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.

Published online: 12 September 2012; doi:10.3114/sim0006. Hard copy: March 2013.

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 Emeriella, 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 wide range 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). Aspergillus (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 aspergillosis, 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
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 germ tubes that are in close vicinity to each other have fused. It has been described that fusion in A. oryzae, A. sojae, A. tamarii, and A. nidulans most often occurs between conidia (> 80%), while fusions between conidia and germ tubes and fusion of germ tubes 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 germ tubes 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 Diepening et al. 2009). Fusion of hyphae was reported to be rare when germ tubes 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 part 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
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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 carboxypolymethylene 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

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*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.
| Name   | Description                              | Developmental stage | Function                                                                 | Page number       |
|--------|------------------------------------------|---------------------|--------------------------------------------------------------------------|-------------------|
| abaA   | ATTS transcription factor                | Asexual development | Regulation of sterigmata formation during conidiophore development       | 9, 10, 12, 14, 19, 24 |
| abr1   | Vermelone dehydratase                    | Spore protection    | Melanin biosynthesis in A. fumigatus                                     | 21, 24 |
| abr2   | Laccase with homology to yA              | Spore protection    | Melanin biosynthesis in A. fumigatus                                     | 21 |
| alb1   | Polyketide synthase                      | Spore protection    | Melanin biosynthesis in A. fumigatus                                     | 21 |
| arp1   | Scytalone dehydratase                    | Spore protection    | Melanin biosynthesis in A. fumigatus                                     | 21 |
| arp2   | Hydroxynaphthalene (HN) reductase        | Spore protection    | Melanin biosynthesis in A. fumigatus                                     | 21 |
| ayg1   | Polyketide carbon backbone modification  | Spore protection    | Melanin biosynthesis in A. fumigatus                                     | 21 |
| brtA   | C2H2 zinc finger transcription factor    | Asexual development | Regulation of stalk development                                           | 9–14, 17–19, 21, 24 |
| bmaA   | Multicopper oxidase                      | Spore protection    | Melanin biosynthesis in A. niger                                         | 3, 9, 21 |
| chB    | Class V endochitinase B                  | Vegetative growth   | Autolysis                                                                | 10 |
| chsA   | Chitin synthase                          | Asexual development | Septum formation in conidiophores                                        | 10 |
| chsC   | Chitin synthase                          | Asexual development | Septum formation in conidiophores                                        | 10 |
| cryA   | Cryptochrome/photolyase                   | Blue light response | Inhibition of sexual development                                          | 19–21 |
| cyA    | Adenylate cyclase                        | Germination         | GanB mediated germination                                                 | 24, 25 |
| dewA   | Hydrophobin                              | Asexual development | Coating of conidia                                                       | 3, 9, 14 |
| fadA   | Go-subunit heterotrimeric G-protein complex | Vegetative growth | Inhibition sexual and asexual development Homothallic cleistothecia and Hüle cell formation | 10–13, 16 |
| fbaA   | Regulator of G-protein signaling         | Asexual development | Inhibition of vegetative growth enabling asexual development             | 10–13, 16 |
| fbaB   | bZIP-type transcription factor           | Asexual development | Regulation of conidiophore formation                                      | 7, 12, 13 |
| fbaC   | C2H2 zinc finger transcription factor    | Asexual development | Regulation of conidiophore formation                                      | 12, 13, 16, 17, 24 |
| fbdD   | c-Myb transcription factor               | Asexual development | Regulation of conidiophore formation                                      | 12–13 |
| fbeE   | Protein involved in conidiophore formation | Asexual development | Regulation of conidiophore formation                                      | 12, 13, 16, 17 |
| flG    | Homology to bacterial glutamine syntetase| Asexual development | Repression sexual development                                             | 8, 9, 11–13, 24 |
| tphA   | Phytochrome                              | Red Light response  | Stimulation asexual development and repression of sexual development in the light | 19–21 |
| fwaA   | Polyketide synthase                      | Spore protection    | Melanin biosynthesis                                                     | 3, 9, 21 |
| ganA   | Go-subunit of heterotrimeric G-protein complex of A. nidulans and A. oryza | Not known | Melanin biosynthesis                                                     | 12, 16 |
| ganB   | Go-subunit of heterotrimeric G-protein complex of A. nidulans and A. oryza | Vegetative growth | Repression asexual development                                           | 11, 12, 16, 23, 24 |
| gacC   | Go-subunit of heterotrimeric G-protein complex of A. oryza | Not known | cAMP dependent Germination                                               | 12 |
| gpaA   | Go-subunit of heterotrimeric G-protein complex of A. fumigatus | Vegetative growth | Promotion vegetative growth                                               | 12 |
| gpaB   | Go-subunit of heterotrimeric G-protein complex of A. fumigatus | Asexual development | Regulation asexual development                                           | 12 |
| gpgA   | Gy-subunit of heterotrimeric G-protein complex | Vegetative growth | Stimulation vegetative growth                                             | 11, 12, 15, 16, 23, 24 |
| gpaA   | G-protein receptor (GPCR)                | Sexual development  | Homothallic cleistothecia formation                                       | 15–17 |
| gprB   | G-protein receptor (GPCR)                | Sexual development  | Homothallic cleistothecia formation                                       | 15–16 |
Table 2. (Continued).

| Name   | Description                              | Developmental stage      | Function                                                                 | Page number |
|--------|------------------------------------------|--------------------------|--------------------------------------------------------------------------|-------------|
| gprD   | G-protein receptor (GPCR)                | Vegetative growth        | Repression sexual development                                            | 15          |
| kapA   | α-importin                               | Light response           | Protein import into nucleus in the dark                                  | 18, 20      |
| lueA   | Transcription factor                     | Light response           | Regulation of asexual development, sexual cleistothecia and Hüle cell formation | 19, 20    |
| lueA   | White collar blue light receptor         | Blue light response      | Stimulation sexual development in the light, repression asexual development in the light | 19, 20 |
| MAT1-1 | α-homeodomain transcription factor       | Sexual development       | Regulation of sexual reproduction                                         | 15          |
| MAT1-2 | High mobility group domain (HMG)-transcription factor | Sexual development | Regulation of sexual reproduction                                         | 15          |
| medA   | Temporal regulation conidiophore formation | Asexual development      | Regulation of conidiophore development                                   | 9, 10, 16, 17, 24 |
| mpkB   | Mitogen activated protein kinase (MAPK) | Sexual development       | Signalling in cleistothecia and Hüle cell formation                       | 14, 16, 17  |
| nosA   | Zn(II)Cys₆ transcription factor         | Sexual development       | Regulation of cleistothecia formation (primordium maturation)            | 16, 17, 19  |
| nsdC   | Zinc finger transcription factor         | Vegetative growth        | Repressing asexual development                                           | 14, 16, 17  |
| nsdD   | GATA-like transcription factor           | Asexual development      | Repressing asexual development                                           | 14, 16-18, 21 |
| olvA   | Homologue of aymA                       | Spore protection         | Melanin biosynthesis in A. niger                                        | 3, 9, 21    |
| phnA   | Phosducin like protein                   | Vegetative growth        | Positive regulation Gβγ stimulating vegetative growth                    | 11          |
| pkaA   | Protein kinase                          | Vegetative growth        | Stimulation vegetative growth                                            | 11, 12, 24  |
| pkaB   | Protein kinase activity                  | Vegetative growth        | Potential backup for pkaA                                               | 11, 12, 24  |
| pptA   | Polyketide synthase                     | Balance Sexual and Asexual development | Production oleic and linoleic acid derived oxylipins (psiBα)  | 17–19       |
| pptB   | Fatty acid oxygenase                     | Balance Sexual and Asexual development | Production oleic and linoleic acid derived oxylipins (psiBβ) regulating asexual and sexual development | 17–19       |
| pptC   | Fatty acid oxygenase                     | Balance Sexual and Asexual development | Production oleic and linoleic acid derived oxylipins (psiBβ) regulating asexual and sexual development | 17–19       |
| pptA   | Polyketide synthase                     | Spore protection         | Melanin biosynthesis in A. niger                                        | 21          |
| rasA   | GTPase of the RAS superfamily           | Asexual development      | Polared growth during germination                                        | 23, 24      |
| regA   | Regulator of G-protein signaling        | Asexual development      | Enhancing intrinsic activity GanB (G-α subunit)                          | 11, 12, 23, 24 |
| rodA   | Hydrophobin                              | Asexual development      | Formation rodlet layer during conidiophore development                   | 3, 9, 14    |
| rodB   | Hydrophobin                              | Asexual development      | Formation rodlet layer during Conidiophore development                   | 14          |
| rolA   | RodA-like Hydrophobin                    | Asexual development      | Cutinase recruitment during conidiophore formation in A. oryzae          | 14          |
| rosA   | Zn(II)Cys₆ transcription factor         | Sexual development       | Cleistothecia formation (VeA dependent)                                 | 16, 17, 21  |
| schA   | Ser/Thr protein kinase                   | Germination              | Signalling leading to germination                                        | 24          |
| sfaD   | Gβ-subunit of heterotrimeric G-protein complex | Vegetative growth | Regulation vegetative growth                                             | 11, 12, 15, 16, 23, 24 |
| sfgA   | Gα4-type Zn(II)Cys₆ type transcription factor | Vegetative growth      | Repression asexual development                                           | 12, 13      |
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 abundant protein secretion is generally assumed to take place in growing zones of the mycelium (Levin et al. 2007a, Levin et al. 2007b, 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.

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
transcription factor FlbB, which is involved in asexual development may contribute to the survival under such conditions. Notably, the changes in the environment. A heterogeneous hyphal population hyphae explore the substrate and they may be exposed to rapid hyphae are heterogeneous at the colony periphery. The leading et al. 2011a). So far, we can only guess why of RNA (de Bekker of hyphae. Individual hyphae each have their own composition RNA itself (Bleichrodt 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 °C and 30 °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 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).
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 ΔfluG 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 ΔfluG 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 ΔfluG 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 bma (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 glA (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 germings (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 ΔbrlA 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 ΔbrlA 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 ΔbrlA 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 ΔfluG 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 Serna 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 *brlAa* and *brlAB*. Both transcripts are essential for proper conidiophore development (Prade & Timberlake 1993) and are controlled at the transcriptional (*brlAa* and *brlAB*) and translation level (*brlAB*) (Han & Adams 2001). Transcript *brlAB* contains a short upstream ORF (μORF) and a downstream ORF that encodes the same polypeptide as *brlAa* but with an N-terminal extension of 23 aa (Prade & Timberlake 1993). Both polypeptides contain two C_H_2 zinc finger DNA binding motifs. The *brlAa* and *brlAB* 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 ΔbrlAB strain that can form secondary conidiophores (*i.e.* a conidiophore that develops from another conidiophore). Asexual development proceeds further in the ΔbrlAB 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 ΔabaA 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 *AbA* with *brlA* is complex (Fig. 4A). Gene *abaA* is activated by *BrlA* and, in turn, *BrlA* stimulates formation of *brlAa* transcripts but represses *brlAB* accumulation (Adams et al. 1998, Andrianopoulos & Timberlake 1994, Han & Adams 2001, Sewall et al. 1990). This is caused by *AbA* binding to a responsive element in the *brlAβ* 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 *AbA* because the over-expression of *brlAB* activates expression of *brlAa* in an *abaA* mutant (Han & Adams 2001) (Fig. 4A). Taken together, both *BrlA* and *AbA* control transcript levels of *brlAa* and *brlAB*. *AbA* 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 (Marshalls & 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 (Marshalls & Timberlake 1991). In addition, *WetA* seems to activate itself (Adams et al. 1998, Boylan et al. 1987, Marshalls & 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.
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 *stuAα* and *stuAβ* 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 *BrlA* (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 Δ*medA* strain. Moreover, the mutant strain shows higher levels of *brlAβ*, but not *brlAα*. As a result, the ratio of *brlAα* and *brlAβ* 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 Δ*medA* strain forms repeated layers of sterigmata and frequent reinited secondary conidiophores (Clutterbuck 1989, Sewall et al. 1990). This phenotype resembles that of a strain of *A. nidulans* in which the chitin synthase genes *chsA* and *chsC* 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 Δ*chsAΔchsC* 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 *BrlA*, *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 Gα subunit *FadA* (fluffy autolytic dominant) of a heterotrimeric G-protein. As a result, the Gα 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 Δ*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* (Δ*fadA* or dominant-interfering *fadA* mutant) can also counteract the autolytic...
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, \textit{fadA}^{G42R}, results in autolytic mutants similar to the flbA mutant (Hicks \textit{et al.} 1997, Yu \textit{et al.} 1996). The constitutively active \textit{fadA}^{G42R} mutant phenotype cannot be suppressed by overexpression of \textit{flbA} (Yu \textit{et al.} 1999).

In its inactive GDP-bound form, FadA of \textit{A. nidulans} forms a heterotrimeric G-protein with the β- and γ-subunits encoded by \textit{sfaD} and \textit{gpgA}, respectively (Rosén \textit{et al.} 1999, Seo \textit{et al.} 2005, Yu \textit{et al.} 1996, Yu \textit{et al.} 1999). When FadA becomes GTP bound, this α-subunit dissociates from SfaD and GpgA (Fig. 5). Deletion of \textit{sfaD} (Rosén \textit{et al.} 1999) or \textit{gpgA} (Seo \textit{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 \textit{sfaD} (Rosén \textit{et al.} 1999) but not \textit{gpgA} (Seo \textit{et al.} 2005) also causes hyper-sporulation. The Δ\textit{sfaD} Δ\textit{gpgA} double mutant shows a phenotype identical to those of the Δ\textit{sfaD} mutant (Seo \textit{et al.} 2005). This shows that Δ\textit{sfaD} is epistatic to Δ\textit{gpgA} and that SfaD can induce inappropriate conidiation even in the absence of GpgA. Notably, constitutive activation of \textit{fadA} in the absence of \textit{sfaD} is sufficient to cause proliferation of undifferentiated hyphae (Seo \textit{et al.} 2005, Wieser \textit{et al.} 1997). Taken together, FadA and SfaD-GpgA have overlapping functions in stimulating vegetative growth (Rosén \textit{et al.} 1999, Seo \textit{et al.} 2005). The phosducin like protein A (PhnA) seems to be involved in positively regulating Gβγ signaling in \textit{A. nidulans}. Deletion of \textit{phnA} results in a phenotype similar to that of a Δ\textit{sfaD} strain (Seo & Yu 2006). This would agree with the role of phosducin like proteins to act as chaperones for Gβγ assembly (Yu 2006). Finally, deletions in \textit{sfaD}, \textit{fadA} or \textit{gpgA} do not suppress conidiation defects in a \textit{fluG} mutant. Therefore, the FadA/SfaD/GpgA vegetative growth-signaling cascade seems to be distinct from that of the FluG pathway (Seo \textit{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 \textit{pkaA} has a major role in the stimulation of vegetative growth and the repression of conidiation (Lafon \textit{et al.} 2005, Lafon \textit{et al.} 2006, Seo \textit{et al.} 2005, Yu & Keller 2005). Inactivation of \textit{pkaA} results in hyper-sporulation and reduced radial growth (Shimizu & Keller 2001). On the other hand, overexpression of \textit{pkaA} leads to decreased sporulation accompanied by a fluffy-like appearance. Deletion of the other \textit{pka} gene in the genome of \textit{A. nidulans}, \textit{pkaB}, causes no apparent phenotype (Ni \textit{et al.} 2005). However, over-expression of \textit{pkaB} reduces conidiation and increases vegetative growth on solid medium. Moreover, it complements the reduced radial growth of the Δ\textit{pkaA} strain. Apparently, PkaB functions as a backup for PkaA (Ni \textit{et al.} 2005, Seo \textit{et al.} 2003).

The FlbA/FadA/SfaD/GpgA pathway seems to be conserved in \textit{A. nidulans}, \textit{A. oryzae} and \textit{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 autophagy phenotype is missing in *A. fumigatus*. Moreover, in both *A. oryzae* and *A. fumigatus* hyphal proliferation is reduced in the Δ*gaoC* 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 Gα 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. 2003, Liebmann et al. 2003, Rosén et al. 1999, Seo et al. 2005, Yu et al. 1996). In contrast to *gaoB*, 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. 2004a, Garzia et al. 2010) (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 Δ*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 Δ*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 in *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 Δ*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, which is necessary for conidiation (Wieser et al. 1994). The brackets show increased vegetative growth and accelerated autolysis and conidiophore formation, a fluffy appearance of the colonies, accelerated vegetative growth, and accelerated autolysis and

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 brackets 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 Δ*flbA* strain. In contrast, fluG expression is stimulated by *FlbB* and *FlbC*. For instance, a Δ*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 Δ*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 *weiA*. Overexpression of *flbC* not only inhibits vegetative growth in a wild-type strain but also in a Δ*abaA* or ΔbrlA 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 (Exebeste 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 (Exebeste et al. 2008). A Δ*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 (Exebeste 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 (Exebeste 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 ΔsfgA 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 *flbA* and *vosA* is delayed in the Δ*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

**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)Cys6 binuclear cluster DNA binding motif (Seo et al. 2006). Mutations in *sfgA* bypass the need for FluG during asexual development. The Δ*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.
cell death. FlbE is localised at hyphal tips. In fact, it co-localises with FlbB. 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 FlbB and FlbE. Localisation of FlbB and FlbE was also lost in a ΔflbE and a ΔflbB 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 FlbB stability is affected by the absence of a functional form of FlbE (Garzia et al. 2009). FlbE may thus protect FlbB from proteolytic degradation. It may do so by physical interaction with FlbB. At least, such an interaction was shown in vivo (Garzia et al. 2009). Taken together, FlbE and FlbB function in close association with each other and are functionally interdependent (Garzia et al. 2009).

The FlbB/FlbE complex is a requisite for flbD expression in the wild-type (Garzia et al. 2010) (Fig. 6). FlbD 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 FlbB/FlbE complex is found at hyphal apices (Garzia et al. 2009). In contrast, FlbD 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 (Exebeeste et al. 2010a, Garzia et al. 2009, 2010). Not only depends flbD expression on the presence of FlbB, FlbD also interacts with this protein (Exebeeste et al. 2010a, Garzia et al. 2009, 2010). The underlying mechanism is so far unknown but might involve a translationally modified form of FlbB (Garzia et al. 2010). Both FlbD and the FlbB/FlbE complex seem to activate brlA expression (Garzia et al. 2010) (Fig. 6). Overexpression of flbD restores the conidiation defect in the ΔflbE strain (Kwon et al. 2010a), suggesting an additive effect of both pathways. Interestingly flbD and flbD transcripts disappear shortly after brlA activation (Exebeeste 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 ΔflbE phenotype in A. nidulans (Kwon et al. 2010a), suggesting a similar role for these orthologues. Indeed, FlbE 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 FlbB protein is in A. fumigatus encoded by two transcripts, flbBa and flbBb. The longest transcript, flbBb, 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 ΔflbB strain of A. fumigatus. FlbC and FlbD 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 ΔsteC 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

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**Development in Aspergillus**

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**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. FlbB and FlbE form a complex that activates brlA leading to asexual development. FlbC activates brlA together with the FlbB/FlbD transcription complex. FlbC also activates FluG and regulatory genes that act downstream of brlA. FlbA activates brlA by inactivating FadA and probably plays a role in repressing FluG. (Adapted from Exebeeste et al. 2010).
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 ΔnsdD 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 (Amanian et al. 2009, Amanian & 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 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 ΔBrlA strain does not express rodA but expression of the hydrophobin gene is not affected in the Δabaa and ΔwetA strains. A ΔrodA strain forms wettable conidiophores and conidia. This is accompanied by the absence of the rodlet layer (Stringer et al. 1991). As a consequence, ΔrodA 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 ΔrodA 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 ΔrodA 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 dewAIs not only abolished in the ΔbrlA strain but also in the Δabaa and ΔwetA 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 ΔdewA 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 ΔdewA 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 wettabiltiy of the ΔdewAΔrodA strain is much more pronounced when compared to the ΔdewA and the ΔrodA 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 ΔrodA conidia of A. fumigatus lacks the rodlet layer. Instead, the surface is granular. In contrast, the surface of ΔrodAΔrodB 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. 2008a, 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-fertilization) 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 cleistothecal 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 red-pigmented 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 red-pigmented spores in each of the 10,000 ascii 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 homodomain 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 gpgA, while it is repressed by MAT1-2 (Szewczyk & Krakmann 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, ΔgprA and ΔgprB strains produce less cleistothecia that are smaller than wild-type fruiting bodies under homothallic conditions. The ΔgprA and ΔgprB cleistothecia produce only 5 % of the ascospores when compared to the wild-type but viability of these spores is not affected. The ΔgprAΔgprB 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 ΔgprD 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 βγ-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
which of the α-subunits is the cognate Gα 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 ΔfadA strain under heterothallic conditions but fadA is essential for homothallic sexual development. Under homothallic conditions, cleistothecia formation is abolished in the ΔfadA strain but Hülle cell formation is not affected (Rosén et al. 1999, Seo et al. 2005). The RGS protein FlbA 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 ΔmedA strain only forms unorganised masses of Hülle cells and does not differentiate cleistothecia or ascogenous tissue (Busby et al. 1996). Sexual development is delayed in the ΔstuA strain and it forms only 1 % of the cleistothecia that are formed by 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 ΔsteC 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 ΔsteC 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 ΔsteC strain strongly indicates that a MAPK cascade functions in GprA/B mediated signaling (Fig. 7).

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 ΔsteA strain does not 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 ΔnsdD 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_{3}H_{1}_{2}S and a C_{2}H_{2}C 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 ΔnsdC 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)$_2$Cys$_6$ 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 ΔrosA 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 ΔrosA strain is even higher under low-glucose or high-osmolality conditions. When grown submersed, Hülle cells are produced in the ΔrosA 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 ΔnosA 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 ΔnosA 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 mm in diameter instead of 300 mm) are produced. These fruiting bodies do produce viable ascospores. Expression of nosA was increased in a ΔrosA 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)$_2$Cys$_6$ transcription factors determines whether vegetative hyphae undergo sexual development.

**THE BALANCE OF SEXUAL AND ASEXUAL 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, BrlA, 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 ΔbrlA strain (Valiun et al. 2000). Repression of steA in uninduced hyphae has been related to the BrlA protein. The regulatory interaction between brlA, 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 brlA involved in asexual development (Fig. 6) but at the same time repress the sexual pathway (Fig. 7) (Kwon et al. 2010a, b). Both a ΔflbC and a ΔflbE 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 psiA and psiβ, 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 enhances asexual sporulation. On the other hand, psiBo and psiC can 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 to psiBo and psiC 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 psiBo, while the latter two genes are involved in the synthesis of psiB (Fig. 8). Deletion of ppoA reduces psiBo 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 psiBo 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 ΔppoB and ΔppoC 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 ΔppoB strain, whereas ppoA is repressed.
Krijgsheld et al. (Tsitsigiannis et al. 2005) (Fig. 8). On the other hand, ppoA mRNA levels are higher in a ΔppoC strain, whereas ppoC mRNA levels are lower in a strain over-expressing ppoA (Tsitsigiannis et al. 2004a).

Deletion of ppoB significantly increases brlA 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 ΔppoB strain when compared to ascospores. Changes in sporulation ratios in the ΔppoA and ΔppoC strains also agree with the relative expression of brlA and nsdD (Tsitsigiannis et al. 2004a, b). Expression of brlA is down-regulated in a ΔppoAΔppoBΔppoC 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 ΔppoAΔppoBΔppoC 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 mitae 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 brlA. On the other hand, both brlA and nsdD activate ppoC, whereas nsdD represses ppoA (Tsitsigiannis et al. 2004a, b). In the other loop Ppo oxylipid 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 ppoB 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 ΔveA 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 ΔveA 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 ΔveA strain, the ΔvelB strain does not show light-dependent development. The ΔvelB 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 ΔveA strain. Over-expression of veA in the ΔvelB 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 veA background inactivation of laeA has no effect on asexual reproduction (Bayram et al. 2008b, Sankaya Bayram et al. 2010). However, conidiophore formation is reduced both in the light and in the dark when laeA is
Expression of the oxylipin gene ppoA is completely abolished in a ΔveA strain (Fig. 8). Both the ΔveA strain (Kim et al. 2002) and the ΔppA strain (Tsitisiannis et al. 2004b) show an increase in the ratio of conidia and ascospore formation. Notably, expression of veA is increased in the ΔppAΔppBΔppC strain, which suggests the existence of a regulatory loop between ppo genes and veA (Tsitisiannis 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 Δfpha 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 Δfpha 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 VeIB 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 cofactor. 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
LreB. Asexual development is somewhat increased in the ΔlreA and ΔlreB 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 ΔlreA ΔfphA strain, the ΔlreB ΔfphA strain, and the ΔlreA ΔlreB ΔfphA 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 ΔlreA and ΔlreB strains when compared to the wild-type. Strains ΔlreA ΔfphA, ΔlreB ΔfphA, and ΔlreA ΔlreB ΔfphA behave like the ΔlreB strain. When exposed to white light, cleistothecium formation is nearly absent in the ΔlreA and ΔlreB 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.

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. (B) 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).
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 assexual and sexual development. Expression increases during late asexual and sexual sporulation, and therefore suggests a role of CryA in late developmental processes. The ΔcryA 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 ΔcryA strain resembles that of the ΔrosA 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 ΔcryA 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 blush-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. 1998). In the case of A. fumigatus, ayy1 (Aspergillus yellowish green) converts YWA1 into 1,3,6,8-tetraydroxynaphthalene (1,3,6,8-THN), which is further modified in the DHN pathway (Fujii et al. 2004). Gene apr2 (Aspergillus reddish-pink) encodes the hydroxynaphthalene (HN) reductase that forms both scytalone and vermelone (Tsai et al. 1999), whereas apr1 encodes the dehydratase that converts scytalone in 1,3,8-THN, which is the precursor for Apr2 (Tsai et al. 1999). The combination of alb1, ayy1, apr1, and apr2 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 blush-green pigment of wild-type A. fumigatus spores. Gene abr1 (Aspergillus brown) is a putative multicopper oxidase that converts vermelone 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 apr1 and apr2. Moreover, spore pigmentation of A. niger is insensitive to the HN reductase (Arp2) inhibitor tricyclazole A. The fwnA (fawn), pptA (phosphopantetheinyl transferase), olvA (olive), and bmA (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 ΔpptA 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 bmA 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 (Rujiter 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, erythritol, 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

\[ \text{Alb1} \]

\[ \text{YWA1} \]

\[ \text{Ayg1} \]

1,3,6,8-THN

\[ \text{Arp2} \]

\[ \text{Scytalone} \]

\[ \text{Arp1} \]

1,3,8-THN

\[ \text{Arp2} \]

\[ \text{Vermelone} \]

\[ \text{Abr1} \]

1,8-DHN

\[ \text{Abr2} \]

\[ \text{Melanin} \]

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 ΔmpdA 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, tspA and tspB, 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 ΔtspA 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 ΔtspA strain. Trehalose also has a role in protection of conidia of A. nidulans. The ΔtspA 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 ΔtspA 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 ΔvelB strain show reduced viability and stress tolerance (Sarikaya Bayram et al. 2010). In both ΔvosA and ΔvelB strains reduced expression of genes involved in the biosynthesis of trehalose is observed (Sarikaya 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 (Sarikaya 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 (Osherov & 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.
Development in Aspergillus

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 Gα-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. 8), 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 ΔganB 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 ΔganB 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<sup>Q208L</sup>) 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 ΔganB strain germinate very poorly, whereas expression of GanB<sup>Q208L</sup> 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<sup>Q208L</sup>, conidia of the ΔrgsA strain germinate even in the absence of a carbon source. This effect is not observed in a ΔganBB<sub>ΔrgsA</sub> or a ΔsfaDΔrgsA strain (Han et al. 2004b, Lafon et al. 2005).

The ΔganB 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 ΔganB 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 heterotrimeric G-protein together with SfaD and GpgA (Fig. 11). Spore germination and trehalose degradation is also affected in conidia of the ΔsfaD and ΔgpgA strains, although not as strong as observed in the ΔganB strain (Lafon et al. 2005). A role of RgsA in GanB signaling is further supported by the finding that trehalose degradation in the ΔrgsA 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 ΔcyA strain is completely devoid of cAMP.
(Fillinger et al. 2002). This indicates that cyaA encodes the unique adenylate cyclase during mycelial growth. Conidia of the ΔcyA 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). Trehalose breakdown is reduced in the ΔpkaA conidia and germ tube outgrowth is affected, but not as strong as in the ΔcyA 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 ΔpkaB strain does not have an apparent phenotype (Ni et al. 2005). However, the ΔpkaAΔpkaB 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 ΔpkaA 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 ΔpkaA background results in a phenotype similar to that of the ΔcyA 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 ΔgapA conidia germinate in the presence of glucose. In contrast, whereas ΔgapA 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 (Osherov & 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 ΔmpkA 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 ΔstuA 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 ΔflbC 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, gerrmings, 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 mycelium. The role of heterogeneity between the leading hyphae will be obtained when the whole culture, or for instance, whole ascocarps or ascospores are used for analysis. This average gene expression or activity of the hyphae may be far from reflecting 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.

ACKNOWLEDGEMENTS

This project was financed by the Kluiver Centre for Genomics of Industrial Fermentation, which is part of the Netherlands Genomics Initiative / Netherlands Organization for Scientific Research.

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