mah & Yu 2006) repress conidiation. Remarkably, in *A. oryzae* it also represses vegetative growth (Ogawa *et al.* 2010), while in *A. fumigatus* vegetative growth is stimulated (Liebmann *et al.* 2004, Shimizu & Keller 2001).

Apart from FadA, two other G α subunits are present in *A. nidulans* (GanA and GanB) and *A. fumigatus* (GpaA and GpaB), and three in *A. oryzae* (GanA, GanB, GaoC) (Chang *et al.* 2004, Lafon *et al.* 2006, Liebmann *et al.* 2003, Rosén *et al.* 1999, Seo *et al.* 2005, Yu *et al.* 1996). In contrast to *ganB*, the functions of *ganA* and *gaoC* have not been established yet (Chang *et al.* 2004, Han *et al.* 2004a, Lafon *et al.* 2006). Like FadA, GanB in its inactive form interacts with SfaD and GpgA (Seo *et al.* 2005) (Fig. 5), which in fact are the only β and γ subunits of trimeric G-proteins in *A. nidulans*, *A. fumigatus*, and *A. oryzae* (Lafon *et al.* 2006). The $\Delta ganB$ strain shows hyper-sporulation in submerged cultures and earlier expression of the *brlA* transcripts. Constitutive activation of GanB results in reduced hyphal growth and a severe defect in asexual sporulation (Chang *et al.* 2004). Like FadA, GanB therefore seems to be involved in repression of *brlA* and inhibition of asexual sporulation (Chang *et al.* 2004).

RgsA (*regulator of G protein signaling*) is a repressor of GanB signaling. Colonies of the $\Delta rgsA$ strain are reduced in size, form more aerial hyphae, and accumulate dark brown pigments (Han *et al.* 2004a). Expression of a constitutively active RgsA results in hyper-sporulation in submerged cultures and earlier expression of the *brlA* transcripts (Han *et al.* 2004a). The presence of glucose is claimed to result in the increase of *rgsA* mRNA levels, and this would result in down-regulation of GanB mediated signaling. In cases of stress or unfavorable carbon sources, *rgsA* levels decrease and as a consequence GanB-GTP signaling is activated (Han *et al.* 2004a). In *A. fumigatus* the outcome of the signaling pathway involving the GanB orthologue GpaB is different. Growth and conidium formation of a $\Delta gpaB$ strain is slightly decreased (Liebmann *et al.* 2004). This and other data show that GpaB signaling in *A. fumigatus* promotes asexual sporulation via PKA. However, the PkaC1 cascade in *A. fumigatus* is complex, since it also promotes vegetative growth, when activated by GpaA (Liebmann *et al.* 2003).

Upstream activators of *brlA*

FluG activates sporulation by a derepression pathway that involves the SfgA protein (Fig. 6). Gene *sfgA* (*suppressor of fluG*) is predicted to encode a transcription factor with a Gal4-type Zn(II)₂Cys₆ binuclear cluster DNA binding motif (Seo *et al.* 2006). Mutations in *sfgA* bypass the need for FluG during asexual development. The $\Delta sfgA$ strain shows hyperactive sporulation in liquid submerged cultures. Overexpression of *sfgA* results in delayed and reduced levels of *brlA* mRNA, and in colonies with reduced conidiation. Apparently, the primary role of FluG is to remove the repressive effects of SfgA (Seo *et al.* 2006). The low FluG levels in young colonies would result in *sfgA*-mediated repression of conidiation.

Once the FluG factor has accumulated above a certain threshold, it inhibits the repression of conidiation by SfgA (Seo *et al.* 2006). SfgA has also been proposed to negatively regulate FlbA (Fig. 6). By this, the repression of SfgA by FluG will result in both activation of conidiation and inhibition of FadA-mediated stimulation of growth.

Apart from *flbA*, four other regulatory genes, *flbB*, *flbC*, *flbD*, and *flbE*, have been identified that act upstream of *brlA* (Wieser *et al.* 1994). All *flb* mutant strains show low *brlA* expression and a fluffy phenotype (Wieser *et al.* 1994). The mutants grow indeterminately and produce masses of aerial hyphae. The *flb* mutants can restore conidiation in *fluG* loss-of-function mutants (Wieser *et al.* 1994). These results indicate that the *flb* genes are involved in responding to the diffusible signaling molecule, produced by FluG, which is necessary for conidiation (Wieser *et al.* 1994). SfgA acts downstream of *fluG* but upstream of *flbA-D*. As mentioned above, FluG is assumed to repress *sfgA* thus releasing the repression of the *flb* genes. Notably, *flb* genes are involved in regulation of *fluG* expression (Ruger-Herreros *et al.* 2011). A repressing function of FlbA on *fluG* expression is indicated by a 7-fold higher *fluG* expression in a $\Delta flbA$ strain. In contrast, *fluG* expression is stimulated by FlbB and FlbC. For instance, a $\Delta flbC$ shows no *fluG* mRNA accumulation (Ruger-Herreros *et al.* 2011).

The *flbC* gene encodes a transcriptional regulator containing two C₂H₂ zinc finger DNA binding domains (Kwon *et al.* 2010a). The $\Delta flbC$ strain shows delayed and reduced conidiation, while overexpression causes restricted hyphal growth and delayed conidiation. In wild type colonies, FlbC is localised in nuclei of vegetative hyphae and in conidiophores (*i.e.* not in conidia). Here, FlbC activates *brlA*, *abaA*, and *vosA* but not *wetA*. Overexpression of *flbC* not only inhibits vegetative growth in a wild-type strain but also in a $\Delta abaA$ or $\Delta brlA$ background. Thus, FlbC plays a direct role in repressing vegetative growth, independent of *brlA* or *abaA* (Fig. 6). The deletion of *flbC* also results in highly enhanced sexual fruiting body formation (see below) (Kwon *et al.* 2010a). Taken together, FlbC has a repressive role in sexual development, but positively regulates germination and asexual development (Kwon *et al.* 2010a). FlbC acts in a pathway parallel to that of *flbB*, *flbD*, and *flbE* (Garzia *et al.* 2010, Wieser & Adams 1995). Absence of FlbC does not affect expression of *flbB* or *flbE* and vice versa. Moreover, double mutants cause additive effects, resulting in a prolonged delay in conidiation (Kwon *et al.* 2010a). It has been proposed that FlbC coordinates activation of *brlA* together with a FlbB/FlbD transcriptional complex (Ettebest *et al.* 2010a, Garzia *et al.* 2010) (Fig. 6). Promoter binding regions of FlbC and FlbB/FlbD may overlap (Garzia *et al.* 2010, Han & Adams 2001).

The *flbB* gene encodes a fungal specific bZIP-type transcription factor (Ettebest *et al.* 2008). A $\Delta flbB$ strain shows defective branching patterns, delayed conidiation with a fluffy appearance and, under stress conditions, a high autolysis rate. Overexpression of *flbB* results in reduced conidiophore vesicle formation, and a reduced number of metulae (Ettebest *et al.* 2008, 2009). FlbB is located within the cytoplasm at the hyphal apex during early vegetative growth. In contrast, it preferentially accumulates at the most apical nucleus during later stages of growth (Ettebest *et al.* 2008). The repressor SfgA may be a key intermediate in the process of nuclear localisation of FlbB. This is indicated by the fact that FlbB is found in all nuclei of $\Delta sfgA$ hyphae, rather than only at the hyphal tip as observed in the wild-type strain. Gene *flbE* encodes a protein without any known conserved domain (Garzia *et al.* 2009). Expression of *brlA* and *vosA* is delayed in the $\Delta flbE$ strain (Kwon *et al.* 2010a). This is accompanied by absence of conidiophore formation, a fluffy appearance of the colonies, accelerated vegetative growth, and accelerated autolysis and

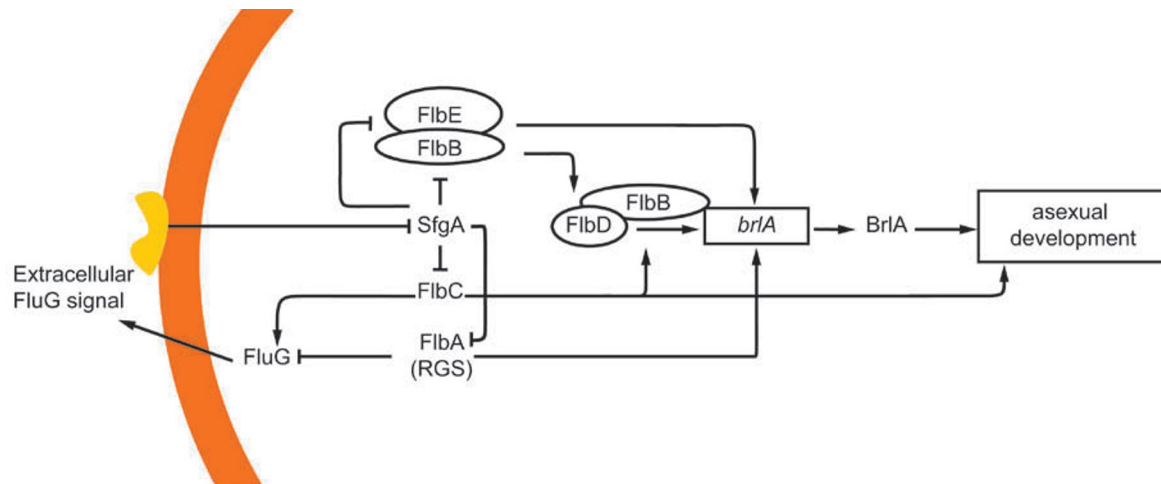


Fig. 6. Model of upstream regulation of *brlA*. FluG is involved in the formation of an extracellular factor that activates an unknown receptor. At a certain concentration of FluG, the general suppressor SfgA is inhibited removing the repression of the *flb* genes. FlibB and FlibE form a complex that activates *brlA* leading to asexual development. FlibC activates *brlA* together with the FlibB/FlibD transcription complex. FlibC also activates *fluG* and regulatory genes that act downstream of BrlA. FlibA activates *brlA* by inactivating FadA and probably plays a role in repressing *fluG*. (Adapted from Etxebeste *et al.* 2010).

cell death. FlibE is localised at hyphal tips. In fact, it co-localises with FlibB. Localisation of these proteins at the hyphal tip depends on the presence of F-actin. This is concluded from the fact that disintegration of the actin filaments causes mis-localisation of FlibB and FlibE. Localisation of FlibB and FlibE was also lost in a $\Delta flbE$ and a $\Delta flbB$ strain, respectively. These results indicate that these proteins depend on each other for proper localisation at the hyphal apex. It has also been shown that FlibB stability is affected by the absence of a functional form of FlibE (Garzia *et al.* 2009). FlibE may thus protect FlibB from proteolytic degradation. It may do so by physical interaction with FlibB. At least, such an interaction was shown *in vivo* (Garzia *et al.* 2009). Taken together, FlibE and FlibB function in close association with each other and are functionally interdependent (Garzia *et al.* 2009).

The FlibB/FlibE complex is a requisite for *flbD* expression in the wild-type (Garzia *et al.* 2010) (Fig. 6). FlibD is a c-Myb transcription factor. Deletion of its encoding gene results in delayed conidiation and a fluffy phenotype (Wieser *et al.* 1994, Wieser & Adams 1995). Overexpression causes sporulation in liquid submerged cultures. This is due to inappropriate activation of *brlA* (Wieser & Adams 1995). As mentioned above, the FlibB/FlibE complex is found at hyphal apices (Garzia *et al.* 2009). In contrast, FlibD is found in all nuclei of vegetative hyphae of *A. nidulans*. Thus, other transcription factors seem also to be involved in the regulation of *flbD* expression (Etxebeste *et al.* 2010a, Garzia *et al.* 2009, 2010). Not only depends *flbD* expression on the presence of FlibB, FlibD also interacts with this protein (Etxebeste *et al.* 2010a, Garzia *et al.* 2009, 2010). The underlying mechanism is so far unknown but might involve a translationally modified form of FlibB (Garzia *et al.* 2010). Both FlibD and the FlibB/FlibE complex seem to activate *brlA* expression (Garzia *et al.* 2010) (Fig. 6). Overexpression of *flbD* restores the conidiation defect in the $\Delta flbE$ strain (Kwon *et al.* 2010a), suggesting an additive effect of both pathways. Interestingly *flbB* and *flbD* transcripts disappear shortly after *brlA* activation (Etxebeste *et al.* 2008, Wieser & Adams 1995). However, the mechanism underlying this effect is independent of *brlA* levels (Garzia *et al.* 2010).

The *flb* genes are conserved in *A. fumigatus*, *A. oryzae* and *A. nidulans* (Kwon *et al.* 2010a, Ogawa *et al.* 2010). The phenotypes of the Δflb strains of *A. oryzae* are similar to those of *A. nidulans*. These results indicate that the functions of these

regulatory genes are conserved between *A. oryzae* and *A. nidulans* (Ogawa *et al.* 2010). The *A. fumigatus flbE* could complement the $\Delta flbE$ phenotype in *A. nidulans* (Kwon *et al.* 2010a), suggesting a similar role for these orthologues. Indeed, FlibE in *A. fumigatus* is necessary for proper control of conidiation and *brlA* and *vosA* expression. However, deletion of *flbE* does not cause an elevated vegetative proliferation or accelerated autolysis or cell death in *A. fumigatus* (Kwon *et al.* 2010a). Inactivation of *flbB* in *A. fumigatus* results in delayed and reduced sporulation, and precocious cell death. Moreover, expression of *brlA* and *abaA* is affected. In contrast to *A. nidulans*, the FlibB protein is in *A. fumigatus* encoded by two transcripts, *flbBa* and *flbB β* . The longest transcript, *flbB β* , is constitutively expressed, while the *flbBa* transcript is found during progression of conidiation (Xiao *et al.* 2010). Both transcripts are needed for proper asexual development. The *flbB* gene of *A. nidulans*, encoding one transcript, only partially complements the $\Delta flbB$ strain of *A. fumigatus*. FlibC and FlibD functions in *A. fumigatus* are probably similar to *A. nidulans*, but characterisation is ongoing at the moment (Xiao *et al.* 2010).

MAPK pathways and downstream targets

A mitogen activated protein kinase (MAPK) module consists of three kinases known as MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK. MAPKKK is phosphorylated in response to external stimuli. Active (*i.e.* phosphorylated) MAPKKK phosphorylates MAPKK, which in turn phosphorylates MAPK. Phosphorylated MAPK enters the nucleus and activates transcription factors by phosphorylation (Dickman & Yarden 1999). A MAPK pathway has been shown to repress asexual development and to stimulate sexual development in *A. nidulans* (see below). This pathway includes MAPKKK SteC (Wei *et al.* 2003). Growth of the $\Delta steC$ strain on minimal medium results in reduced growth and hyphae are more curled and more branched than the wild-type. Conidiophore development is initiated as in wild-type, but the length distribution of the stalks is affected. Metulae and phialide morphology is normal in the majority of the conidiophores. In about 2 % of the conidiophores, however, only a few metulae arise from the vesicle and these metulae do not differentiate normally. In addition, secondary conidiophores grow out of the vesicle. Finally, the mutant produces 1–2 % of very large conidia in addition to wild-type-like spores. A translational fusion of SteC and GFP localises in metulae, phialides and young

conidia but not in conidiophore stalks. The targets of SteC during asexual development have not yet been identified. A functional study of MAPKK genes in *A. nidulans* is lacking and inactivation of the MAPK gene, *mpkB*, does not affect sporulation (Paoletti *et al.* 2007). Similarly, the putative target of *mpkB*, *steA*, is not involved in asexual development (Vallim *et al.* 2000). In contrast, a potential other target of *mpkB*, *nsdD* (*never in sexual development*), does affect conidiation (Han *et al.* 2001). A $\Delta nsdD$ strain starts to conidiate earlier. On the other hand, over-expression of *nsdD* suppresses formation of conidiophores. Similarly, deletion of the transcription factor gene *nsdC* results in derepression of conidiation. In this case, the effect depends on the carbon source. Derepression is observed on carbon sources favoring sexual development. This indicates that NsdC acts as a repressor of asexual development under those conditions (Kim *et al.* 2009).

The role of hydrophobins in asexual development

Regulators activate target genes that fulfill a structural or enzymatic role in the formation of asexual structures. Genes have been identified that are upregulated in conidiophores and / or conidia (Bleichrodt *et al.* 2013, van Leeuwen *et al.* 2013a, b). Hydrophobin genes are examples of such target genes. Hydrophobins mediate the escape of hyphae into the air and make aerial structures such as conidiophores and conidia hydrophobic (Wösten 2001). This hydrophobicity ensures that reproductive structures do not fall back in the substrate under humid conditions and serves dispersal of conidia by wind or vectors. Hydrophobins may also affect the cell wall architecture (van Wetter *et al.* 2000) and mediate attachment to hydrophobic substrates (Wösten 2001). In the case of *A. fumigatus* it has also been shown that hydrophobins prevent immune recognition by the host (Aimanianda *et al.* 2009, Aimanianda & Latgé 2010, Bruns *et al.* 2010, Dagenais *et al.* 2010, Paris *et al.* 2003). Moreover, the hydrophobin RolA of *A. oryzae* recruits cutinase by adsorbing to the substrate of the enzyme. As a result, the substrate is efficiently degraded (Takahashi *et al.* 2005).

The *A. nidulans*, *A. fumigatus*, *A. oryzae*, and *A. niger* genomes contain 6, 4-5, 2, and 8 hydrophobin genes, respectively (Jensen *et al.* 2010). One or more of the hydrophobins in each species enable hyphae to grow into the air by lowering the surface tension of the aqueous environment (see above). The aerial structures are then coated with hydrophobins to make them hydrophobic. So far, it has not been established which hydrophobins line aerial hyphae and the conidiophore stalk and vesicle of aspergilli. However, hydrophobins have been identified that coat sterigmata and conidia. The hydrophobin gene *rodA* (*rodlet*) of *A. nidulans* is expressed during the final stages of conidiophore formation. It is not expressed by vegetative hyphae and conidia (Boylan *et al.* 1987, Stringer *et al.* 1991). Expression of *rodA* is mediated by BrlA but not by AbaA or WetA. This is based on the finding that a $\Delta brlA$ strain does not express *rodA* but expression of the hydrophobin gene is not affected in the $\Delta abaA$ and $\Delta wetA$ strains. A $\Delta rodA$ strain forms wettable conidiophores and conidia. This is accompanied by the absence of the rodlet layer (Stringer *et al.* 1991). As a consequence, $\Delta rodA$ conidia adhere to each other in water. This affects their dispersal by air flow. The rodlet layer is also absent at the surface of metulae and phialides of the $\Delta rodA$ strain (Stringer *et al.* 1991). Experimental data imply that the RodA protein is produced by the sterigmata and diffuses to the outer surface of these structures as well as to that of the conidia to form the rodlet layer. *Aspergillus fumigatus* contains an ortholog of *rodA*.

Inactivation of this gene results in a phenotype similar to that in *A. nidulans* (Paris *et al.* 2003, Thau *et al.* 1994). Moreover, *rodA* of *A. fumigatus* is involved in attachment of spores to particular substrates. The adhesion of the $\Delta rodA$ conidia is reduced in the case of collagen and bovine serum albumin but is not affected in the case of pneumocytes, fibrinogen, and laminin.

The *dewA* (*detergent wettable*) hydrophobin gene is expressed in sporulating cultures but not in cultures that grow vegetatively (Boylan *et al.* 1987, Stringer & Timberlake 1995). Unlike RNA of *rodA*, transcripts of *dewA* are present in conidia (Breakspear & Momany 2007). In agreement, immuno-detection showed that DewA hydrophobin is specifically present in cell walls of conidia, especially in mature spores. Expression of *dewA* is not only abolished in the $\Delta brlA$ strain but also in the $\Delta abaA$ and $\Delta wetA$ strains. Forced expression of *brlA* or *abaA* has only resulted in *dewA* expression in strains with an intact copy of *wetA*. Thus, *dewA* is regulated by *brlA* and *abaA* via *wetA* (Stringer & Timberlake 1995). Conidia of $\Delta dewA$ are still covered with the rodlet layer. Thus, DewA is not essential for the rodlet layer on spores. Yet, when present, it may be part of it. The conidia of the $\Delta dewA$ strain do not wet in water. However, they wet more easily compared to wild-type spores when detergent is added to the water. A role of DewA in surface hydrophobicity is also indicated from the fact that wettability of the $\Delta dewA \Delta rodA$ strain is much more pronounced when compared to the $\Delta dewA$ and the $\Delta rodA$ strains. *Aspergillus fumigatus* also contains a hydrophobin that is present at the surface of conidia but whose presence is not essential for the rodlet layer of conidia (Paris *et al.* 2003). This hydrophobin, RodB, is different in sequence when compared to DewA. As mentioned above, the surface of $\Delta rodA$ conidia of *A. fumigatus* lacks the rodlet layer. Instead, the surface is granular. In contrast, the surface of $\Delta rodA \Delta rodB$ conidia is amorphous. Taken together, RodB of *A. fumigatus* may be part of the rodlet layer of conidia when RodA is present. In the absence of RodA, RodB forms a granular structure and does not form rodlets.

SEXUAL DEVELOPMENT

About one-third of the described species of *Aspergillus* have a known sexual stage (Geiser 2009). However, analysis of genome sequences suggests that most, if not all, *Aspergillus* species should be able to reproduce sexually (Dyer & Paoletti 2005). Indeed, *A. fumigatus*, *A. flavus*, *A. parasiticus*, and *A. nomius* have been shown to have a sexual stage (Horn *et al.* 2009a, b, 2011, O'Gorman *et al.* 2009). In the case of *A. fumigatus*, this was 145 years after this fungus was described for the first time. Clearly, the conditions under which sexual reproduction takes place differs between aspergilli; some of which being more restrictive (e.g. *A. fumigatus*) than others (*A. nidulans*).

Aspergillus species with a sexual state can be either homothallic or heterothallic. Homothallic species undergo sexual reproduction without the need to cross with a compatible partner. *Aspergillus nidulans* (Paoletti *et al.* 2007) and *Neosartorya fischeri* (Rydholm *et al.* 2007) are known to be homothallic. It should be noted that *A. nidulans* can also be heterothallic and can thus cross with a partner. In contrast, *A. flavus* (Horn *et al.* 2009a), *A. parasiticus* (Horn *et al.* 2009b), *A. fumigatus* (O'Gorman *et al.* 2009), and *A. nomius* (Horn *et al.* 2011) have been shown to be exclusively heterothallic. The genomic sequences of *A. niger* (Pel *et al.* 2007) and *A. oryzae* (Rokas & Galagan 2008) predict that these fungi are either heterothallic or truly asexual (Pál *et al.* 2008). The sequenced

A. niger strains only contain the *MAT1-1* mating type locus (see below) but otherwise seem to encompass a complete set of genes enabling sexual development.

Sexual development in *A. nidulans* is a highly complex process, ultimately resulting in fruiting bodies of 125–200 μm in diameter that are called cleistothecia. Sexual development is influenced by both environmental and intrinsic signals, and occurs only when all prerequisites are met. The process of sexual development starts after 40–50 h of cultivation at 37 °C in the center of the colony and mature cleistothecia can be found after approximately 96 h (Seo *et al.* 2004). The production of Hülle cells (see below) represents the first sign of sexual development. Subsequently, hyphae fuse to form a dikaryon. As mentioned, in the case of *A. nidulans* these hyphae may originate from the same colony (self-fertilisation) or from another individual (out-crossing). The hyphae that fuse are morphologically similar. Thus, male antheridia and female ascogonia cannot be distinguished (Benjamin 1955). A population of dikaryotic cells originates from a single cell fusion event, and gives rise to a single cleistothecium (Champe *et al.* 1994). The Hülle cells surround the dikaryotic hyphae and form an increasingly packed “nest”. They differentiate into thick-walled globose cells (Champe & Simon 1992, Pöggeler *et al.* 2006) that are believed to provide protection and nutrition (Zonneveld 1977). An intertwined network of ascogenous dikaryotic hyphae develops within the cleistothecial shell. Nuclear fusion takes place inside the ultimate branches of the dikaryotic hyphae, which represent the young asci. This is immediately followed by meiosis and a post-meiotic mitosis, thus resulting in eight nuclei. These nuclei are then separated from each other by membranes, giving rise to eight red-pigmented spores in each of the 10.000 asci within one fruiting body (Pöggeler *et al.* 2006). A second post-meiotic mitosis makes that mature ascospores are binucleate (Braus *et al.* 2002).

Regulation of sexual development

Mating type loci

Strains of heterothallic ascomycetes contain either a *MAT1-1* or a *MAT1-2* mating type locus. These loci are not related in sequence, and are therefore called ideomorphs. The *MAT1-1* locus contains a gene encoding an α -domain transcription factor, while the *MAT1-2* encodes a high mobility group-domain (HMG) transcription factor (Turgeon & Yoder 2000). The homothallic species *A. nidulans* contains both the *MAT1-1* and *MAT1-2* mating type loci, which reside on different chromosomes (Paoletti *et al.* 2007). Inactivation of *MAT1-1* or *MAT1-2* does not affect vegetative growth or asexual sporulation. Moreover, Hülle cells and cleistothecia are formed under conditions inducing the sexual cycle. However, the cleistothecia are lower in number and smaller than those of the wild-type strain (Paoletti *et al.* 2007). In addition, no ascospores are formed within the cleistothecia. This and the fact that fruiting body development can be induced by placing both transcriptional activator genes under an inducible promoter shows that *MAT1-1* and *MAT1-2* are the master switches of sexual reproduction.

In filamentous ascomycetes, the *MAT* loci determine cell type identity and may confer nuclear recognition and proliferation (Coppin *et al.* 1997, Shiu & Glass 2000). In addition, *MAT* loci regulate expression of a pheromone-signaling system. This system, which is generally involved in the detection of a mating partner, has been best studied in *Saccharomyces cerevisiae* (Banuett 1998). In *S. cerevisiae* the α - and α -cells each produce a peptide pheromone and a receptor for the pheromone of the other

partner. Binding of the pheromone to the receptor that is produced by the compatible partner triggers G protein-mediated signaling via a mitogen activated protein kinase (MAPK) cascade. As a result, a homeodomain transcription factor gene is activated that induces cell cycle arrest, and activates the mating process. Components of a pheromone-signaling pathway similar to that of *S. cerevisiae* have been detected in *A. nidulans* and other sequenced *Aspergillus* species, including pheromone and pheromone receptor genes (Pál *et al.* 2008). Notably, expression of the pheromone and pheromone receptor genes is not affected when the *MAT* genes are inactivated in *A. nidulans*. Apparently, these genes are not under control of the mating type loci (Paoletti *et al.* 2007). They may be induced by environmental conditions that stimulate sexual reproduction. In contrast, *MAT1-1* of *A. fumigates* does stimulate the pheromone gene *ppgA*, while it is repressed by *MAT1-2* (Szewczyk & Krappmann 2010).

Pheromones, pheromone receptors, and intracellular signalling

In *A. nidulans* only one putative pheromone gene has been identified, which has homology to the gene encoding the α -pheromone of *S. cerevisiae* (Dyer *et al.* 2003). Until now, it has not been confirmed that the encoded product binds to a pheromone receptor(s). Alternatively, psi factor (see below) may bind to the receptor protein(s) (Seo *et al.* 2004). Receptor proteins have been identified in *A. nidulans* on basis of homology with the pheromone receptor genes of *S. cerevisiae* (Seo *et al.* 2004). Inactivation of these genes, *gprA* and *gprB* (G protein receptor), does not affect growth rate, Hülle cell formation, and asexual sporulation. However, $\Delta gprA$ and $\Delta gprB$ strains produce less cleistothecia that are smaller than wild-type fruiting bodies under homothallic conditions. The $\Delta gprA$ and $\Delta gprB$ cleistothecia produce only 5 % of the ascospores when compared to the wild-type but viability of these spores is not affected. The $\Delta gprA\Delta gprB$ strain does not form cleistothecia at all under homothallic conditions but Hülle cells are still formed. Notably, sexual development is not impaired under heterothallic conditions; *i.e.* when strains are crossed in which either or both *gprA* and *gprB* have been inactivated. Heterokaryon formation in this case can be selected by using parental strains that have different auxotrophic markers. Taken together, pheromone receptors genes, and therefore the pheromone signaling pathway, is only required for homothallic sexual development (Seo *et al.* 2004). Genes *gprA* and *gprB* represent two out of sixteen genes encoding seven-transmembrane-spanning G protein coupled receptor proteins (GPCR) (Lafon *et al.* 2006). Gene *gprD* is another GPCR gene. Inactivation of *gprD* results in a strain with impaired vegetative growth and exhibiting extremely enhanced sexual development (Han *et al.* 2004a, b). Deletion of *gprA* and *gprB* rescue the growth defects of the $\Delta gprD$ strain and fruiting bodies are no longer formed. Thus, GprD functions downstream of GprA/B (Han *et al.* 2004a, b).

In general, binding of pheromones sensitises the pheromone receptors. As a result, the receptors physically interact with a heterotrimeric G protein. This induces the exchange of GDP for GTP on the α -subunit of the heterotrimeric G-protein, resulting in its dissociation from the $\beta\gamma$ -subunits (Fig. 7). Genes *sfaD* and *gpgA* are the only β and γ subunit encoding genes in the genome of *A. nidulans*. Inactivation of *sfaD* or *gpgA* abolishes and severely affects cleistothecia formation under homothallic and heterothallic conditions, respectively. Moreover, under both conditions more Hülle cells are formed (Rosén *et al.* 1999, Seo *et al.* 2005). The genome of *A. nidulans* contains three α -subunit encoding genes; *ganA*, *ganB*, and *fadA* (see above). It has not fully been established

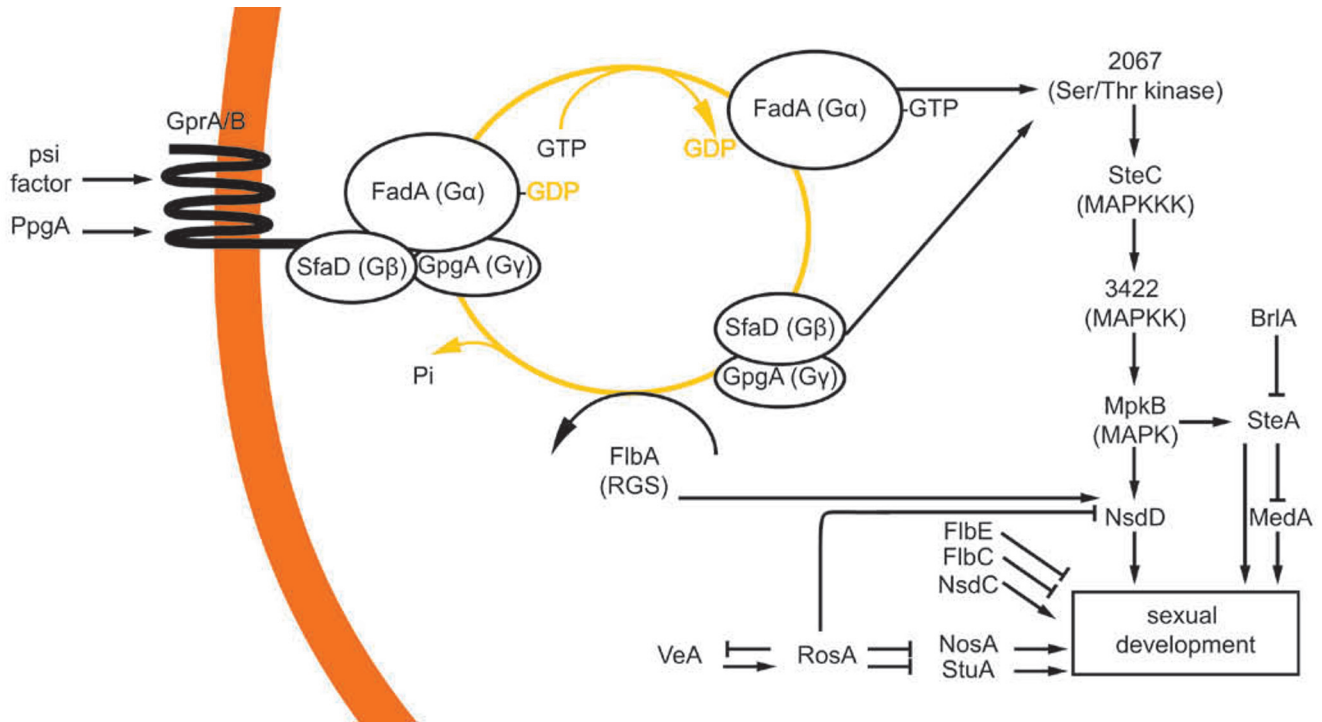


Fig. 7. GprA/B mediated signalling resulting in sexual development of *A. nidulans*. Signalling involves the heterotrimeric G-protein consisting of SfaD, GpgA and FadA. FadA bound to GTP activates a MAP kinase cascade (hypothetical protein AN2067.2, SteC, hypothetical protein AN3422.2 and MpkB). This in turn, activates the regulators NsdD and SteA that induce sexual development. In addition, SteA inhibits MedA that is also involved in activating sexual development. SteA, NsdD, and NosA also activate the sexual development program, while FibC and FibE are repressors of this pathway. RosA is a transcriptional inhibitor of *veA*, *nsdD*, *nosA* and *stuA*. (Adapted from Seo *et al.* 2004, Yu 2006).

which of the α -subunits is the cognate G α protein of GprA and GprB. A role of GanA and GanB in sexual development, if any, has not yet been reported. On the other hand, the role of *fadA* in sexual development has been studied. Cleistothecia formation is not affected in a Δ *fadA* strain under heterothallic conditions but *fadA* is essential for homothallic sexual development. Under homothallic conditions, cleistothecia formation is abolished in the Δ *fadA* strain but Hülle cell formation is not affected (Rosén *et al.* 1999, Seo *et al.* 2005). The RGS protein FibA that controls FadA-mediated signaling (see above) also plays a role in fruiting body development (Han *et al.* 2001). Inactivation of *flbA* completely abolishes cleistothecia and Hülle cell formation under homothallic conditions. The developmental modifier genes *medA* and *stuA*, which play a crucial role in asexual development, also function in sexual differentiation (Fig. 7). The Δ *medA* strain only forms unorganised masses of Hülle cells and does not differentiate cleistothecia or ascogonic tissue (Busby *et al.* 1996). Sexual development is delayed in the Δ *stuA* strain and it forms only 1 % of the cleistothecia that are formed by the wild-type. Moreover, the cleistothecial shell of the Δ *stuA* strain is much more fragile when compared to the wild-type (Wu & Miller 1997). Thus, *medA* and *stuA* are activators of sexual development.

Activation of the heterotrimeric G-protein by pheromone binding results in activation of a mitogen activated protein kinase pathway (MAPK). As mentioned above, the MAPK module consists of three kinases known as MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK. The MAPKKK SteC is involved in formation of cleistothecia (Wei *et al.* 2003). The Δ *steC* strain grows slower, forms more branched hyphae, and has an altered conidiophore morphology. Moreover, heterokaryon formation is inhibited. This is probably due to the inability of hyphae to fuse, possibly by the fact that hyphal recognition is blocked (Wei *et al.* 2003). The Δ *steC* strain also has a block in cleistothecium development. Only a few small nest-like structures and single Hülle cells are being produced by

this strain. The fact that deletion of both *gprA* and *gprB* results in a phenotype similar to that of the Δ *steC* strain strongly indicates that a MAPK cascade functions in GprA/B mediated signaling (Fig. 7). This would be similar to that found in the yeast mating response. In fact, the *A. nidulans* genome is predicted to contain all the major components of such a yeast MAPK cascade (Seo *et al.* 2004, Yu 2006) (Fig. 7). However, so far the role of all these genes has not yet been confirmed. For instance, a functional study of MAPKK genes in *A. nidulans* is lacking. However, it has been shown that inactivation of the MAPK gene, *mpkB*, abolishes sexual reproduction. Both Hülle cells and cleistothecia are not formed (Paoletti *et al.* 2007). This indicates that MpkB is part of the GprA/B mediated signaling pathway that results in formation of cleistothecia and ascospores.

Transcriptional activators

A target of MpkB seems to be SteA (Vallim *et al.* 2000). This homeodomain-C₂H₂-zinc finger transcription factor is homologous to Ste12, which in *S. cerevisiae* is activated by the MAPK signaling pathway involved in mating. A Δ *steA* strain does neither form cleistothecia nor primordial structures but Hülle cell formation is not affected. Expression of *steA* does not depend on *medA* and *stuA*. In contrast, *medA* but not *stuA* is repressed by *steA*. This suggests that *steA* and *stuA* are part of independent pathways regulating sexual development and that *medA* acts downstream of *steA* (Vallim *et al.* 2000) (Fig. 7). Another target of MpkB could be *nsdD* (*never in sexual development*). This gene encodes a putative GATA-like transcription factor with a type IVb zinc finger DNA-binding domain (Han *et al.* 2001). Inactivation of *nsdD* abolishes formation of cleistothecia. In contrast, over-expression of the gene results in increased formation of fruiting bodies on solid medium and Hülle cells are formed even in submerged culture. As a result of over-expression the phenotype of the *veA1* mutation (see below) is overruled. This indicates that *nsdD* acts downstream of this gene or functions in an overlapping

pathway (Han *et al.* 2001). The *nsdD* gene of *A. fumigatus* has a similar function as its counterpart in *A. nidulans*. The $\Delta nsdD$ mutant of *A. fumigatus* accumulates dark pigments and sexual development is abolished (Szewczyk & Krappmann 2010). A third gene encoding a transcriptional regulator involved in sexual development is *nsdC* (*never in sexual development*) (Kim *et al.* 2009). This gene encodes a putative transcription factor carrying a novel type of zinc-finger DNA-binding domain consisting of two C_2H_2 's and a C_2HC motif. Inactivation of *nsdC* completely abolishes fruiting body formation. On the other hand, overexpression of the gene promotes cleistothecia formation, even under repressing stress conditions (Kim *et al.* 2009). The phenotype of the $\Delta nsdC$ strain is not affected by over-expression of *steA* or *nsdD*. These results suggest that NsdC, NsdD, and SteA are non-redundant proteins and thus are all essential for fruiting body development.

Gene *nosA* (*number of sexual spores*) (Vienken & Fischer 2006) and *rosA* (*repressor of sexual development*) (Vienken *et al.* 2005) encode putative $Zn(II)_2Cys_6$ transcription factors that share 43 % amino acid identity. Transcript levels of these genes are usually very low but the genes are up-regulated during carbon starvation and during asexual development. RosA is an inhibitor and NosA an activator of sexual development. Asexual and sexual development is not affected under standard laboratory conditions when *rosA* is inactivated in a strain with a *veA1* mutation (see below). However, the number of cleistothecia increases when compared to the wild-type strain when the $\Delta rosA$ strain contains a fully functional *veA* gene (Vienken *et al.* 2005). These data indicate that RosA function depends on VeA. The difference in the number of cleistothecia between the wild-type strain and the $\Delta rosA$ strain is even higher under low-glucose or high-osmolarity conditions. When grown submerged, Hülle cells are produced in the $\Delta rosA$ strain, while sexual development in the wild-type is completely absent under this condition (Vienken *et al.* 2005). Asexual development is not affected in a $\Delta nosA$ strain with a *veA1* mutation. However, this strain was unable to undergo sexual development (Vienken & Fischer 2006). Asexual development in the light is also not affected when the $\Delta nosA$ strain contains a fully functional *veA* gene. On the other hand, sexual development in the dark is blocked in the primordial stage in this mutant strain. Differentiated cells such as ascus mother cells and Hülle cells are hardly formed and only a few very small cleistothecia (about 30 μm in diameter instead of 300 μm) are produced. These fruiting bodies do produce viable ascospores. Expression of *nosA* was increased in a $\Delta rosA$ strain (Vienken & Fischer 2006). Similarly *nsdD*, *veA*, and *stuA* are up-regulated in this mutant strain (Vienken *et al.* 2005). Taken together, RosA represses sexual development, whereas NosA is required for primordium maturation. The balance between these two $Zn(II)_2Cys_6$ transcription factors determines whether vegetative hyphae undergo sexual development.

THE BALANCE OF SEXUAL AND ASEQUAL DEVELOPMENT

There is a balance between sexual and asexual development. *Aspergillus nidulans* forms conidia in the light, whereas ascospores are preferentially produced in the dark (Adams *et al.* 1998, Bayram *et al.* 2010, Mooney *et al.* 1990, Purschwitz *et al.* 2006). Several light sensors and regulatory proteins are involved in light-regulated development. Moreover, the balance between sexual and asexual reproduction is the result of psi factor and cross-talk between

regulatory pathways that are involved in these developmental pathways. The light-regulated pathway, the regulation by psi factor, and the cross-talk between regulatory pathways of asexual and sexual reproduction are part of a complex regulatory network.

The role of SteA, BriA, FlbC, and FlbE

SteA is assumed to be the transcription factor that is activated by MpkB (Fig. 7) thereby promoting sexual development. Expression of *steA* is increased in undifferentiated hyphae of a $\Delta briA$ strain (Vallim *et al.* 2000). Repression of *steA* in uninduced hyphae has been related to the BriA β protein. The regulatory interaction between *briA*, *medA*, and *steA* is a clear example of cross-talk between the developmental programs of asexual and sexual sporulation. FlbC and FlbE also play a role in the cross-talk between the asexual and sexual developmental pathway. FlbC and FlbE are upstream activators of *briA* involved in asexual development (Fig. 6) but at the same time repress the sexual pathway (Fig. 7) (Kwon *et al.* 2010a, b). Both a $\Delta flbC$ and a $\Delta flbE$ strain show elevated cleistothecium formation and abundant formation of Hülle cells (Kwon *et al.* 2010a, b).

The role of psi factor

Aspergillus nidulans produces oleic- and linoleic-acid-derived oxylipins that are known as psi factor (precocious sexual inducer). These hormone-like molecules modulate the timing and balance between sexual and asexual spore development (Calvo *et al.* 2002). Psi factor is mainly a mixture of secreted hydroxylated linoleic (18 : 2) and oleic (18 : 1) molecules termed psi α and psi β , respectively (Champe *et al.* 1987, Champe & el-Zayat 1989). The positioning of the hydroxy groups distinguishes the psi compounds as psiA (5',8'-dihydroxy-), psiB (8'-hydroxy-), and psiC (designating a lactone ring at the 5' position of psiA) (Mazur *et al.* 1990, 1991). Purified psiA α enhances asexual sporulation. On the other hand, psiB α and psiC α stimulate sexual reproduction and inhibit asexual spore development (Champe *et al.* 1987, Champe & el-Zayat 1989). It has therefore been proposed that the ratio of psiA α to psiB α and psiC α determines whether asexual or sexual sporulation dominates (Champe *et al.* 1987, Champe & el-Zayat 1989). Gene *ppoA* (Tsitsigiannis *et al.* 2004a), *ppoB* (Tsitsigiannis *et al.* 2005), and *ppoC* (Tsitsigiannis *et al.* 2004b), encode putative fatty acid oxygenases. The former gene is required for the production of psiB α , while the latter two genes are involved in the synthesis of psiB β (Fig. 8). Deletion of *ppoA* reduces psiB α levels and, as a consequence, increases the ratio of asexual to sexual spore numbers 4-fold. On the other hand, over-expression of *ppoA* results in elevated levels of psiB α and decreases the ratio of asexual to sexual spore numbers 6-fold. An increased ratio of sexual to asexual spore numbers is also observed after deletion of *ppoC* (Tsitsigiannis *et al.* 2004a), whereas an opposite phenotype is observed after inactivation of *ppoB* (Tsitsigiannis *et al.* 2005). This is unexpected since the $\Delta ppoB$ and $\Delta ppoC$ strains produce similar psiB profiles (Tsitsigiannis *et al.* 2004a, 2005). Several explanations for this phenomenon have been proposed, one of which is the possibility that the products of the *ppo* genes have more than one substrate. This would generate oxylipins, of unknown nature, that would also affect differentiation (Garscha *et al.* 2007). Another explanation may be related to the finding that the products of the fatty acid oxygenases affect the expression of *ppo* genes and thereby impact the composition of psi factor. Gene *ppoC* is upregulated in a $\Delta ppoB$ strain, whereas *ppoA* is repressed

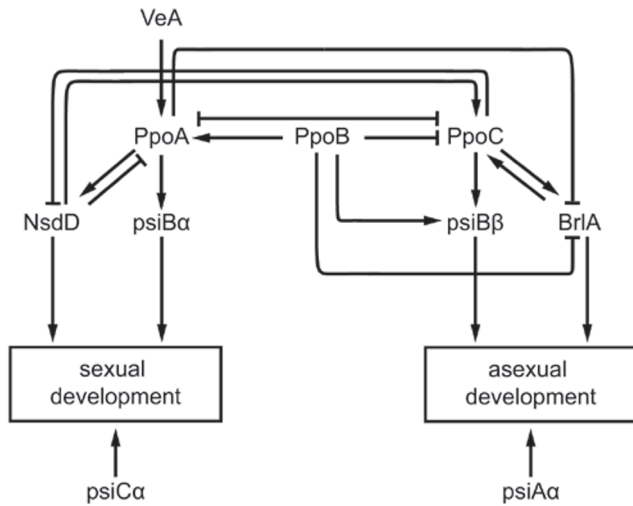


Fig. 8. Oxylipins, known as psi factor, regulate timing and balance between sexual and asexual development. The hormone like structures psiBa and psiCα stimulate sexual development, whereas psiAα and psiBβ stimulate asexual development. Psi factor results from *ppoA*, *ppoB*, and *ppoC* activity. Expression of these genes is regulated by the products resulting from the Ppo proteins and by BriA and NsdD. In turn, the products resulting from the Ppo genes regulate expression of *briA* and *nsdD*.

(Tsitsigiannis *et al.* 2005) (Fig. 8). On the other hand, *ppoA* mRNA levels are higher in a Δ *ppoC* strain, whereas *ppoC* mRNA levels are lower in a strain over-expressing *ppoA* (Tsitsigiannis *et al.* 2004a).

Deletion of *ppoB* significantly increases *briA* expression but has a minor effect on expression of the regulatory genes of sexual reproduction *nsdD* and *veA* (Tsitsigiannis *et al.* 2005) (Fig. 8). This would explain why the net formation of conidia is upregulated in a Δ *ppoB* strain when compared to ascospores. Changes in sporulation ratios in the Δ *ppoA* and Δ *ppoC* strains also agree with the relative expression of *briA* and *nsdD* (Tsitsigiannis *et al.* 2004a, b). Expression of *briA* is down-regulated in a Δ *ppoA* Δ *ppoB* Δ *ppoC* strain, whereas higher levels of *nsdD* and *veA* transcripts are found. This correlates with the increased number of ascospores when compared with the conidia. Notably, sexual development precedes asexual development in the Δ *ppoA* Δ *ppoB* Δ *ppoC* strain, whereas the opposite is observed in the wild-type. Taken together, *ppo* genes determine timing and ratio of sexual and asexual reproduction (Tsitsigiannis *et al.* 2005). The transcripts of *ppoA* and *ppoC* are developmentally regulated in differentiated tissues (Tsitsigiannis *et al.* 2004a, b). Moreover, PpoA has been localised in metulae of conidiophores and Hülle cells (Tsitsigiannis *et al.* 2004b). These observations suggest that Ppo enzymes and/or their substrates are spatially and temporally regulated in reproductive tissues. This would result in a temporal and spatial distribution profile of the oxylipins, which in turn orchestrates the sexual and asexual sporulation schedule (Tsitsigiannis *et al.* 2005). This schedule is associated with two feedback loops (Fig. 8). Genes *ppoC* and *ppoA* regulate the expression of *nsdD* and *briA*. On the other hand, both *briA* and *nsdD* activate *ppoC*, whereas *nsdD* represses *ppoA* (Tsitsigiannis *et al.* 2004a, b). In the other loop Ppo oxylipin products would antagonistically signal generation of Ppo substrates (Tsitsigiannis *et al.* 2004a, 2005).

Aspergillus niger (Wadman *et al.* 2009) and *A. fumigatus* (Garscha *et al.* 2007) produce the same oxylipins as *A. nidulans*. The *A. niger* genome contains the *ppo* genes *ppoA*, *ppoC*, and *ppoD*. A *ppoB* homologue is not present (Wadman *et al.* 2009). *Aspergillus niger* strains in which the *ppoA* or *ppoD* gene are inactivated are not affected in oxylipin production and sporulation.

In contrast, a multi-copy *ppoC* strain has reduced conidia formation. This shows that oxylipins also play a role in *A. niger* development but it could well be that the function of the individual psi factor components is different.

The role of light

Regulatory genes involved in light response

The *veA1* (*velvet*) mutant of *A. nidulans* was isolated and characterised for the first time in 1965 (Käfer 1965). Sexual development in such strains (among which many lab strains) is generally retarded and reduced, while asexual development is more pronounced (Yager 1992). Deletion of *veA* has a more dramatic effect. A Δ *veA* strain does not produce cleistothecia not even in the dark (Kim *et al.* 2002). On the other hand, over-expression of *veA* results in reduced asexual development and increased production of cleistothecia, even under conditions where the wild-type hardly forms these sexual structures (*i.e.* in liquid medium or on solid medium containing a high concentration of potassium chloride). Taken together, *VeA* is a positive regulator of sexual development, while simultaneously suppressing asexual development (Kim *et al.* 2002). In contrast, the *veA* gene of *A. fumigatus* (Krappmann *et al.* 2005) and *A. parasiticus* (Calvo *et al.* 2004) have been shown to stimulate asexual reproduction. However, it should be noted that the positive effect on conidia formation is especially clear under adverse environmental conditions. The influence of these conditions on the phenotype of Δ *veA* of *A. nidulans* has not yet been studied.

The *veA* gene of *A. nidulans* is expressed throughout culturing both in liquid and solid medium but its expression increases during sexual development. The *veA* gene encodes a velvet protein that contains a nuclear localisation signal (NLS) (Kim *et al.* 2002, Stinnett *et al.* 2007). *VeA* is predominantly present in the cytoplasm in the light (white or blue light), but migrates to the nucleus in the dark or when exposed to red light. Migration of *VeA* to the nucleus depends on the interaction between its NLS and the importin α , KapA (Stinnett *et al.* 2007) (Fig. 9). The velvet protein encoded by the *veA1* allele is predicted to encode a truncated *VeA* that lacks the first 36 N-terminal amino acids (Kim *et al.* 2002). As a consequence, it misses a non-functional NLS. Indeed, interaction of KapA with the mutant protein is reduced. The *VeA1* protein locates mainly in the cytoplasm both in the dark and in the light. This explains why development is affected in the *veA1* strain (Stinnett *et al.* 2007).

Apart from KapA, *VeA* interacts with the regulator of secondary metabolism *LaeA* (Bok & Keller 2004) and the *VeA*-like protein *VelB* (Bayram *et al.* 2008b, Bok & Keller 2004) (Fig. 9). *VeA* interacts with *LaeA* in the nucleus, whereas the complex of *VeA* and *VelB* can be found both in the cytoplasm and in the nucleus (Bok & Keller 2004) (Fig. 9). The interaction with *VeA* explains why *VelB* can migrate into the nucleus despite the absence of an apparent NLS (Bayram *et al.* 2008b). Like the Δ *veA* strain, the Δ *veIB* strain does not show light-dependent development. The Δ *veIB* strain is unable to form cleistothecia in the light and in the dark. The effect on asexual sporulation, however, is not as strong as in the Δ *veA* strain. Over-expression of *veA* in the Δ *veIB* strain and vice versa does not complement the developmental defects. These data show that the *VeA/VelB* complex mediates light regulated sexual and asexual development. The binding partner *LaeA* also has a role in asexual and sexual development. In a *veA1* background inactivation of *laeA* has no effect on asexual reproduction (Bayram *et al.* 2008b, Sarikaya Bayram *et al.* 2010). However, conidiophore formation is reduced both in the light and in the dark when *laeA* is

inactivated in a strain with a functional *veA* gene (Sarıkaya Bayram *et al.* 2010). The $\Delta laeA$ strain in the wild-type background produces five times less conidia in the light when compared to the wild-type. The absolute number of conidia of the $\Delta laeA$ strain is similar in the light and in the dark. An opposite effect is observed for cleistothecia production. Fruiting body formation is markedly increased in the $\Delta laeA$ strain when grown in the light. As a consequence, the number of cleistothecia in the mutant strain is similar in the light and the dark. These results are explained by experiments that suggest that the $\Delta laeA$ strain is entirely blind, which results in a dark-phenotype (low number of conidiophores and high number of cleistothecia) independent of illumination. A $\Delta laeA\Delta veA$ strain shows a ΔveA phenotype (*i.e.* absence of cleistothecia formation both in light and dark). Thus, *veA* is epistatic to $\Delta laeA$ (Sarıkaya Bayram *et al.* 2010). Taken together, *LaeA* is a negative regulator of sexual development when *A. nidulans* is grown in the light. *LaeA* also has a role in the formation of Hülle cells in the dark. Dark grown colonies of the $\Delta laeA$ strain form less of these cells that feed the cleistothecia. Consequently, the diameter of the cleistothecia as well as the number of ascospores is reduced 5-fold when compared to the wild-type (Sarıkaya Bayram *et al.* 2010).

LaeA plays an important role in regulating the levels of the *VeA*-like proteins *VelB* and *VosA* in a light-dependent way. When a wild-type colony is exposed to light, the amount of *VosA* and *VelB* (see below) is decreased. As a consequence, the amount of *VosA/VelB* complex is reduced, which releases the repression on asexual development (Fig. 9). In parallel, the reduction of *VelB* in the light affects sexual development. In the $\Delta laeA$ strain, high amounts of *VosA* and *VelB* are found in the light, and as a consequence asexual developmental is inhibited, whereas sexual development is promoted (Sarıkaya Bayram *et al.* 2010). In addition, *LaeA* controls levels of *VeA* and inhibits a post-translational modification of this protein (Sarıkaya Bayram *et al.* 2010). The function of the post-translational modification is not yet clear.

LaeA regulates directly or indirectly downstream regulatory genes involved in sexual and asexual development (Sarıkaya Bayram *et al.* 2010). Expression of regulatory genes of sexual development, *steA* and *nosA* (Vallim *et al.* 2000, Vienken & Fischer 2006), is transiently reduced during vegetative growth of $\Delta laeA$ strains. Of interest, the $\Delta nosA$ strain has a similar phenotype as $\Delta laeA$ strains with respect to fruiting body formation (Vienken & Fischer 2006). Hülle cell formation and, as a consequence, the size of cleistothecia is strongly reduced. This indicates that Hülle cell formation is regulated by *laeA* via *nosA*. In agreement, over-expression of *nosA* in a $\Delta laeA$ strain rescues, albeit moderately, the small cleistothecia phenotype (Sarıkaya Bayram *et al.* 2010). A $\Delta laeA$ strain also shows reduced levels of transcripts of *abaA* but not of *brlA*. The effect on *abaA* expression may well explain the reduced ability of $\Delta laeA$ strains to form conidia. Similar to *LaeA*, *VeA* affects expression of downstream genes involved in asexual development. The higher conidiation profile in a ΔveA strain is accompanied by a change in expression of *brlA* (Kato *et al.* 2003). In both the ΔveA strain and wild-type colonies the *brlA β* transcript is present. However, in the ΔveA strain the *brlA α* transcript is dominant (Kato *et al.* 2003). The relation of reduced *veA* levels and an increase in the ratio between the *brlA α* and *brlA β* transcripts is also observed when expression of these genes is analysed after a light exposure of 30 and 60 minutes (Ruger-Herreros *et al.* 2011). Taken together, these data indicate that *veA* affects the *brlA α /brlA β* transcript ratio (Kato *et al.* 2003). Other developmental genes that are linked to *veA* are the psi factor oxilipin genes that control the balance between asexual and sexual reproduction (see above).

Expression of the oxilipin gene *ppoA* is completely abolished in a ΔveA strain (Fig. 8). Both the ΔveA strain (Kim *et al.* 2002) and the $\Delta ppoA$ strain (Tsitsigiannis *et al.* 2004b) show an increase in the ratio of conidia and ascospore formation. Notably, expression of *veA* is increased in the $\Delta ppoA\Delta ppoB\Delta ppoC$ strain, which suggests the existence of a regulatory loop between *ppo* genes and *veA* (Tsitsigiannis *et al.* 2005).

Photoreceptors involved in light response

Light has to be sensed in order to enable regulatory proteins to activate or inhibit developmental pathways. Large numbers of conidia are produced in white light. Asexual sporulation also occurs in red (680 nm) or blue (450 nm) light but this process is reduced when compared to white light. When red and blue light are combined, formation of conidia is similar to that in white light (Purschwitz *et al.* 2008). Both blue and red light alone effectively inhibit sexual development to an extent similar to white light. Three light sensors have been identified in *A. nidulans*. *FphA* is a phytochrome that represents a red-light receptor. Blue light is sensed by the white-collar complex consisting of *LreA* and *LreB*, while the photolyase/cryptochrome *CryA* senses blue light as well as UVA (Bayram *et al.* 2010). Development is regulated via interplay of these receptors.

FphA (*fungal phytochrome*) represents the phytochrome of *A. nidulans*. It contains an N-terminal photosensory module (GAF and PHY domain) that harbors the chromophore. The C-terminal part of *FphA* contains a histidine kinase related domain, which is expected to be involved in the regulatory output. In addition, it contains a predicted ATPase domain and two NLS sequences. *FphA* is responsible for the effect of red light on development of *A. nidulans* (Blumenstein *et al.* 2005). Conidiation is slightly reduced when *fphA* is inactivated (Purschwitz *et al.* 2008). Moreover, whereas a wild-type strain produces conidia in red light, the $\Delta fphA$ strain still reproduces sexually. Yet, the number of cleistothecia is only 10 % of that found in the dark (Blumenstein *et al.* 2005). Under the latter condition, formation of cleistothecia is similar in the wild-type and the $\Delta fphA$ strain (Purschwitz *et al.* 2008). These data show that *FphA* is an activator of asexual development and a repressor of sexual reproduction. *FphA* is located in the cytoplasm and in the nucleus (Blumenstein *et al.* 2005, Purschwitz *et al.* 2008). *FphA* that resides in the nucleus interacts with the *LreB* subunit of the white-collar complex that signals blue light (see below) (Purschwitz *et al.* 2008) (Fig. 9). Nuclear-located *FphA* also has a physical interaction with *VeA* (Purschwitz *et al.* 2008). The light control of *VeA* might be activated during development via a direct interaction with *FphA*. *VeA* is highly phosphorylated, and *FphA* has a light driven histidine kinase activity (Brandt *et al.* 2008). It might therefore be that *FphA* phosphorylates *VeA*. However, up to now no downstream phosphor transfer initiated by *FphA* has been observed (Bayram *et al.* 2010, Purschwitz *et al.* 2009). Moreover, it is not clear whether photoreceptor-linked *VeA* also interacts with *VelB* and/or *LaeA*.

LreA and *LreB* (*light response*) form the white-collar blue light receptor, and are orthologues of the best characterised white collar proteins WC-1 and WC-2 of *N. crassa* (Purschwitz *et al.* 2008). The *LreA* protein contains a light-, oxygen-, or voltage-sensitive (LOV) domain that harbors the flavin adenine dinucleotide co-factor. It also contains two protein-protein interaction domains called PER-ARNT-SIM (PAS) domains. In addition, *LreA* is characterised by a NLS and a GATA-type zinc-finger DNA binding domain. *LreB* is a smaller protein than *LreA*. It contains a NLS sequence, only one PAS domain, and the GATA-type zinc-finger DNA binding domain. The light sensing domain is thus lacking in

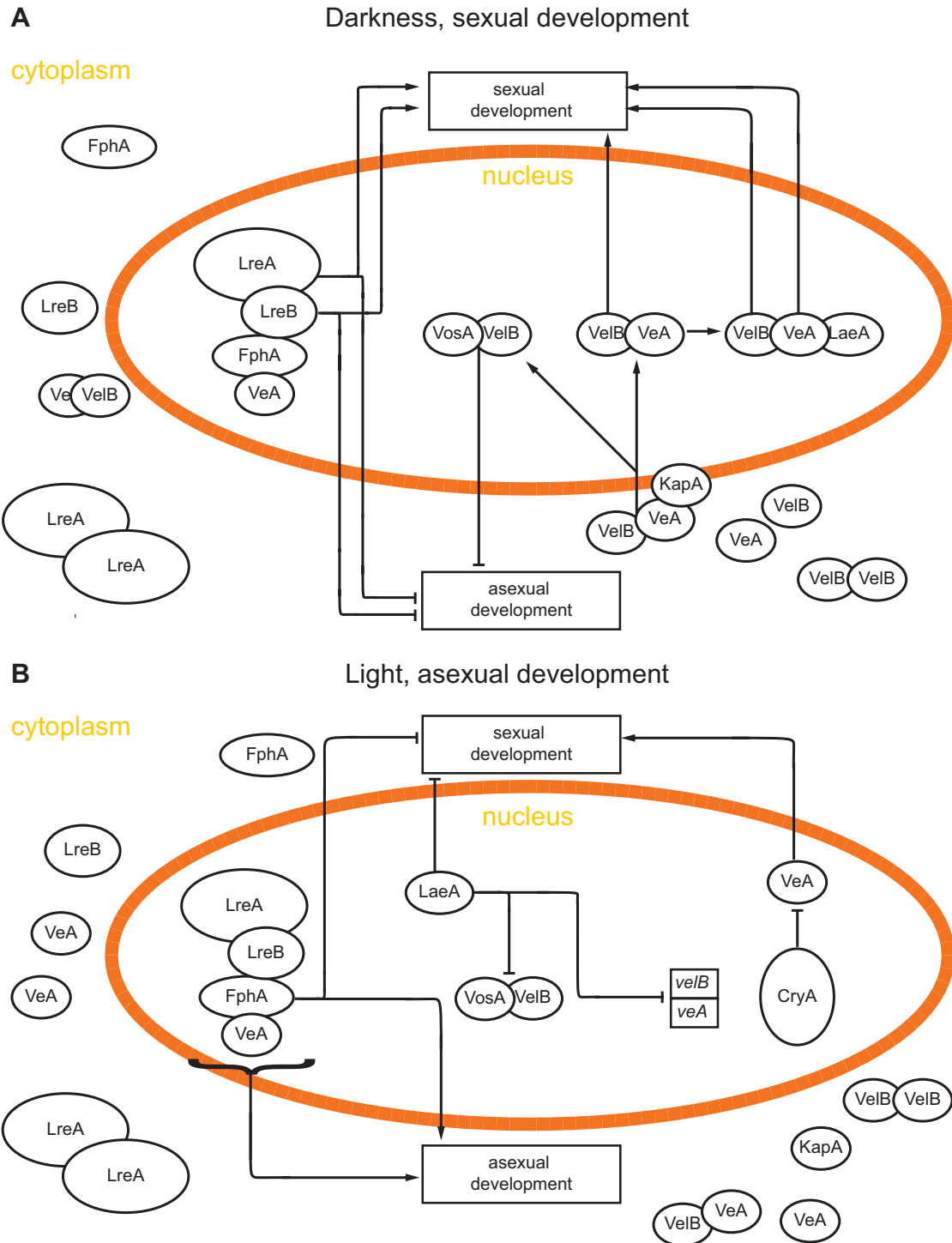


Fig. 9. Light-regulated development in *A. nidulans*. (A) In the dark VelB enters the nucleus together with VeA and α -importin KapA. In the nucleus, VeA and VelB act as a dimeric complex or as a trimeric complex together with LaeA to positively regulate sexual development. VelB also forms a complex with VosA that negatively regulates asexual development. In the light, activity of LaeA results in reduced levels of VelB and VosA. As a consequence, the inhibition of asexual development by the VelB-VosA complex is released. Moreover, the reduction of VelB levels abolishes stimulation of sexual development. Light is detected by the red light receptor FphA and the blue light receptor proteins LreA and LreB. These light receptors form a complex in the nucleus together with VeA. The cryptochrome/photolyase CryA also plays a role in light regulated development. Like FphA, it is a repressor of sexual development. (Adapted from Sarikaya Bayram *et al.* 2010).

LreB. Asexual development is somewhat increased in the $\Delta lreA$ and $\Delta lreB$ strains, irrespective of the presence of light. This finding suggests that LreA and LreB repress conidia formation (Purschwitz *et al.* 2008). Notably, the number of conidia in the $\Delta lreA\Delta fphA$ strain, the $\Delta lreB\Delta fphA$ strain, and the $\Delta lreA\Delta lreB\Delta fphA$ strain is strongly decreased when compared to the wild-type. Yet, both in the dark and in the light a basal level of conidiation is observed in these strains. Cleistothecia production in the dark is reduced by 70 % and 30 %, respectively, in the $\Delta lreA$ and $\Delta lreB$ strains when

compared to the wild-type. Strains $\Delta lreA\Delta fphA$, $\Delta lreB\Delta fphA$, and $\Delta lreA\Delta lreB\Delta fphA$ behave like the $\Delta lreB$ strain. When exposed to white light, cleistothecium formation is nearly absent in the $\Delta lreA$ and $\Delta lreB$ strains. This effect is suppressed by inactivation of *fphA* in these strains. Taken together, LreA and LreB act as activators of the sexual cycle and their activity is repressed by light through the action of FphA (Purschwitz *et al.* 2008). LreB also has a function in activation of the asexual cycle by induction of the *brlA* gene (Ruger-Herreros *et al.* 2011). It may be that LreB directly controls *brlA*

expression via its DNA binding domain (Fig. 9). Another possibility is that the photoreceptor complex activates other transcriptional regulators that in turn bind to the *brlA* promoter.

CryA of *A. nidulans* is a combined cryptochrome/photolyase that resides in the nucleus. It repairs UV induced DNA damage. It also regulates fruiting body formation under both UVA and blue light conditions (350–370 and 450 nm) but does not have a known DNA binding domain (Bayram *et al.* 2008a). The *cryA* (cryptochrome) gene has a basal expression during vegetative growth and early asexual and sexual development. Expression increases during late asexual and sexual sporulation, and therefore suggests a role of CryA in late developmental processes. The Δ *cryA* strain shows no phenotype on solid medium under standard laboratory conditions but in submerged cultures Hülle cells, but not cleistothecia, are formed (Bayram *et al.* 2008a). These data suggest that CryA is a negative regulator of sexual development. The phenotype of the Δ *cryA* strain resembles that of the Δ *rosA* strain (Vienken *et al.* 2005). Hülle cells are also formed in submerged cultures when *veA* or *nsdD* are over-expressed (Han *et al.* 2001, Kim *et al.* 2002). Expression levels of *veA*, *nsdD*, and *rosA* are increased in the Δ *cryA* strain. This suggests that the cryptochrome regulates these transcription factors (Bayram *et al.* 2008a) (Fig. 9). These and other expression data indicate that CryA represses *veA* expression, whereas *VeA* stimulates *nsdD* expression. *NsdD* subsequently activates expression of its own negative regulator *rosA* (Bayram *et al.* 2008a). The *rosA/nsdD* feedback loop results in the presence of *NsdD* during development of Hülle cells and not during later stages of sexual development. In this way, *NsdD* can fulfill its function in the early stages of sexual reproduction.

DORMANCY AND GERMINATION

Dormancy

Spores should not germinate on the conidiophore or in the ascocarp but rather when they have been dispersed. Moreover, spores should only germinate when environmental conditions are favorable for fungal growth. This requires mechanisms to keep spores dormant. During dormancy, spores may be exposed to various stress conditions such as UV-radiation, drought, and relatively high temperatures. Conidia of *Aspergillus* are moderately resistant to these stress situations due to a number of resistance mechanisms.

Volatiles that prevent germination of spores on reproductive structures

The volatile 1-octen-3-ol is produced by fungi as a degradation product of linoleic acid. It inhibits germination of conidia of *Penicillium paneum* and *A. nidulans* (Chitarra *et al.* 2004, Herrero-Garcia *et al.* 2011). The critical concentration of 1-octen-3-ol needed to inhibit germination is obtained when spores are present at a high density. This situation is met on a conidiophore and in this situation the volatile acts as a self-inhibitor. Upon spore dispersal, the concentration of 1-octen-3-ol drops. As a result, outgrowth of the conidia is no longer self-inhibited but only depends on the environmental conditions.

Resistance of conidia against UV

Conidia of the genus *Aspergillus* survive UV due to the presence of melanin or melanin-like pigments in their cell wall. The melanin-

(like) pigment is also a virulence factor (Tsai *et al.* 1999). Melanin contained in the cell wall of *Aspergillus* conidia is synthesised via the 1,8-dihydroxynaphthalene (1,8-DHN) pathway (Fig. 10), which is conserved in the Aspergilli (Baker 2008, Tsai *et al.* 1999). The DHN pathway results in brown or black melanin. Absence of enzymatic steps or modification of melanin precursors results in pigments with different colours.

A cluster of six genes has been identified that is involved in the production of the bluish-green pigment in the conidia of *A. fumigatus* (Tsai *et al.* 1999). Inactivation of one of each genes in the cluster results in spores with different colours. Gene *alb1* (*albino*) encodes the polyketide synthase of the DHN pathway (Fig. 10). This polyketide synthase produces the heptaketide naphthopyrone YWA1 (Watanabe *et al.* 2000), which is the precursor for the green spore pigment of *A. nidulans* (Watanabe *et al.* 1999). In the case of *A. fumigatus*, *ayg1* (*Aspergillus yellowish green*) converts YWA1 into 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN), which is further modified in the DHN pathway (Fujii *et al.* 2004). Gene *arp2* (*Aspergillus reddish-pink*) encodes the hydroxynaphthalene (HN) reductase that forms both scytalone and vermeline (Tsai *et al.* 1999), whereas *arp1* encodes the dehydratase that converts scytalone in 1,3,8-THN, which is the precursor for *Arp2* (Tsai *et al.* 1999). The combination of *alb1*, *ayg1*, *arp1*, and *arp2* are predicted to produce a brown-black melanin. The two other genes in the cluster are assumed to be required for the production of the bluish-green pigment of wild-type *A. fumigatus* spores. Gene *abr1* (*Aspergillus brown*) is a putative multicopper oxidase that converts vermeline to 1,8-DHN (Pihet *et al.* 2009). Subsequently, 1,8-DHN is polymerised by the laccase encoded by *abr2* (Sugareva *et al.* 2005).

In contrast to other Aspergilli such as *A. nidulans* and *A. fumigatus*, there is little evidence that supports the involvement of the DHN pathway in *A. niger* (Jørgensen *et al.* 2011). The *A. niger* genome lacks clear orthologs for *arp1* and *arp2*. Moreover, spore pigmentation of *A. niger* is insensitive to the HN reductase (*Arp2*) inhibitor tricyclazole. The *fwnA* (*fawn*), *pptA* (*phosphopantetheinyl transferase*), *olvA* (*olive*), and *brnA* (*brown*) genes have been shown to be involved in the production of the characteristic black spore pigment of *A. niger*. Gene *fwnA* encodes a polyketide synthase. Inactivation of this gene results in fawn-coloured conidia. Conidia of the Δ *pptA* strain are white due to the absence of phosphopantetheinyl transferase activity. This activity is required for activation of polyketide synthases (PKSs) and non-ribosomal peptide synthases. The proteins encoded by *olvA* and *brnA* are homologous to the *A. fumigatus* *AygA* and *Abr1* proteins, respectively.

Resistance to drought and high temperature

Dormant conidia of *A. nidulans* survive up to 6 weeks at room temperature in liquid (Fillinger *et al.* 2001) but even much longer when kept in a dried state. In a dried state, the mRNA pool of the conidia of *A. fumigatus* hardly changes even after a year of storage (Lamarre *et al.* 2008). Conidia of *Aspergillus* also survive relatively high temperatures. For example, conidia of *A. niger* survive 1 h at 50 °C (Ruijter *et al.* 2003). Compatible solutes are assumed to protect conidia against drought and heat stress. These molecules include the disaccharide trehalose and the polyols mannitol, glycerol, erithreitol, and arabinitol. The compatible solutes do not affect functioning of proteins and membranes when they accumulate to high concentrations inside the cell. Conidia of *A. nidulans*, and *A. oryzae* contain 0.7–1.4 pg trehalose and 0.5–0.8 pg mannitol (d'Enfert & Fontaine 1997, Sakamoto *et al.* 2009). Together they

Acetyl CoA + Malonyl CoA

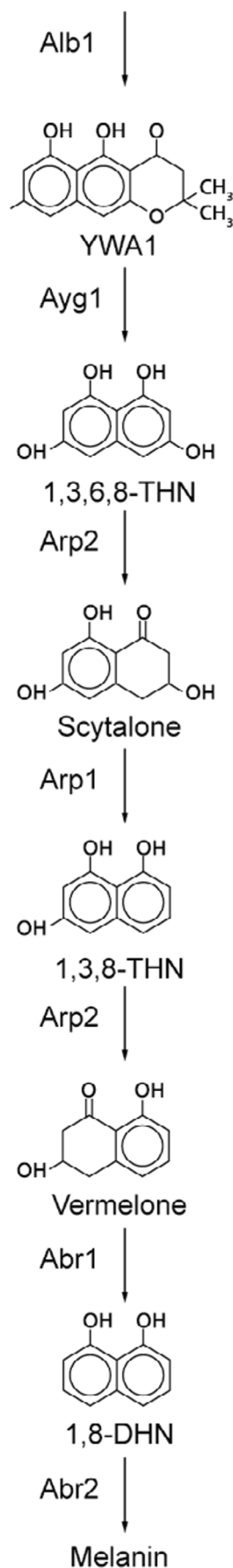


Fig. 10. Synthesis of melanin by means of the DHN pathway. Proteins of *A. fumigatus* responsible for each of the steps are indicated. Note that absence of particular enzymes and/or modification of melanin precursors will result in melanin-like pigments with colours other than brown/black. (Adapted from Fujii *et al.* 2004, Tsai *et al.* 1999, Pihet *et al.* 2009).

account for 4–6 % of the wet weight of these spores. Mannitol is the most abundant compatible solute in the case of *A. niger*. Mannitol and trehalose represent 10.9 % and 3.6 % of the dry weight of conidia, respectively (Ruijter *et al.* 2003, Witteveen & Visser 1995).

Conidia of an *A. niger* strain in which the mannitol 1-phosphate dehydrogenase gene *mpdA* is inactivated have increased trehalose (11.5 % dry weight) and reduced mannitol levels (4.0 % dry weight). Despite the fact that the total amount of compatible solutes remains unaltered, mutant conidia show 90 % viability loss after 1 h incubation at 50 °C when compared to the wild-type. The $\Delta mpdA$ conidia are also more sensitive to other stress conditions including freeze-thawing, drying, and hypochlorite treatment. Absence of trehalose also affects heat resistance of *A. niger* conidia (Wolschek & Kubicek 1997). *Aspergillus niger* contains two genes, *tpsA* and *tpsB*, that encode trehalose-6-phosphate synthase. This enzyme catalyses the first step in trehalose biosynthesis. Trehalose content is reduced by more than 50 % in the $\Delta tpsA$ strain, which is accompanied by increased sensitivity to heat stress. About 3 times more conidia of the wild-type survive incubation at 55 °C when compared to the $\Delta tpsA$ strain. Trehalose also has a role in protection of conidia of *A. nidulans*. The $\Delta tpsA$ strain of *A. nidulans* is incapable of producing trehalose (Fillinger *et al.* 2001). Consequently, conidia of the mutant strain are unable to germinate at 44 °C in a glucose medium, whereas wild-type spores do grow out to form a mycelium. Prolonged incubation at 44 °C also abolishes the ability of the $\Delta tpsA$ to germinate at lower temperatures. A role for trehalose to protect conidia against heat stress is also indicated by the fact that inactivation of the neutral trehalase gene *treB* promotes heat resistance of germinating conidia. This is explained by the fact that these conidia can not degrade trehalose and are therefore longer protected against temperature stress (d'Enfert *et al.* 1999).

VosA and VelB regulate the trehalose biosynthetic genes in conidia and ascospores. Levels of *vosA* transcripts and VosA protein are high in both spore types. Inactivation of *vosA* results in the lack of trehalose in spores, which is accompanied by a rapid loss of spore viability, and a dramatic reduction in tolerance of conidia to heat and oxidative stress (Tao & Yu 2011). Similarly, spores of a $\Delta velB$ strain show reduced viability and stress tolerance (Sarıkaya Bayram *et al.* 2010). In both $\Delta vosA$ and $\Delta velB$ strains reduced expression of genes involved in the biosynthesis of trehalose is observed (Sarıkaya Bayram *et al.* 2010). It has been proposed that a heterodimer of VelB and VosA activates expression of genes involved in trehalose biosynthesis and by this plays a role in viability and stress tolerance of spores (Sarıkaya Bayram *et al.* 2010).

Proteins and metabolites other than polyols and sugars may also protect dormant conidia. High numbers of transcripts are observed in conidia of *A. niger* of a gene encoding a LEA-like protein (*late embryogenesis abundant*) and of two small heat shock proteins (van Leeuwen *et al.* 2013a). Conidia of *A. oryzae* accumulate the amino acid glutamic acid (Sakamoto *et al.* 2009) up to approximately one percent of the wet weight.

Germination

Three stages can be distinguished during germination of conidia. In the first phase of germination, dormancy is broken by environmental cues such as the presence of water and air either or not in combination with inorganic salts, amino acids or fermentable sugars (Osharov & May 2001). Spores grow isotropically in the second phase of germination. This process that is also known as swelling is observed between 2 and 6 h after inoculation of *A.*

niger at 25 °C (van Leeuwen *et al.* 2013a, b). During this stage, the diameter of the spore increases two fold or more due to water uptake. This is accompanied by a decrease in the microviscosity of the cytoplasm (Dijksterhuis *et al.* 2007). Moreover, molecules are directed to the cell cortex to enable addition of new plasma membrane and cell wall (Momany 2002). In the third phase of germination, a germ tube is formed by polarised growth. To this end, the morphogenetic machinery is redirected to the site of polarisation (d'Enfert 1997, Harris 2006, Momany 2002, Harris & Momany 2004). Polarised growth of *A. niger* can be observed 6 h after inoculation at 25 °C (van Leeuwen *et al.* 2013a, b). At a later stage, the growth speed of the germ tube increases.

Transcripts of about one third of the genes can be detected by micro-arrays in dormant conidia of *A. niger* (van Leeuwen *et al.* 2012a, b). Transcripts representing the functional gene classes protein synthesis and protein fate are enriched in the RNA pool. A strong drop in the amount of RNA is observed in the first two hours of germination (van Leeuwen *et al.* 2013a, b). Notably, transcripts belonging to the functional gene classes protein synthesis and its subcategories translation and initiation are over-represented in the up-regulated genes at 2 h. Moreover, the categories transcription (including rRNA synthesis and rRNA processing), energy (respiration), cell cycle & DNA processing are overrepresented in the up-regulated genes at this time point. Up-regulation of genes involved in protein synthesis has also been shown in germinating conidia of *A. fumigatus* (Lamarre *et al.* 2008). The importance of protein synthesis in early stages of germination is also indicated by the fact that the protein synthesis inhibitor cycloheximide prevents isotropic growth, while inhibitors of the cytoskeleton and DNA- and RNA synthesis do not affect this process (Oshero & May 2000). The total number of genes that are expressed in germinating conidia of *A. niger* gradually increase between 2 and 8 h after inoculation at 25 °C (van Leeuwen *et al.* 2013a). After 4 h of germination, the functional categories metabolism and cell cycle and DNA processing are over-represented in the up-regulated genes. The latter suggests that the conidium prepares itself for mitosis, which occurs a few hours later. No functional gene classes are over- or under-represented in the differentially expressed genes at 6 h and 8 h (van Leeuwen *et al.* 2013a).

Upon activation of conidia of *A. nidulans* and *A. niger*, the compatible solute trehalose is converted to glucose (d'Enfert *et al.* 1999, van Leeuwen *et al.* 2013b). Similarly, mannitol levels quickly drop during the first 2-3 h of germination. Intracellular trehalose is degraded by the action of the neutral trehalase TreB (d'Enfert *et al.* 1999). The $\Delta treB$ strain of *A. nidulans* still contains 1.2 pg of trehalose after 3 h of germination and also shows a reduction in the degradation of mannitol when compared to the wild-type. Germ tube formation is not affected in the $\Delta treB$ strain of *A. nidulans* in the presence of an external C-source. However, it is delayed in the case the concentration of the external C-source is very low. Apparently, degradation of trehalose is needed to generate energy during germination. Interestingly, germinating $\Delta treB$ -spores resist a heat shock of 50 °C for 30 min, whereas more than 80 % of the wild-type spores have died after this treatment. This suggests that trehalose has a protective effect. However, experiments with a $\Delta tpsA$ strain that is not able to synthesise trehalose show that the situation is more complex. Three-hours-old germlings of this mutant strain show accumulation defects of trehalose after subjection to heat stress or oxidative stress. The isotropically growing wild-type spores accumulate 0.8 pg trehalose as a response to the stress, but the $\Delta tpsA$ strain was not able to do so. Remarkably, there was no effect on the sensitivity of these germlings for a second heat shock at 50 °C (Fillinger *et al.* 2001).

Regulation of germination of conidia

cAMP and RasA signalling

Initiation and completion of germination requires the sensing of external signals. To this end, conidia of *A. nidulans* use signaling via cAMP/protein kinase A (Fig. 11), and independently, signaling via RasA. In general, signalling via cAMP/protein kinase A (PKA) is initiated by an external signal through a heterotrimeric G-protein. Sensing of the external signal leads to the activation of the G α -subunit of the heterotrimeric G-protein, which activates adenylate cyclase. This enzyme produces cAMP that can bind to the regulatory subunit of PKA. As a result the regulatory subunit dissociates from the catalytic subunit (PKAc). Active PKAc phosphorylates proteins and in this way controls their activity. As described, the heterotrimeric G protein GanB-SfaD-GpgA represses conidia formation in *A. nidulans* (Fig. 5), but this heterotrimeric G-protein is also a carbon source sensor involved in early cAMP-dependent germination in *A. nidulans* (Lafon *et al.* 2005). Conidia of a $\Delta ganB$ strain show a reduced rate of swelling and germ tube formation (Chang *et al.* 2004). Wild-type conidia start to swell 2 h after inoculation. Of these conidia, 8 % and > 90 % had formed germ tubes 4 and 6 h post inoculation, respectively. Germ tubes are hardly observed after 4 h in the case of the $\Delta ganB$ strain, and less than 50 % of these spores have formed a germ tube after 10 h of inoculation. In contrast, expression of a constitutive active version of GanB (GanB^{Q208L}) promotes germ tube formation. In this case, germination of conidia even takes place in the absence of carbon source (Chang *et al.* 2004). Gene *ganB* is also involved in the germination process of ascospores. Ascospores of the $\Delta ganB$ strain germinate very poorly, whereas expression of GanB^{Q208L} results in precocious ascospore germination, even in the absence of carbon source. Taken together, GanB plays a positive role during germination, probably through carbon source sensing. The RgsA protein of *A. nidulans* negatively regulates GanB signaling (Han *et al.* 2004b) (Fig. 11). Like a strain expressing GanB^{Q208L}, conidia of the $\Delta rgsA$ strain germinate even in the absence of a carbon source. This effect is not observed in a $\Delta ganB\Delta rgsA$ or a $\Delta sfaD\Delta rgsA$ strain (Han *et al.* 2004b, Lafon *et al.* 2005).

The $\Delta ganB$ strain of *A. nidulans* shows a > 3-fold reduction in trehalose degradation during spore germination (Lafon *et al.* 2005). Breakdown of this disaccharide is a direct outcome of activation of the cAMP/PKA pathway during early germination (d'Enfert *et al.* 1999). A role of GanB in cAMP/PKA signaling has been proven by measuring cAMP levels in germinating spores. Addition of glucose to dormant wild-type spores results in a rapid and transient increase in cAMP levels. This increase is not observed in the $\Delta ganB$ strain (Lafon *et al.* 2005). Thus, GanB regulates cAMP synthesis in response to glucose at the start of germination. As described above GanB forms a heterotrimer G-protein together with SfaD and GpgA (Fig. 11). Spore germination and trehalose degradation is also affected in conidia of the $\Delta sfaD$ and $\Delta gpgA$ strains, although not as strong as observed in the $\Delta ganB$ strain (Lafon *et al.* 2005). A role of RgsA in GanB signaling is further supported by the finding that trehalose degradation in the $\Delta rgsA$ strain is increased. This effect is abolished by inactivation of *ganB* in this strain but also by inactivation of *sfaD* (Lafon *et al.* 2005). These data indicate that glucose-stimulated activation of the cAMP/PKA pathway by GanB requires a functional G-protein formed by GanB, SfaD, and GpgA (Fig. 11).

CyaA represents the adenylate cyclase of the cAMP/PKA pathway that is regulated by GanB (Fig. 11). In contrast to the wild-type, mycelium of the $\Delta cyaA$ strain is completely devoid of cAMP

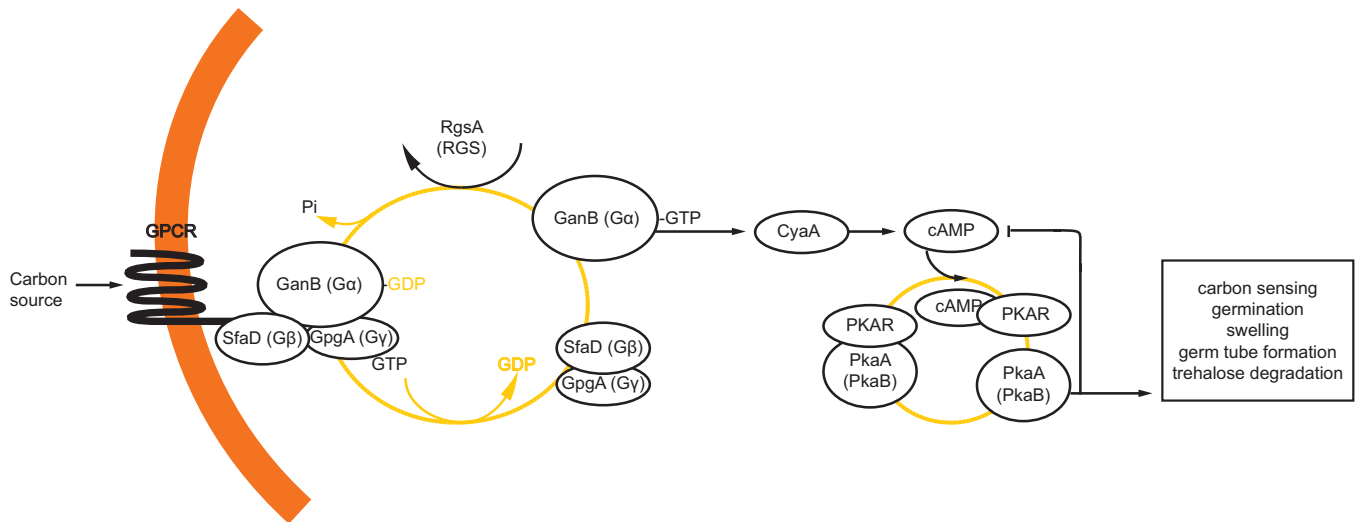


Fig. 11. The cAMP/protein kinase A signaling pathway involved in germination of spores in *A. nidulans*. The presence of a carbon source is sensed by a GPCR that activates the G α subunit GanB. GanB-GTP activates adenylate cyclase CyaA that produces cyclic adenosine-monophosphate (cAMP). cAMP binds to the regulatory subunit of PKA (PKAR), thus releasing the catalytic subunit PkaA. Active PkaA phosphorylates downstream targets resulting in swelling, germ tube formation and trehalose degradation. PkaA and PkaB have an overlapping role in spore germination in the presence of glucose but an opposite role in germination in the absence of a carbon source.

(Fillinger *et al.* 2002). This indicates that *cyaA* encodes the unique adenylate cyclase during mycelial growth. Conidia of the $\Delta cyaA$ strain do not degrade trehalose during the onset of germination. Moreover, germ tube outgrowth is affected. As mentioned above, cAMP produced by adenylate cyclase activates the catalytic subunit of PKA (PKAc) by releasing the regulatory subunit. In the case of *A. nidulans*, *pkaA* encodes the primary PKAc. Trehalose breakdown is reduced in the $\Delta pkaA$ conidia and germ tube outgrowth is affected, but not as strong as in the $\Delta cyaA$ conidia (Fillinger *et al.* 2002). Germination of conidia is also affected in *A. fumigatus* (Liebmann *et al.* 2004) and *A. niger* (Saudohar *et al.* 2002) when their closest homologue of *pkaA* is inactivated. *Aspergillus nidulans* contains a secondary *pka* gene, *pkaB*, which encodes a catalytic subunit of PKA. The $\Delta pkaB$ strain does not have an apparent phenotype (Ni *et al.* 2005). However, the $\Delta pkaA\Delta pkaB$ strain is lethal, indicating that PkaB is involved in hyphal growth and/or spore germination. Approximately 10-fold up-regulation of *pkaB* mRNA levels rescues the defects in germination of conidia of the $\Delta pkaA$ strain in the presence of glucose (note that the level of up-regulation is important for the phenotypic outcome). In contrast, up-regulation of *pkaB* completely abolishes spore germination in the absence of an external carbon source. Taken together, these data indicate that PkaA and PkaB have an overlapping role in spore germination in the presence of glucose but an opposite role in germination in the absence of a carbon source (Ni *et al.* 2005). Other Ser/Thr protein kinases also contribute to spore germination in *A. nidulans*. Inactivation of the Ser/Thr protein kinase gene, *schA*, in the $\Delta pkaA$ background results in a phenotype similar to that of the $\Delta cyaA$ conidia. This indicates that PkaA and SchA are activated by cAMP produced by CyaA.

The Ras signaling pathway operates independently from the cAMP/PKA signaling pathway during germination of conidia of *A. nidulans* (Fillinger *et al.* 2002). Conidia of *A. nidulans* strains expressing a dominant active form of RasA (RasA^{G17V}) do not proceed to polarised growth. Instead, swelling continues resulting in giant swollen spores (Som & Kolaparthi 1994). This suggests that high RasA activity prevents the switch from isotropic to polarised growth. There are indications that the RasA activity is regulated by a GTPase-activating protein GapA. By stimulating hydrolysis of the GTP bound to RasA it loses its activity (Harispe *et al.* 2008). Both

wild-type and $\Delta gapA$ conidia germinate in the presence of glucose. In contrast, whereas $\Delta gapA$ conidia also swell in the absence of a carbon source, the wild-type does not. A similar phenotype has been reported for *A. nidulans* expressing RasA^{G17V}. This suggests that RasA plays a role in carbon source sensing during conidiation (Osheroev & May 2000), and that this is regulated by GapA. RasA has been suggested to function via activation of a mitogen-activated protein kinase pathway (Fillinger *et al.* 2002). This pathway may well include the mitogen-activated protein kinase MpkA since conidia of the $\Delta mpkA$ strain have a defect in germination (Bussink & Osmani 1999).

Regulation by *stuA* and *flbC*

Genes *fluG*, *brlA*, *abaA*, *wetA*, *medA*, *stuA*, and *vosA* play a central role in conidiophore and conidia formation in aspergilli (Fig. 4). The developmental modifier StuA is a transcriptional regulator involved in proper spatial distribution of AbaA and BrlA (Miller *et al.* 1992, Wu & Miller 1997). Conidiophores of the $\Delta stuA$ strain of *A. fumigatus* are extremely malformed and the number of conidia that are formed is strongly reduced (Sheppard *et al.* 2005). Moreover, these conidiophores are twice the size of wild-type spores. Interestingly, they germinate faster but the underlying mechanism is not known yet. Several transcriptional activators act upstream of BrlA in the regulation of asexual development (Fig. 6). One of these regulators is FlbC, which also has a role in germination (Kwon *et al.* 2010a). Polarised growth has taken place for up to 40 % and 100 % of wild-type conidia 4 h and 6 h post-inoculation, respectively. In contrast, spores of the $\Delta flbC$ strain only show swelling after 4 h, whereas up to 40 % of the spores have formed germ tubes 6 h after inoculation. These findings show that FlbC has a role in germination.

CONCLUSIONS

The genus *Aspergillus* represents a diverse group of fungi that are among the most abundant fungi in the world. The success of aspergilli is explained by the fact that they are not very selective with respect to their abiotic growth conditions, that they can degrade a wide variety of organic molecules, and by the fact that they produce high numbers of asexual and sexual spores that are dispersed over short and

long distances. We have now a strong framework of understanding of molecular mechanisms underlying growth and development of *Aspergillus* but the picture is far from complete. Signalling cascades and transcription factors that are involved in germination, in formation of a vegetative mycelium, and in asexual and sexual development are only partly known. There is clear evidence that these regulatory processes are similar but not identical between *Aspergillus* species. The consequences of these differences for the success of the *Aspergillus* species in nature are not known. Moreover, the question why sexual reproduction in a large group of aspergilli is more restrictive than in other representatives of this genus should be answered, as well as the question what the consequences are for the fitness of the different species.

Expression profiles of conidiophores, conidia, ascocarps, ascospores, germlings, and the vegetative mycelium are clearly distinct. Heterogeneity in gene expression is also found between micro-colonies of a liquid culture of *Aspergillus*. Heterogeneity is even observed between and within zones of the vegetative mycelium. The role of heterogeneity between the leading hyphae that explore the substrate is not known but it is tempting to speculate that the existence of hyphal types increases the fitness of the colony. Heterogeneity between hyphae also has implications how RNA, protein, and metabolite profiles from whole cultures should be interpreted. An average gene expression or activity of the hyphae will be obtained when the whole culture, or for instance, whole ascocarps or conidiophores are used for analysis. This average may by far not reflect the composition or activity of particular cellular or hyphal types within the mycelium or tissue that is being analysed. As a consequence, regulatory mechanisms may not be identified or mis-interpreted. Therefore, single cells or particular cell types should be analysed to improve our understanding of growth and development of *Aspergillus* and other fungi.

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REFERENCES

- Adams TH, Boylan MT, Timberlake WE (1988). *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. *Cell* **54**: 353–362.
- Adams TH, Wieser JK, Yu JH (1998). Asexual sporulation in *Aspergillus nidulans*. *Microbiology and Molecular Biology Reviews* **62**: 35–54.
- Agnihotri VP (1964). Studies on *Aspergilli*. XVI. Effect of pH, temperature, and carbon and nitrogen interaction. *Mycopathologia et Mycologia Applicata* **24**: 305–314.
- Aguirre J (1993). Spatial and temporal controls of the *Aspergillus brlA* developmental regulatory gene. *Molecular Microbiology* **8**: 211–8.
- Aimanianda V, Bayry J, Bozza S, Knemeyer O, Perruccio K, et al. (2009). Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* **460**: 1117–1121.
- Aimanianda V, Latgé JP (2010). Fungal hydrophobins form a sheath preventing immune recognition of airborne conidia. *Virulence* **1**: 185–187.
- Al-Doory Y (1984). Chapter 3. In: *Mould Allergy*. (Al-Doory Y, Domsom JF, eds) Lea and Febiger, Philadelphia: 287.
- Andrianopoulos A, Timberlake WE (1994). The *Aspergillus nidulans abaA* gene encodes a transcriptional activator that acts as a genetic switch to control development. *Molecular and Cellular Biology* **14**: 2503–2515.
- Astoreca A, Magnoli C, Ramirez ML, Combina M, Dalcero A (2007). Water activity and temperature effects on growth of *Aspergillus niger*, *A. awamori* and *A. carbonarius* isolated from different substrates in Argentina. *International Journal of Food Microbiology* **119**: 314–318.
- Ayerst G (1969). The effects of moisture and temperature on growth and spore germination in some fungi. *Journal of Stored Products Research* **5**: 127–141.
- Bahn YS, Xue C, Idnurm A, Rutherford JC, Heitman J, Cardenas ME (2007). Sensing the environment: lessons from fungi. *Nature Reviews Microbiology* **5**: 57–69.
- Baker SE (2008). *Aspergillus* genomics and DHN-melanin conidial pigmentation. In: *Aspergillus in the genomics era*. (Varga J, Samson RA, eds) Wageningen Academic Publishers, Wageningen: 73–82.
- Banuet F (1998). Signalling in the yeasts: an informational cascade with links to the filamentous fungi. *Microbiology and Molecular Biology Reviews* **62**: 249–274.
- Bayram Ö, Biesemann C, Krappmann S, Galland P, Braus GH (2008a). More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. *Molecular Biology of the Cell* **19**: 3254–3262.
- Bayram Ö, Krappmann S, Ni M, Bok JW, Helmstaedt K, et al. GH (2008b). VeIB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. *Science* **320**: 1504–1506.
- Bayram Ö, Braus GH, Fischer R, Rodriguez-Romero J (2010). Spotlight on *Aspergillus nidulans* photosensory systems. *Fungal Genetics and Biology* **47**: 900–908.
- Bekker C de, Bruning O, Jonker MJ, Breit TM, Wösten HAB (2011a). Single cell transcriptomics of neighboring hyphae of *Aspergillus niger*. *Genome Biology* **12**: R71.
- Bekker C de, Veluw GJ van, Vinck A, Wiebenga LA, Wösten HAB (2011b). Heterogeneity of *Aspergillus niger* microcolonies in liquid shaken cultures. *Applied and Environmental Microbiology* **77**: 1263–1267.
- Benjamin CR (1955). Ascocarps of *Aspergillus* and *Penicillium*. *Mycologia* **47**: 669–687.
- Bennett JW (2010). An overview of the genus *Aspergillus*. In: *Aspergillus: Molecular Biology and Genomics*. (Machida M, Gomi K, eds) Caister Academic Press, Portland: 1–17.
- Beuchat LR (1986). Extraordinary heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* ascospores in fruit products. *Journal of Food Science* **51**: 1506–1510.
- Bhargava S, Wenger KS, Marten MR (2003). Pulsed feeding during fed-batch *Aspergillus oryzae* fermentation leads to improved oxygen mass transfer. *Biotechnology Progress* **19**: 1091–1094.
- Bleichrodt R (2012). Intercompartmental streaming in *Aspergillus*. PhD Thesis, University of Utrecht.
- Bleichrodt R, Vinck A, Leeuwen MR van, Dijksterhuis J, Grijpstra J, Wösten HAB (2013). Cytoplasmic streaming in vegetative mycelium and aerial structures of *Aspergillus niger*. *Studies in Mycology* **74**: 31–46.
- Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, et al. (2005). The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. *Current Biology* **15**: 1833–1838.
- Bok JW, Keller NP (2004). LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryotic Cell* **3**: 527–535.
- Boylan MT, Mirabito PM, Willett CE, Zimmerman CR, Timberlake WE (1987). Isolation and physical characterization of three essential conidiation genes from *Aspergillus nidulans*. *Molecular and Cellular Biology* **7**: 3113–3118.
- Brakhage AA (2005). Systemic fungal infections caused by *Aspergillus* species: epidemiology, infection process and virulence determinants. *Current Drug Targets* **6**: 875–886.
- Brandt S, von Stetten D, Gunther M, Hildebrandt P, Frankenberg-Dinkel N (2008). The fungal phytochrome FphA from *Aspergillus nidulans*. *The Journal of Biological Chemistry* **283**: 34605–34614.
- Braus GH, Krappmann S, Eckert SE (2002). Sexual development in ascomycetes - fruit body formation of *Aspergillus nidulans*. In: *Molecular Biology of Fungal Development*. (Osiewacz HD, ed.) Dekker, New York: 215–244.
- Breakspear A, Momany M (2007). *Aspergillus nidulans* conidiation genes *dewa*, *fluG*, and *stuA* are differentially regulated in early vegetative growth. *Eukaryotic Cell* **6**: 1697–1700.
- Bruns S, Knemeyer O, Hasenberg M, Aimanianda V, Nietzsche S, Thywissen A, et al. (2010). Production of extracellular traps against *Aspergillus fumigatus* in vitro and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA. *PLoS Pathogens* **6**: e1000873.
- 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.
- Bussink HJ, Osmani SA (1999). A mitogen-activated protein kinase (MPKA) is involved in polarized growth in the filamentous fungus, *Aspergillus nidulans*. *FEMS Microbiology Letters* **173**: 117–125.
- Cai JJ, Woo PC, Lau SK, Smith DK, Yuen KY (2006). Accelerated evolutionary rate may be responsible for the emergence of lineage-specific genes in ascomycota. *Journal of Molecular Evolution* **63**: 1–11.
- Calvo AM, Wilson RA, Bok JW, Keller NP (2002). Relationship between secondary metabolism and fungal development. *Microbiology and Molecular Biology Reviews* **66**: 447–459.
- Calvo AM, Bok JW, Brooks W, Keller NP (2004). *veA* is required for toxin and sclerotial production in *Aspergillus parasiticus*. *Applied and Environmental Microbiology* **70**: 4733–4739.

- Carlsen M, Spohr AB, Nielsen J, Villadsen J (1996). Morphology and physiology of an alpha-amylase producing strain of *Aspergillus oryzae* during batch cultivations. *Biotechnology and Bioengineering* **49**: 266–276.
- Champe SP, Rao P, Chang A (1987). An endogenous inducer of sexual development in *Aspergillus nidulans*. *Journal of General Microbiology* **133**: 1383–1387.
- Champe SP, el-Zayat AA (1989). Isolation of a sexual sporulation hormone from *Aspergillus nidulans*. *Journal of Bacteriology* **171**: 3982–3988.
- Champe SP, Simon LD (1992). Cellular differentiation and tissue formation in the fungus *Aspergillus nidulans*. In: *Morphogenesis: an analysis of the development of biological form*. (Rossomando EF, Alexander S, eds) Marcel Dekker INC., New York: 63–91.
- Champe SP, Nagle DL, Yager LN (1994). Sexual sporulation. *Progress in Industrial Microbiology* **29**: 429–454.
- Chang MH, Chae KS, Han DM, Jahng KY (2004). The GanB Galpha-protein negatively regulates asexual sporulation and plays a positive role in conidial germination in *Aspergillus nidulans*. *Genetics* **167**: 1305–1315.
- Chipeta ZA, du Preez JC, Christopher L (2008). Effect of cultivation pH and agitation rate on growth and xylanase production by *Aspergillus oryzae* in spent sulphite liquor. *Journal of Industrial Microbiology & Biotechnology* **35**: 587–594.
- Chitarra GS, Abee T, Rombouts FM, Posthumus MA, Dijksterhuis J (2004). Germination of *Penicillium paneum* conidia is regulated by 1-octen-3-ol, a volatile self-inhibitor. *Applied and Environmental Microbiology* **70**: 2823–2829.
- Clutterbuck AJ (1969). A mutational analysis of conidial development in *Aspergillus nidulans*. *Genetics* **63**: 317–327.
- Clutterbuck AJ (1977). The genetics of conidiation in *Aspergillus nidulans*. In: *Genetics and Physiology of Aspergillus*. (Smith JE, Pateman JA, eds) Academic Press, London: 305–317.
- Coppin E, Debuchy R, Arnaise S, Picard M (1997). Mating types and sexual development in filamentous ascomycetes. *Microbiology and Molecular Biology Reviews* **61**: 411–428.
- Dagenais TR, Giles SS, Amanianda V, Latgé JP, Hull CM, Keller NP (2010). *Aspergillus fumigatus* LaeA-mediated phagocytosis is associated with a decreased hydrophobin layer. *Infection and Immunity* **78**: 823–829.
- d'Enfert C (1997). Fungal Spore Germination: Insights from the Molecular Genetics of *Aspergillus nidulans* and *Neurospora crassa*. *Fungal Genetics and Biology* **21**: 163–172.
- d'Enfert C, Fontaine T (1997). Molecular characterization of the *Aspergillus nidulans* *treA* gene encoding an acid trehalase required for growth on trehalose. *Molecular Microbiology* **24**: 203–216.
- d'Enfert C, Bonini BM, Zapella PD, Fontaine T, da Silva AM, Terenzi HF (1999). Neutral trehalases catalyze intracellular trehalose breakdown in the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa*. *Molecular Microbiology* **32**: 471–483.
- Denning DW (1998). Invasive aspergillosis. *Clinical Infectious Diseases* **26**: 781–803.
- Dickman MB, Yarden O (1999). Serine/threonine protein kinases and phosphatases in filamentous fungi. *Fungal Genetics and Biology* **26**: 99–117.
- Diepingen AD van, Pal K, Lee TAJ van der, Hoekstra RF, Debets AJM (2009). The *het-c* heterokaryon incompatibility gene in *Aspergillus niger*. *Mycological research* **113**: 222–229. Dijksterhuis J, Nijse J, Hoekstra FA, Golovina EA (2007). High viscosity and anisotropy characterize the cytoplasm of fungal dormant stress-resistant spores. *Eukaryotic Cell* **6**: 157–170.
- D'Souza CA, Lee BN, Adams TH (2001). Characterization of the role of the FluG protein in asexual development of *Aspergillus nidulans*. *Genetics* **158**: 1027–1036.
- Dyer PS, Paoletti M, Archer DB (2003). Genomics reveals sexual secrets of *Aspergillus*. *Microbiology* **149**: 2301–2303.
- Dyer PS, Paoletti M (2005). Reproduction in *Aspergillus fumigatus*: sexuality in a supposedly asexual species? *Medical Mycology* **43**: S7–S14.
- Dynesen J, Nielsen J (2003). Surface hydrophobicity of *Aspergillus nidulans* conidia and its role in pellet formation. *Biotechnology Progress* **19**: 1049–1052.
- Etxebeste O, Ni M, Garzia A, Kwon NJ, Fischer R, et al. (2008). Basic-zipper-type transcription factor F1bB controls asexual development in *Aspergillus nidulans*. *Eukaryotic Cell* **7**: 38–48.
- Etxebeste O, Herrero-García E, Araújo-Bazán L, Rodríguez-Urra AB, Garzia A, et al. (2009). The bZIP-type transcription factor F1bB regulates distinct morphogenetic stages of colony formation in *Aspergillus nidulans*. *Molecular Microbiology* **73**: 775–789.
- Etxebeste O, Garzia A, Espeso EA, Ugalde U (2010a). *Aspergillus nidulans* asexual development: making the most of cellular modules. *Trends in Microbiology* **18**: 569–576.
- Etxebeste O, Ugalde U, Espeso EA (2010b). Adaptive and developmental responses to stress in *Aspergillus nidulans*. *Current Protein & Peptide Science* **11**: 704–718.
- Fillinger S, Chaveroche MK, Dijk P van, Vries RP de, Ruijter GJ, et al. (2001). Trehalose is required for the acquisition of tolerance to a variety of stresses in the filamentous fungus *Aspergillus nidulans*. *Microbiology* **147**: 1851–1862.
- Fillinger S, Chaveroche MK, Shimizu K, Keller NP, d'Enfert C (2002). cAMP and ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. *Molecular Microbiology* **44**: 1001–1016.
- Finkelstein DB, Rambossek J, Crawford MS, Soliday CL, McAda PC (1989). Protein secretion in *Aspergillus niger*. In: *Genetics and Molecular Biology of Industrial Microorganisms*. (Hershberger CL, Queener SW, Hegeman G, eds) American Society of Microbiology, Washington DC: 295–300.
- Fischer R, Kües U (2006). Asexual Sporulation in Mycelial Fungi. In: *The Mycota I: Growth, Differentiation and Sexuality*. (Kües U, Fischer R, eds) Springer Berlin Heidelberg, New York: 263–292.
- Fujii I, Yasuoka Y, Tsai HF, Chang YC, Kwon-Chung KJ, Ebizuka Y (2004). Hydrolytic polyketide shortening by Ayg1p, a novel enzyme involved in fungal melanin biosynthesis. *The Journal of Biological Chemistry* **279**: 44613–44620.
- Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, et al. (2005). Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* **438**: 1105–1115.
- Garscha U, Jerneren F, Chung D, Keller NP, Hamberg M, Oliv EH (2007). Identification of dioxygenases required for *Aspergillus* development. Studies of products, stereochemistry, and the reaction mechanism. *The Journal of Biological Chemistry* **282**: 34707–34718.
- Garzia A, Etxebeste O, Herrero-García E, Fischer R, Espeso EA, Ugalde U (2009). *Aspergillus nidulans* F1bE is an upstream developmental activator of conidiation functionally associated with the putative transcription factor F1bB. *Molecular Microbiology* **71**: 172–184.
- Garzia A, Etxebeste O, Herrero-García E, Ugalde U, Espeso EA (2010). The concerted action of bZip and cMyb transcription factors F1bB and F1bD induces *brfA* expression and asexual development in *Aspergillus nidulans*. *Molecular Microbiology* **75**: 1314–1324.
- Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, et al. (2000). Genomic expression programs in the response of yeast cells to environmental changes. *Molecular Biology of the Cell* **11**: 4241–4257.
- Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, et al. (2006). Proteome survey reveals modularity of the yeast cell machinery. *Nature* **440**: 631–636.
- Geiser DM, Klich MA, Frisvad JC, Peterson SW, Varga J, Samson RA (2007). The current status of species recognition and identification in *Aspergillus*. *Studies in Mycology* **59**: 1–10.
- Geiser DM (2009). Sexual structures in *Aspergillus*: morphology, importance and genomics. *Medical Mycology* **47**: S21–S26.
- Gibson AM, Baranyi J, Pitt JI, Eyles MJ, Roberts TA (1994). Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. *International Journal of Food Microbiology* **23**: 419–431.
- 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*. *Molecular Microbiology* **41**: 299–309.
- Han KH, Seo JA, Yu JH (2004a). Regulators of G-protein signalling in *Aspergillus nidulans*: RgsA downregulates stress response and stimulates asexual sporulation through attenuation of GanB (Galpha) signalling. *Molecular Microbiology* **53**: 529–540.
- Han KH, Seo JA, Yu JH (2004b). A putative G protein-coupled receptor negatively controls sexual development in *Aspergillus nidulans*. *Molecular Microbiology* **51**: 1333–1345.
- Han S, Adams TH (2001). Complex control of the developmental regulatory locus *brfA* in *Aspergillus nidulans*. *Molecular Genetics and Genomics* **266**: 260–270.
- Harispe L, Portela C, Scazzocchio C, Penalva MA, Gorfinkiel L (2008). Ras GTPase-activating protein regulation of actin cytoskeleton and hyphal polarity in *Aspergillus nidulans*. *Eukaryotic Cell* **7**: 141–153.
- Harris SD, Momany M (2004). Polarity in filamentous fungi: moving beyond the yeast paradigm. *Fungal Genetics and Biology* **41**: 391–400.
- Harris SD (2006). Cell polarity in filamentous fungi: shaping the mold. *International Review of Cytology* **251**: 41–77.
- Hawksworth DL (2011). Naming *Aspergillus* species: progress towards one name for each species. *Medical Mycology* **49**: S70–S76.
- Herrero-García E, Garzia A, Cordobes S, Espeso EA, Ugalde U (2011). 8-Carbon oxylipins inhibit germination and growth, and stimulate aerial conidiation in *Aspergillus nidulans*. *Fungal Biology* **115**: 393–400.
- Hicks JK, Yu JH, Keller NP, Adams TH (1997). *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA G alpha protein-dependent signaling pathway. *The EMBO Journal* **16**: 4916–4923.
- Horn BW, Moore GG, Carbone I (2009a). Sexual reproduction in *Aspergillus flavus*. *Mycologia* **101**: 423–429.
- Horn BW, Ramirez-Prado JH, Carbone I (2009b). The sexual state of *Aspergillus parasiticus*. *Mycologia* **101**: 275–280.
- Horn BW, Moore GG, Carbone I (2011). Sexual reproduction in aflatoxin-producing *Aspergillus nomius*. *Mycologia* **103**: 174–183.
- Ichinomiya M, Ohta A, Horiuchi H (2005). Expression of asexual developmental regulator gene *abaA* is affected in the double mutants of classes I and II chitin

- synthase genes, *chsC* and *chsA*, of *Aspergillus nidulans*. *Current Genetics* **48**: 171–183.
- Ishitani C, Sakaguchi KI (1956). Hereditary variation and recombination in Koji molds (*Aspergillus oryzae* and *Asp. sojae*) v. heterocaryosis. *Journal of General and Applied Microbiology* **2**: 345–399.
- Jennings DH (1984). Water flow through mycelia. In: *The ecology and physiology of fungal mycelia*. (Jennings DH, Rayner ADM, eds) Cambridge University Press, Cambridge: 143–164.
- Jennings DH (1987). Translocation of solutes in fungi. *Biological Reviews* **62**: 215–243.
- Jensen BG, Andersen MR, Pedersen MH, Frisvad JC, Sondergaard I (2010). Hydrophobins from *Aspergillus* species cannot be clearly divided into two classes. *BMC Research Notes* **3**: 344.
- Jørgensen TR, Park J, Arentshorst M, Welzen AM van, Lamers G, VanKuyk PA, et al. (2011). The molecular and genetic basis of conidial pigmentation in *Aspergillus niger*. *Fungal Genetics and Biology* **48**: 544–553.
- Käfer E (1965). Origins of translocations in *Aspergillus nidulans*. *Genetics* **52**: 217–232.
- Kato N, Brooks W, Calvo AM (2003). The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by *veA*, a gene required for sexual development. *Eukaryotic Cell* **2**: 1178–1186.
- Kim H, Han K, Kim K, Han D, Jahng K, Chae K (2002). The *veA* gene activates sexual development in *Aspergillus nidulans*. *Fungal Genetics and Biology* **37**: 72–80.
- Kim HR, Chae KS, Han KH, Han DM (2009). The *nsdC* gene encoding a putative C₂H₂-type transcription factor is a key activator of sexual development in *Aspergillus nidulans*. *Genetics* **182**: 771–783.
- Krappmann S, Bayram Ö, Braus GH (2005). Deletion and allelic exchange of the *Aspergillus fumigatus veA* locus via a novel recyclable marker module. *Eukaryotic Cell* **4**: 1298–1307.
- Kwon NJ, Garzia A, Espeso EA, Ugalde U, Yu JH (2010a). F1bC is a putative nuclear C₂H₂ transcription factor regulating development in *Aspergillus nidulans*. *Molecular Microbiology* **77**: 1203–1219.
- Kwon NJ, Shin KS, Yu JH (2010b). Characterization of the developmental regulator F1bE in *Aspergillus fumigatus* and *Aspergillus nidulans*. *Fungal Genetics and Biology* **47**: 981–993.
- Lacey J (1980). The microflora of graindust. In: *Occupational pulmonary disease: focus on graindust and health*. (Doseman JA, Cotton DA, eds) Academic Press, New York: 417–440.
- Lafon A, Seo JA, Han KH, Yu JH, d'Enfert C (2005). The heterotrimeric G-protein GanB(alpha)-SfaD(beta)-GpgA(gamma) is a carbon source sensor involved in early cAMP-dependent germination in *Aspergillus nidulans*. *Genetics* **171**: 71–80.
- Lafon A, Han KH, Seo JA, Yu JH, d'Enfert C (2006). G-protein and cAMP-mediated signaling in aspergilli: a genomic perspective. *Fungal Genetics and Biology* **43**: 490–502.
- Lamarre C, Sokol S, Debeauvais JP, Henry C, Lacroix C, Glaser P, Coppee JY, Francois JM, Latgé JP (2008). Transcriptomic analysis of the exit from dormancy of *Aspergillus fumigatus* conidia. *BMC Genomics* **9**: 417.
- Lee BN, Adams TH (1994a). Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation, leads to activation of *brlA* and premature initiation of development. *Molecular Microbiology* **14**: 323–334.
- Lee BN, Adams TH (1994b). The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. *Genes & Development* **8**: 641–651.
- Lee BN, Adams TH (1996). *FluG* and *flbA* function interdependently to initiate conidiophore development in *Aspergillus nidulans* through *brlA* beta activation. *The EMBO Journal* **15**: 299–309.
- Leeuwen MR van, Krijgheld P, Bleichrodt R, Menke H, Stam H, Stark J, Wösten HAB, Dijksterhuis J (2013a). Germination of conidia of *Aspergillus niger* is accompanied by major changes in RNA profiles *Studies in Mycology* **74**: xx–xx. (This issue)
- Leeuwen MR van, Krijgheld P, Wyatt TT, Golovina EA, Menke H, et al. (2013b). The effect of natamycin on the transcriptome of germinating conidia: “Nothing is what it seems” *Studies in Mycology* **74**: xx–xx. (This issue)
- Leong SL, Hocking AD, Scott ES (2006). Effect of temperature and water activity on growth and ochratoxin A production by Australian *Aspergillus carbonarius* and *A. niger* isolates on a simulated grape juice medium. *International Journal of Food Microbiology* **110**: 209–216.
- Leong SL, Hien LT, An TV, Trang NT, Hocking AD, Scott ES (2007). Ochratoxin A-producing *Aspergilli* in Vietnamese green coffee beans. *Letters in Applied Microbiology* **45**: 301–306.
- Levin AM, Vries RP de, Conesa A, Bekker C de, Talon M, et al. (2007a). Spatial differentiation in the vegetative mycelium of *Aspergillus niger*. *Eukaryotic Cell* **6**: 2311–2322.
- Levin AM, Vries RP de, Wösten HAB (2007b). Localization of protein secretion in fungal colonies using a novel culturing technique; the ring-plate system. *Journal of Microbiological Methods* **69**: 399–401.
- Li B, Nierras CR, Warner JR (1999). Transcriptional elements involved in the repression of ribosomal protein synthesis. *Molecular and Cellular Biology* **19**: 5393–5404.
- Liebmann B, Gattung S, Jahn B, Brakhage AA (2003). cAMP signaling in *Aspergillus fumigatus* is involved in the regulation of the virulence gene *pksP* and in defense against killing by macrophages. *Molecular Genetics and Genomics* **269**: 420–435.
- Liebmann B, Müller M, Braun A, Brakhage AA (2004). The cyclic AMP-dependent protein kinase a network regulates development and virulence in *Aspergillus fumigatus*. *Infection and Immunity* **72**: 5193–5203.
- Mah JH, Yu JH (2006). Upstream and downstream regulation of asexual development in *Aspergillus fumigatus*. *Eukaryotic Cell* **5**: 1585–1595.
- Marshall MA, Timberlake WE (1991). *Aspergillus nidulans wetA* activates spore-specific gene expression. *Molecular and Cellular Biology* **11**: 55–62.
- Masai K, Maruyama J, Sakamoto K, Nakajima H, Akita O, Kitamoto K (2006). Square-plate culture method allows detection of differential gene expression and screening of novel, region-specific genes in *Aspergillus oryzae*. *Applied Microbiology and Biotechnology* **71**: 881–891.
- Mazur P, Meyers HV, Nakanishi K, el-Zayat AA, Champe SP (1990). Structural elucidation of sporogenic fatty acid metabolites from *Aspergillus nidulans*. *Tetrahedron Letters* **31**: 3837–3840.
- Mazur P, Nakanishi K, El-Zayat AA, Champe SP (1991). Structure and synthesis of sporogenic psi factors from *Aspergillus nidulans*. *Journal of the Chemical Society, Chemical Communications* **20**: 1486–1487.
- Mehra S, Jaitly A (1995). pH and temperature optima for growth and sporulation in some common fungi from city waste. *Mycoscience* **36**: 243–246.
- Metz B, Kossen NWF (1977). The growth of molds in the form of pellets- a literature review. *Biotechnology and Bioengineering* **19**: 781–799.
- Meyer V, Wu B, Ram AFJ (2011). *Aspergillus* as a multi-purpose cell factory: current status and perspectives. *Biotechnology Letters* **33**: 469–476.
- Miller KY, Toennis TM, Adams TH, Miller BL (1991). Isolation and transcriptional characterization of a morphological modifier: the *Aspergillus nidulans* stunted (*stuA*) gene. *Molecular and General Genetics* **227**: 285–292.
- Miller KY, Wu J, Miller BL (1992). *StuA* is required for cell pattern formation in *Aspergillus*. *Genes & Development* **6**: 1770–1782.
- Mirabito PM, Adams TH, Timberlake WE (1989). Interactions of three sequentially expressed genes control temporal and spatial specificity in *Aspergillus* development. *Cell* **57**: 859–868.
- Mogensen J, Nielsen HB, Hofmann G, Nielsen J (2006). Transcription analysis using high-density micro-arrays of *Aspergillus nidulans* wild-type and *creA* mutant during growth on glucose or ethanol. *Fungal Genetics and Biology* **43**: 593–603.
- Momany M (2002). Polarity in filamentous fungi: establishment, maintenance and new axes. *Current Opinion in Microbiology* **5**: 580–585.
- Mooney JL, Hassett DE, Yager LN (1990). Genetic analysis of suppressors of the *veA1* mutation in *Aspergillus nidulans*. *Genetics* **126**: 869–874.
- Moukha SM, Wösten HAB, Asther M, Wessels JG (1993). In situ localization of the secretion of lignin peroxidases in colonies of *Phanerochaete chrysosporium* using a sandwiched mode of culture. *Journal of General Microbiology* **139**: 969–978.
- Nasseri S, Assadi MM, Sepehr MN, Rostami K, Shariat M, Nadafi K (2002). Chromium removal from tanning effluent using biomass of *Aspergillus oryzae*. *Pakistan Journal of Biological Sciences* **5**: 1056–1059.
- Ni M, Riersen S, Seo JA, Yu JH (2005). The *pkaB* gene encoding the secondary protein kinase A catalytic subunit has a synthetic lethal interaction with *pkaA* and plays overlapping and opposite roles in *Aspergillus nidulans*. *Eukaryotic Cell* **4**: 1465–1476.
- Ni M, Yu JH (2007). A novel regulator couples sporogenesis and trehalose biogenesis in *Aspergillus nidulans*. *PLoS One* **2**: e970.
- Nielsen PV, Beuchat LR, Frisvad JC (1988). Growth of and fumitremorgin production by *Neosartorya fischeri* as affected by temperature, light, and water activity. *Applied and Environmental Microbiology* **54**: 1504–1510.
- Ogawa M, Tokuoaka M, Jin FJ, Takahashi T, Koyama Y (2010). Genetic analysis of conidiation regulatory pathways in koji-mold *Aspergillus oryzae*. *Fungal Genetics and Biology* **47**: 10–18.
- O’Gorman CM, Fuller HT, Dyer PS (2009). Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* **457**: 471–474.
- Ogundero VW (1981). Cultural and nutritional studies of zoopathogenic fungi associated with livestock feeds in Nigeria. *Zeitschrift für Allgemeine Mikrobiologie* **21**: 255–259.
- Oshero N, May G (2000). Conidial germination in *Aspergillus nidulans* requires RAS signaling and protein synthesis. *Genetics* **155**: 647–656.
- Oshero N, May GS (2001). The molecular mechanisms of conidial germination. *FEMS Microbiology Letters* **199**: 153–160.
- Pál K, Diepingen AD van, Varga J, Debets AJ, Hoekstra RF (2008). Sexual genes in the asexual filamentous fungus *Aspergillus niger* and related *Aspergilli*. In: *Aspergillus in the genomics era*. (Varga J, Samson RA, eds) Wageningen Academic Publishers, Wageningen: 107–128.

- Panasenko V (1967). Ecology of microfungi. *The Botanical Review* 189–215.
- Paoletti M, Seymour FA, Alcocer MJ, Kaur N, Calvo AM, et al. (2007). Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. *Current Biology* 17: 1384–1389.
- Paris S, Debeauvais JP, Cramer R, Carey M, Charles F, et al. (2003). Conidial hydrophobins of *Aspergillus fumigatus*. *Applied and Environmental Microbiology* 69: 1581–1588.
- Pawar NV, Patil VB, Kamble SS, Dixit GB (2008). First Report of *Aspergillus niger* as a Plant Pathogen on *Zingiber officinale* from India. *Plant Disease* 92: 1368–1368.
- Peer AF van, Bekker C de, Vinck A, Wösten HAB, Lugones LG (2009a). Phleomycin increases transformation efficiency and promotes single integrations in *Schizophyllum commune*. *Applied and Environmental Microbiology* 75: 1243–1247.
- Peer AF van, Müller WH, Boekhout T, Lugones LG, Wösten HAB (2009b). Cytoplasmic continuity revisited: closure of septa of the filamentous fungus *Schizophyllum commune* in response to environmental conditions. *PLoS One* 4: e5977.
- Pel HJ, Winde JH de, Archer DB, Dyer PS, Hofmann G, et al. (2007). Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nature Biotechnology* 25: 221–231.
- Penalva MA, Arst HN (2004). Recent advances in the characterization of ambient pH regulation of gene expression in filamentous fungi and yeasts. *Annual Review of Microbiology* 58: 425–451.
- Pihet M, Vandeputte P, Tronchin G, Renier G, Saulnier P, et al. (2009). Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. *BMC Microbiology* 9:177.
- Pitt JI (1981). Food storage and biodeterioration. In: *Biology of Conidial Fungi*. (Cole GT, Kendrick B, eds) Academic Press, New York: 111–142.
- Pitt JI (1994). The current role of *Aspergillus* and *Penicillium* in human and animal health. *Journal of Medical and Veterinary Mycology* 32 Suppl 1: 17–32.
- Pöggeler S, Nowrousian M, Kück U (2006). Fruiting body development in Ascomycetes. In: *The Mycota I: Growth, Differentiation, and Sexuality*. (Kües U, Fischer R, eds) Springer Verlag, Berlin: 325–355.
- Prade RA, Timberlake WE (1993). The *Aspergillus nidulans* *brlA* regulatory locus consists of overlapping transcription units that are individually required for conidiophore development. *The EMBO Journal* 12: 2439–2447.
- Purschwitz J, Müller S, Kastner C, Fischer R (2006). Seeing the rainbow: light sensing in fungi. *Current Opinion in Microbiology* 9: 566–571.
- Purschwitz J, Müller S, Kastner C, Schoser M, Haas H, et al. (2008). Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. *Current Biology* 18: 255–259.
- Purschwitz J, Müller S, Fischer R (2009). Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the White Collar protein LreB. *Molecular Genetics and Genomics* 281: 35–42.
- Roca MG, Davide LC, Mendes-Costa MC, Wheals A (2003). Conidial anastomosis tubes in *Colletotrichum*. *Fungal Genetics & Biology* 40: 138–145.
- Roca MG, Art J, Jeffrey CE, Read ND (2005a). Cell biology of conidial anastomosis tubes in *Neurospora crassa*. *Eukaryotic Cell* 4: 911–919.
- Roca MG, Read ND, Wheals AE (2005b). Conidial anastomosis tubes in filamentous fungi. *FEMS Microbiology Letters* 249: 191–198.
- Rokas A, Galagan JE (2008). *Aspergillus nidulans* genome and a comparative analysis of genome evolution in *Aspergillus*. In: *The Aspergilli: Genomics, Medical Aspects, Biotechnology, and Research Methods*. (Osmani SA, Goldman GH, eds) Taylor & Francis, Boca Raton: 43–55.
- Roncal T, Ugalde U (2003). Conidiation induction in *Penicillium*. *Research in Microbiology* 154: 539–546.
- Rosén S, Yu JH, Adams TH (1999). The *Aspergillus nidulans* *sfaD* gene encodes a G protein beta subunit that is required for normal growth and repression of sporulation. *The EMBO Journal* 18: 5592–5600.
- Ruger-Herreros C, Rodríguez-Romero J, Fernández-Barranco R, Olmedo M, Fischer R, et al. (2011). Regulation of conidiation by light in *Aspergillus nidulans*. *Genetics* 188: 809–822.
- Ruijter GJ, Bax M, Patel H, Flitter SJ, Vondervoort PJ van de, et al. (2003). Mannitol is required for stress tolerance in *Aspergillus niger* conidia. *Eukaryotic Cell* 2: 690–698.
- Rydholm C, Dyer PS, Lutzoni F (2007). DNA sequence characterization and molecular evolution of MAT1 and MAT2 mating-type loci of the self-compatible ascomycete mold *Neosartorya fischeri*. *Eukaryotic Cell* 6: 868–874.
- Sakamoto K, Iwashita K, Yamada O, Kobayashi K, Mizuno A, et al. (2009). *Aspergillus oryzae* *atfA* controls conidial germination and stress tolerance. *Fungal Genetics and Biology* 46: 887–897.
- Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (2000). Identification of the common food- and airborne fungi, *Neosartorya fischeri*. In: *Introduction to food- and airborne fungi*. (Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O, eds) Centraalbureau Voor Schimmelcultures, Utrecht: 44–47.
- Samson RA, Varga J (2009). What is a species in *Aspergillus*? *Medical Mycology* 47: S13–S20.
- Sarikaya Bayram Ö, Bayram Ö, Valerius O, Park HS, Irriger S, et al. (2010). LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. *PLoS Genetics* 6: e1001226.
- Saudohar M, Bencina M, Vondervoort PJ van de, Panneman H, Legisa M, et al. (2002). Cyclic AMP-dependent protein kinase is involved in morphogenesis of *Aspergillus niger*. *Microbiology* 148: 2635–2645.
- Serna I de la, Ng D, Tyler BM (1999). Carbon regulation of ribosomal genes in *Neurospora crassa* occurs by a mechanism which does not require Cre-1, the homologue of the *Aspergillus* carbon catabolite repressor, CreA. *Fungal Genetics and Biology* 26: 253–269.
- Seo JA, Guan Y, Yu JH (2003). Suppressor mutations bypass the requirement of *fluG* for asexual sporulation and sterigmatocystin production in *Aspergillus nidulans*. *Genetics* 165: 1083–1093.
- 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*. *Molecular Microbiology* 53: 1611–1623.
- Seo JA, Han KH, Yu JH (2005). Multiple roles of a heterotrimeric G-protein gamma-subunit in governing growth and development of *Aspergillus nidulans*. *Genetics* 171: 81–89.
- Seo JA, Guan Y, Yu JH (2006). FluG-dependent asexual development in *Aspergillus nidulans* occurs via derepression. *Genetics* 172: 1535–1544.
- Seo JA, Yu JH (2006). The phosphatase-like protein PhnA is required for G(beta gamma)-mediated signaling for vegetative growth, developmental control, and toxin biosynthesis in *Aspergillus nidulans*. *Eukaryotic Cell* 5: 400–410.
- Sewall TC, Mims CW, Timberlake WE (1990). *abaA* controls phialide differentiation in *Aspergillus nidulans*. *Plant Cell* 2: 731–739.
- Sheppard DC, Doedt T, Chiang LY, Kim HS, Chen D, et al. (2005). The *Aspergillus fumigatus* StuA protein governs the up-regulation of a discrete transcriptional program during the acquisition of developmental competence. *Molecular Biology of the Cell* 16: 5866–5879.
- Shimizu K, Keller NP (2001). Genetic involvement of a cAMP-dependent protein kinase in a G protein signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans*. *Genetics* 157: 591–600.
- Shin KS, Kwon NJ, Yu JH (2009). G(beta gamma)-mediated growth and developmental control in *Aspergillus fumigatus*. *Current Genetics* 55: 631–641.
- Shiu PK, Glass NL (2000). Cell and nuclear recognition mechanisms mediated by mating type in filamentous ascomycetes. *Current Opinion in Microbiology* 3: 183–188.
- Singh S, Sandhu D (1982). Growth response of some thermophilous fungi at different incubation temperatures. *Proceedings: Plant Sciences* 91: 153–158.
- Skromme I, Sanchez O, Aguirre J (1995). Starvation stress modulates the expression of the *Aspergillus nidulans* *brlA* regulatory gene. *Microbiology* 141: 21–28.
- Som T, Kolaparthi VS (1994). Developmental decisions in *Aspergillus nidulans* are modulated by Ras activity. *Molecular and Cellular Biology* 14: 5333–5348.
- Stevens DA, Kan VL, Judson MA, Morrison VA, Dummer S, et al. (2000). Practice guidelines for diseases caused by *Aspergillus*. *Clinical Infectious Diseases* 30: 696–709.
- Stinnett SM, Espeso EA, Cobeno L, Araújo-Bazán L, Calvo AM (2007). *Aspergillus nidulans* VeA subcellular localization is dependent on the importin alpha carrier and on light. *Molecular Microbiology* 63: 242–255.
- Stringer MA, Dean RA, Sewall TC, Timberlake WE (1991). Rodletless, a new *Aspergillus* developmental mutant induced by directed gene inactivation. *Genes & Development* 5: 1161–1171.
- Stringer MA, Timberlake WE (1995). *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall. *Molecular Microbiology* 16: 33–44.
- Sugareva V, Härtl A, Brock M, Hübner K, Rohde M, et al. (2006). Characterisation of the laccase-encoding gene *abr2* of the dihydroxy-naphthalene-like melanin gene cluster of *Aspergillus fumigatus*. *Archives of Microbiology* 186: 345–355.
- Szewczyk E, Krappmann S (2010). Conserved regulators of mating are essential for *Aspergillus fumigatus* cleistothecium formation. *Eukaryotic Cell* 9: 774–783.
- Takahashi T, Maeda H, Yoneda S, Ohtaki S, Yamagata Y, et al. (2005). The fungal hydrophobin RolA recruits polyesterase and laterally moves on hydrophobic surfaces. *Molecular Microbiology* 57: 1780–1796.
- Tao L, Yu JH (2011). AbaA and WetA govern distinct stages of *Aspergillus fumigatus* development. *Microbiology* 157: 313–326.
- Thau N, Monod M, Crestani B, Rolland C, Tronchin G, et al. (1994). Rodletless mutants of *Aspergillus fumigatus*. *Infection and Immunity* 62: 4380–4388.
- Timberlake WE (1980). Developmental gene regulation in *Aspergillus nidulans*. *Developmental Biology* 78: 497–510.
- Tsai HF, Wheeler MH, Chang YC, Kwon-Chung KJ (1999). A developmentally regulated gene cluster involved in conidial pigment biosynthesis in *Aspergillus fumigatus*. *Journal of Bacteriology* 181: 6469–6477.
- Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller NP (2004a). Endogenous lipogenic regulators of spore balance in *Aspergillus nidulans*. *Eukaryotic Cell* 3: 1398–1411.

- Tsitsigiannis DI, Zarnowski R, Keller NP (2004b). The lipid body protein, PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. *The Journal of Biological Chemistry* **279**: 11344–11353.
- Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller NP (2005). Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*. *Microbiology* **151**: 1809–1821.
- Turgeon BG, Yoder OC (2000). Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genetics and Biology* **31**: 1–5.
- Twumasi-Boateng K, Yu Y, Chen D, Gravelat FN, Nierman WC, Sheppard DC (2009). Transcriptional profiling identifies a role for BrIa in the response to nitrogen depletion and for StuA in the regulation of secondary metabolite clusters in *Aspergillus fumigatus*. *Eukaryotic Cell* **8**: 104–115.
- Valik L, Pieckova E (2001). Growth modelling of heat-resistant fungi: the effect of water activity. *International Journal of Food Microbiology* **63**: 11–17.
- Vallim MA, Miller KY, Miller BL (2000). *Aspergillus* SteA (sterile12-like) is a homeodomain-C₂H₂-Zn²⁺ finger transcription factor required for sexual reproduction. *Molecular Microbiology* **36**: 290–301.
- Varga J, Frisvad JC, Samson RA (2011). Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. *Studies in Mycology* **69**: 57–80.
- Vecht-Lifshitz SE, Magdassi S, Braun S (1990). Pellet formation and cellular aggregation in *Streptomyces tendae*. *Biotechnology and Bioengineering* **35**: 890–896.
- Veluw GJ van, Teertstra WR, Bekker C de, Vinck A, Beek N van, et al. (2013). Heterogeneity in liquid shaken cultures of *Aspergillus niger* inoculated with melanised conidia or conidia of pigmentation mutants. *Studies in Mycology* **74**: 47–57.
- Vienken K, Scherer M, Fischer R (2005). The Zn(II)₂Cys₆ putative *Aspergillus nidulans* transcription factor repressor of sexual development inhibits sexual development under low-carbon conditions and in submerged culture. *Genetics* **169**: 619–630.
- Vienken K, Fischer R (2006). The Zn(II)₂Cys₆ putative transcription factor NosA controls fruiting body formation in *Aspergillus nidulans*. *Molecular Microbiology* **61**: 544–554.
- Vinck A, Terlouw M, Pestman WR, Martens EP, Ram AFJ, et al. (2005). Hyphal differentiation in the exploring mycelium of *Aspergillus niger*. *Molecular Microbiology* **58**: 693–699.
- Vinck A, de Bekker C, Ossin A, Ohm RA, Vries RP de, Wösten HAB (2011). Heterogenic expression of genes encoding secreted proteins at the periphery of *Aspergillus niger* colonies. *Environmental Microbiology* **13**: 216–225.
- Wadman MW, Vries RP de, Kalkhove SIC, Veldink GA, Vliegthart JF (2009). Characterization of oxylipins and dioxygenase genes in the asexual fungus *Aspergillus niger*. *BMC Microbiology* **9**: 59.
- Warner JR (1999). The economics of ribosome biosynthesis in yeast. *Trends in Biochemical Sciences* **24**: 437–440.
- Watanabe A, Fujii I, Sankawa U, Mayorga ME, Timberlake WE, Ebizuka Y (1999). Re-identification of *Aspergillus nidulans* wa gene to code for a polyketide synthase of naphthopyrone. *Tetrahedron Letters* **40**: 91–94.
- Watanabe A, Fujii I, Tsai H, Chang YC, Kwon-Chung KJ, Ebizuka Y (2000). *Aspergillus fumigatus* alb1 encodes naphthopyrone synthase when expressed in *Aspergillus oryzae*. *FEMS Microbiology Letters* **192**: 39–44.
- 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*. *Molecular Microbiology* **47**: 1577–1588.
- Wessels JG (1989). The cell wall and protein secretion in fungi. In: *Proceedings of the EMBO-A LKO Workshop on Molecular Biology of Filamentous Fungi*. (Nevalainen H, Penttilä M, eds).
- Wessels JG (1990). Role of cell wall architecture in fungal tip growth generation. In: *Tip Growth in Plant and Fungal Walls*. (Heath IB, ed.) Academic Press, San Diego: 1–29.
- Wetter MA van, Wösten HAB, Sietsma JH, Wessels JG (2000). Hydrophobin gene expression affects hyphal wall composition in *Schizophyllum commune*. *Fungal Genetics and Biology* **31**: 99–104.
- Wieser J, Lee BN, Fondon JW, Adams TH (1994). Genetic requirements for initiating asexual development in *Aspergillus nidulans*. *Current Genetics* **27**: 62–69.
- Wieser J, Adams TH (1995). *fibD* encodes a Myb-like DNA-binding protein that coordinates initiation of *Aspergillus nidulans* conidiophore development. *Genes & Development* **9**: 491–502.
- Wieser J, Yu JH, Adams TH (1997). Dominant mutations affecting both sporulation and sterigmatocystin biosynthesis in *Aspergillus nidulans*. *Current Genetics* **32**: 218–224.
- Williams JP, Hallsworth JE (2009). Limits of life in hostile environments: no barriers to biosphere function? *Environmental Microbiology* **11**: 3292–3308.
- Witteveen CF, Visser J (1995). Polyol pools in *Aspergillus niger*. *FEMS Microbiology Letters* **134**: 57–62.
- Wolschek MF, Kubicek CP (1997). The filamentous fungus *Aspergillus niger* contains two “differentially regulated” trehalose-6-phosphate synthase-encoding genes, *tpsA* and *tpsB*. *The Journal of Biological Chemistry* **272**: 2729–2735.
- Wösten HAB, Moukha SM, Sietsma JH, Wessels JG (1991). Localization of growth and secretion of proteins in *Aspergillus niger*. *Journal of General Microbiology* **137**: 2017–2023.
- Wösten HAB, Richter M, Willey JM (1999a). Structural proteins involved in emergence of microbial aerial hyphae. *Fungal Genetics and Biology* **27**: 153–160.
- Wösten HAB, Wetter MA van, Lugones LG, Mei HC van der, Busscher HJ, Wessels JG (1999b). How a fungus escapes the water to grow into the air. *Current Biology* **9**: 85–88.
- Wösten HAB, Willey JM (2000). Surface-active proteins enable microbial aerial hyphae to grow into the air. *Microbiology* **146**: 767–773.
- Wösten HAB (2001). Hydrophobins: multipurpose proteins. *Annual Review of Microbiology* **55**: 625–646.
- Wu J, Miller BL (1997). *Aspergillus* asexual reproduction and sexual reproduction are differentially affected by transcriptional and translational mechanisms regulating *stunted* gene expression. *Molecular and Cellular Biology* **17**: 6191–6201.
- Xiao P, Shin KS, Wang T, Yu JH (2010). *Aspergillus fumigatus* *fibB* encodes two basic leucine zipper domain (bZIP) proteins required for proper asexual development and gliotoxin production. *Eukaryotic Cell* **9**: 1711–1723.
- Yager LN (1992). Early developmental events during asexual and sexual sporulation in *Aspergillus nidulans*. *Biotechnology* **23**: 19–41.
- Yamada O, Lee BR, Gomi K, Limura Y (1999). Cloning and functional analysis of the *Aspergillus oryzae* conidiation regulator gene *brIa* by its disruption and misscheduled expression. *Journal of Bioscience and Bioengineering* **87**: 424–429.
- Yu JH, Wieser J, Adams TH (1996). The *Aspergillus* FlbA RGS domain protein antagonizes G protein signaling to block proliferation and allow development. *The EMBO Journal* **15**: 5184–5190.
- Yu JH, Rosén S, Adams TH (1999). Extragenic suppressors of loss-of-function mutations in the *Aspergillus* FlbA regulator of G-protein signaling domain protein. *Genetics* **151**: 97–105.
- Yu JH, Keller NP (2005). Regulation of secondary metabolism in filamentous fungi. *Annual Review of Phytopathology* **43**: 437–458.
- Yu JH (2006). Heterotrimeric G protein signaling and RGSs in *Aspergillus nidulans*. *Journal of Microbiology* **44**: 145–154.
- Zonneveld BJM (1977). Biochemistry and ultrastructure of sexual development in *Aspergillus nidulans*. In: *Genetics and Physiology of Aspergillus*. (Smith JE, Pateman JA, eds) Academic Press, London: 59–80.