REVIEW ARTICLE

Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins

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Abstract

Filamentous fungi produce a number of small bioactive molecules as part of their secondary metabolism ranging from benign antibiotics such as penicillin to threatening mycotoxins such as aflatoxin. Secondary metabolism can be linked to fungal developmental programs in response to various abiotic or biotic external triggers. The velvet family of regulatory proteins plays a key role in coordinating secondary metabolism and differentiation processes such as asexual or sexual sporulation and sclerotia or fruiting body formation. The velvet family shares a protein domain that is present in most parts of the fungal kingdom from chytrids to basidiomycetes. Most of the current knowledge derives from the model *Aspergillus nidulans* where VeA, the founding member of the protein family, was discovered almost half a century ago. Different members of the velvet protein family interact with each other and the nonvelvet protein LaeA, primarily in the nucleus. LaeA is a methyltransferase-domain protein that functions as a regulator of secondary metabolism and development. A comprehensive picture of the molecular interplay between the velvet domain protein family, LaeA and other nuclear regulatory proteins in response to various signal transduction pathway starts to emerge from a jigsaw puzzle of several recent studies.

Introduction

Fungi represent one of the largest groups of eukaryotic organisms on earth, with a conservative estimate of 1.5 million mostly unknown species (Hawksworth & Rossman, 1997). They are metabolically versatile and serve as biofactories for the industrial production of various proteins and metabolites. The fungi have the potential to synthesize numerous secondary metabolites, including antibiotics such as penicillin, which was discovered almost a century ago (Fleming, 1929), but also very potent toxins such as the carcinogenic aflatoxin (Georgianna & Payne, 2009). Secondary metabolites are small, low-molecular-weight bioactive natural products that are not only produced by numerous members of the fungal kingdom, but also by plants or bacteria. Many genes for the synthesis of secondary metabolites are arranged in gene clusters, which are coordinately regulated by cluster-specific transcription factors (Brakhage & Schroeckh, 2010; Evans *et al.*, 2010). Fungal secondary metabolites include nonribosomal peptides such as the penicillins, polyketides such as aflatoxin, hybrids of these two classes as well as terpenes, indole alkaloids or oxylipins (Keller *et al.*, 2005; Hoffmeister & Keller, 2007; Christensen & Kolomiets, 2010).

Fungal spores are dispersed in the air or by water and can therefore easily reach all parts of this planet, including potential hosts as our crops. Molds spoil approximately 10% of the world's annual harvest and farmers in developing countries often are not aware of the threat of mycotoxins (Normile, 2010). Recent studies suggest that the world will need 70–100% more food to feed approximately 9 billion people by the middle of this century. Most likely, more food will need to be produced from the same amount of land due to competition for land from other human activities (Godfray *et al.*, 2010). Hence, a better control of fungal mycotoxin production is required.

The control of secondary metabolism in fungi is often coordinated to fungal growth and development (Calvo *et al.*, 2002; Yu & Keller, 2005; Braus *et al.*, 2010). The evolutionary success of fungi is based on their ability to explore and conquer new ecological niches. A versatile aerobic heterotrophic primary and secondary metabolism is one

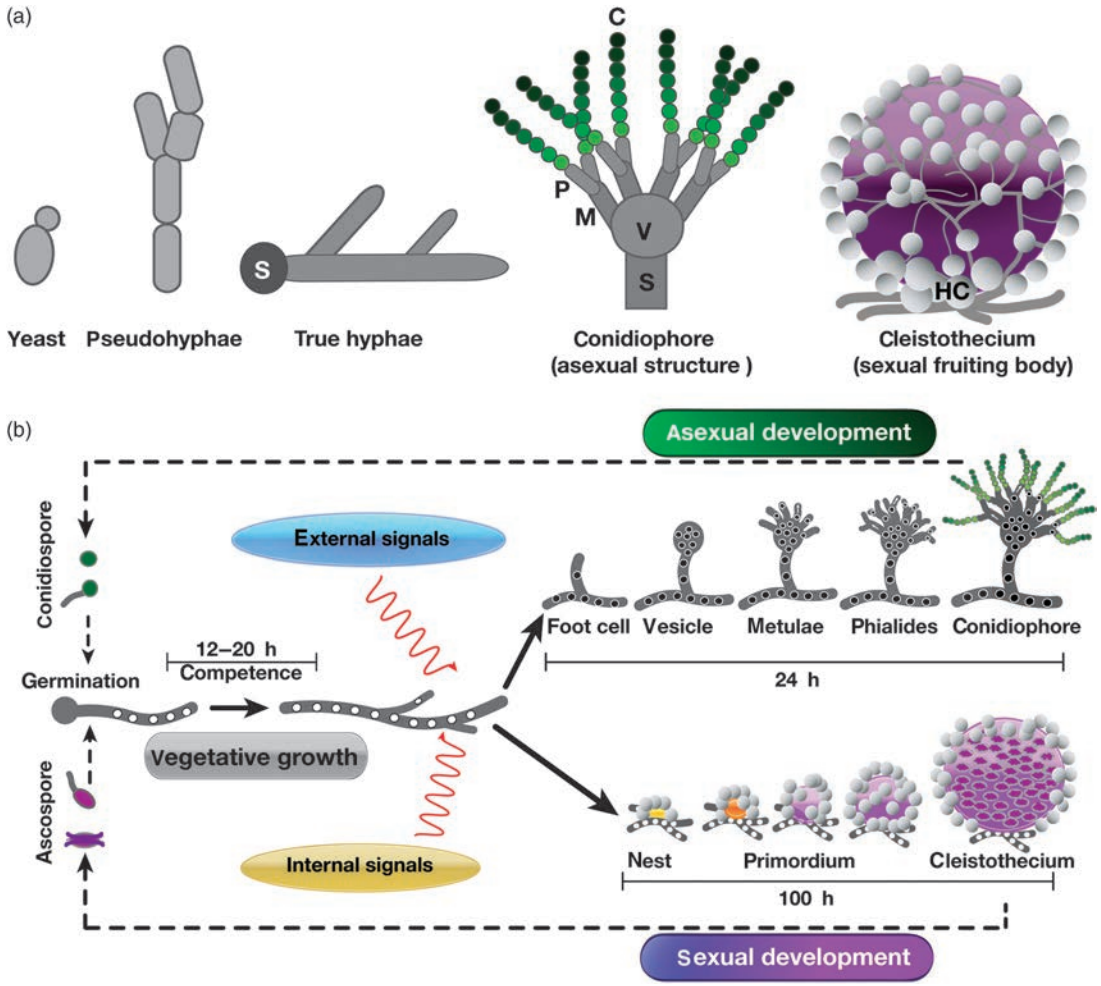

Fig. 1. Fungal cell types and life cycle of the filamentous model fungus *Aspergillus nidulans*. (a) Yeast form: unicellular fungal growth mode; pseudohyphae (elongated cells): filamentous growth form with individual cells; true hyphae: filaments (often separated by permeable septae); conidiophore: composed asexual structure of *A. nidulans*; cleistothecium: spherical closed sexual fruiting body of *Aspergillus* species. S, stalk; V, vesicle; M, metulae; P, phialides; C, conidia; HC, Hülle cells. (b) Life cycle of the model fungus *A. nidulans* from vegetative filamentous growth to asexual or sexual alternatives of development.

prerequisite combined with the specific fungal growth mode, which are normally multicellular hyphae (Fig. 1a). The hyphae are the tube-like structures that are produced after the germination of a fungal spore by the repetition of elongated cellular units. True hyphae are the basic growth units of most fungi and expand at the apex of the tip cell. Polar tip growth includes expansion of the plasma membrane and biosynthesis of cell wall components and requires the apical body of the *Spitzenkörper* as the vesicle supply center (Steinberg, 2007; Harris, 2009). Filamentous fungi are able to adhere to and invade substrates and the size of the organism is not predetermined as in a unitary organism like a human being. Therefore, an undisturbed environment as the soil of an old forest allows the formation of giant filamentous organisms that are assumed to be among the largest and oldest individuals on earth (Smith *et al.*, 1992). Hyphae of higher fungi such as the ascomycetes or the basidiomycetes usually possess septae as cross-walls at rather regular intervals. The septal units are interconnected via pores through which the cytoplasm and, in case of ascomycetes, also entire nuclei can migrate towards the growing tip as a part of a sophisticated transport and communication system of continuous interconnected cellular units (Steinberg, 2007).

Yeasts as single-cell fungi represent only a small proportion of the fungal kingdom and have been evolved in different taxonomic groups (Fig. 1a). Some of them can undergo a transition from the round single-cell yeast form to a filamentous growth mode. The yeast form reflects primarily growth on surfaces or in liquid. Fungal dimorphism between the yeast and the filamentous growth form depends on environmental conditions and can represent

different phases of a fungal life cycle (Gimeno *et al*., 1992; Mösch, 2000; Klein & Tebbets, 2007; Morrow & Fraser, 2009). True hyphae can be formed by dimorphic fungi as the human pathogen *Candida albicans* or the plant pathogen *Ustilago maydis*. There are also pseudohyphae consisting of elongated cells that adhere to each other after cell division is completed without forming interconnected cellular units. Pseudohyphae are typical for diploid budding yeasts of *Saccharomyces cerevisiae*, but can be also formed by *C. albicans* (Fig. 1a).

Filamentous fungi, with the exception of the dimorphic fungi, have to develop vegetative hyphae before they can proceed to other developmental programs. Therefore, vegetative hyphae have to reach a *competence* state, which defines the differentiation capability of a fungal hypha under environmental signals (Axelrod *et al*., 1973). The competence time is dependent on the growth rate of a given fungus. *Aspergillus nidulans* requires for developmental competence between 12 and 20 h after germination of a fungal spore (Fig. 1b). Developmental competence results in susceptibility to environmental signals such as availability of nutrients, fungal pheromones, stress conditions, solid surface, oxygen supply and the ratio between carbon dioxide and oxygen or light of different wavelengths. The combination of developmental competence and environmental signals does not only lead to further fungal differentiation of the hyphae, but also to changes in secondary metabolism. An impairment of a developmental program often coincides with the loss of function in the production of certain secondary metabolites that serve for nutrient acquisition, protection of the fungus or to communicate with the environment (Braus *et al*., 2002, 2010; Bayram *et al*., 2010; Rodriguez-Romero *et al*., 2010). Fungal developmental programs include the transition to asexual spore formation and to sexual fruiting bodies (Fig. 1a). This corresponds to a drastic change in lifestyle from modular filamentous to unitary fruiting bodies and reconstruction requires a functional protein degradation and assembly machinery (Braus *et al*., 2010; Helmstaedt *et al*., 2011). Fruiting bodies comprise highly specialized cells for tissue formation and meiospore development (Pöggeler *et al*., 2006). Abolishment of the potential to form the sexual fruiting bodies of *A. nidulans*, which are closed cleistothecia, coincides with the loss of production of the aflatoxin precursor sterigmatocystin (Kato *et al*., 2003; Bayram *et al*., 2008b).

Asexual spores are conidia, which are single cells, comprising one or several nuclei and can be directly formed from hyphae or indirectly by phialides as spore-forming cells. Phialides can be parts of conidiophores that facilitate the dispersal of the conidia. Conidiophore formation is often initiated in competent hyphae in response to environmental signals starting with an extrusion of a specialized cell from vegetative hyphae (Adams *et al*., 1998; Etxebeste *et al*., 2010; Rodriguez-Romero *et al*., 2010). The numbers of cells required for asexual spore formation vary considerably. *Neurospora crassa* transforms vegetative hyphae into conidiophores. *Aspergillus* species insert additional steps, producing first foot cells from vegetative hyphae, which then grow and lead to a stalk. This ends up with a swollen head called a vesicle. The vesicle produces in a budding-like process short finger-like metulae as a first layer. The phialides form a second layer that generates by mitotic cell divisions the asexual conidia in a process, which is reminiscent of pseudohyphae formation of *S. cerevisiae* (Fig. 1b). The AbaA transcription factor for *A. nidulans* conidia formation and Tec1 for yeast pseudohyphae can be exchanged. These key transcription factors are also encoded by homolog genes, suggesting that the molecular control mechanism for both processes is similar (Andrianopoulos & Timberlake, 1994; Gavrias *et al*., 1996).

Asexual development is typical for most fungi, whereas the sexual reproduction cycle is less common and often not (yet) discovered. Fungi, which do not possess a known sexual cycle, had been classified as *fungi imperfecti* in the past. Comparative genomics suggests that many of the so-called *fungi imperfecti* have at least remnants of a sexual cycle genes and there are examples where this cycle could be activated (O'Gorman *et al*., 2009; Lee *et al*., 2010). Sexual sporulation is normally a more energy-consuming process than asexual sporulation in terms of the number of offspring. However, it may yield more diverse progenies as a consequence of crossing over and recombination during meiosis. Sexual development of self-sterile (heterothallic) fungi such as *N. crassa* requires two partners expressing compatible mating types. The hyphae of the two partners are fused by plasmogamy, followed by karyogamy that fuses the two nuclei. Consecutive meiosis and mitosis finally result in sexual progenies. Self-fertile (homothallic) fungi such as *A. nidulans* or the *N. crassa*-related *Sordaria macrospora* can even undergo the sexual program in the absence of a partner by fusing their own hyphae (Braus *et al*., 2002; Pöggeler *et al*., 2006; Busch & Braus, 2007).

Fruiting bodies require different specialized cells and occur in different shapes as for example the flask-shaped perithecia of *N. crassa* and *S. macrospora* or the closed cleistothecia of *A. nidulans* (Fig. 1a and b) (Busch & Braus, 2007). Specialized cell types serve as reproductive tissues, which are the generative ascogenous hyphae that form the ascospores. The maturing cleistothecium is surrounded by web-like sterile vegetative hyphae. Several aspergilli produce a second cell type that was originally described as chlamydospores. They are thick-walled Hülle cells whose function is to protect and nourish the maturing cleistothecium (Pantazopoulou *et al*., 2007; Sarikaya Bayram *et al*., 2010).

The outer layer of a fruiting body like a cleistothecium covers and protects the ascospores and supports their

survival under harsh conditions. A similar outer layer is present in sclerotia, where a hard outer rind tissue covers a mass of loosely interwoven hyphae at the center. Sclerotia are highly melanized vegetative structures in plant pathogenic fungi. Most sclerotia have lost the sexual propagation ability during evolution, except for *Claviceps*, which has a sexual cycle. This suggests that the sexual program and the formation of the outer layer are distinct programs that can be separated and are linked during sexual fruiting body formation. Sclerotia share with cleistothecia the long-term survival function under inappropriate environmental conditions such as desiccation, temperature or climatic fluctuations (Coley-Smith & Cooke, 1971; Calvo, 2008; Aliferis & Jabaji, 2010).

The molecular mechanisms for the coordination of fungal secondary metabolism and development are the focus of this review (Bayram *et al*., 2008b, 2010). The ecological rationale for this connection is still elusive. An attractive hypothesis is the protection of fungal reproductive structures against competitors or predators in the soil such as earthworms, nematodes, snails, insects, bacteria or other fungi. Secondary metabolites might serve as a chemical shield of the fungus against rivals. This is supported by animal experiments, which showed that fungivor insects prefer those fungi that cannot produce secondary metabolites (Rohlfs *et al*., 2007; Rohlfs & Churchill, 2011).

The velvet (VeA) family of fungal regulatory proteins and the coordination of secondary metabolism and development

VeA protein of *A. nidulans*: the founding member of the velvet family

The velvet family of regulatory proteins is fungal specific, but highly conserved among ascomycetes and basidiomycetes (Ni & Yu, 2007). The founding member of this group is the *A. nidulans* velvet protein VeA, which was described four and half decades ago. Strains carrying the *veA1* point mutation produced more conidia and fewer fruiting bodies than wild-type strains with an intact VeA protein (Kaefer, 1965). The finding that wild-type fungi require red light to induce conidiation, whereas *veA1* mutants allow the formation of conidiation in the absence of light suggested almost three decades later that the velvet protein VeA played a crucial role in red light-induced conidiation (Mooney & Yager, 1990). In 2002, the *veA* gene was cloned by multicopy complementation of the *A. nidulans veA1* mutant strain (Kim *et al*., 2002). When the *veA* gene was deleted, the deletion phenotype differed from the original *veA1* mutation. *veA*Δ strains could not produce any sexual fruiting bodies even under favorable dark conditions. Overexpression of *veA* led to constitutive sexual fruiting body formation irrespective of light or dark conditions. The *veA1* allele carries a single point mutation in the start codon (ATG) of *veA*, resulting in ATT. Because of this mutation, mRNA translation starts at the second AUG, leading to an N-terminally truncated mutant protein lacking the first 37 amino acids. VeA protein localization within the cell depends on illumination. The cellular VeA subpopulation increases in light in the cytoplasm, whereas darkness results in nuclear accumulation of VeA (Stinnett *et al*., 2007; Bayram *et al*., 2008b). Localization of the truncated VeA1 mutant protein is unresponsive to light and results in a significantly larger cytoplasmic than nuclear localization of the protein. In 2003, it was shown that *veA*Δ mutants could not produce mycotoxins of the aflatoxin family such as sterigmatocystin or the antibiotic penicillin due to the lack of transcripts for the corresponding biosynthetic enzymes. This established a connection between fungal control of secondary metabolism and development with the *velvet A* (*veA*)-encoded protein as a key factor (Kato *et al*., 2003).

VeA in other fungi

VeA homologs have been primarily studied in ascomycetes. Deletion of the *veA* gene in other aspergilli, such as the toxin producers *Aspergillus parasiticus* or *Aspergillus flavus*, further corroborated VeA as a molecular link between secondary metabolism and development (Calvo *et al*., 2004; Duran *et al*., 2007; Amaike & Keller, 2009). *veA*Δ mutants of *A. flavus* or *A. parasiticus* result in the loss of aflatoxin or aflatoxin precursor production associated with the loss of the potential to develop sclerotia (Calvo *et al*., 2004; Duran *et al*., 2007). The *veA*Δ mutant of the opportunistic human pathogen *Aspergillus fumigatus* leads to a change in the interplay between metabolism and asexual spore formation, which results in nitrogen source-dependent sporulation (Krappmann *et al*., 2005).

The role of VeA homologs in other ascomycetes is diverse, but always related to development and secondary metabolism. Deletion of the *veA* homolog *FvVE1* of the heterothallic plant pathogen and mycotoxic fungus *Fusarium verticilloides* (Li *et al*., 2006) results in an increased ratio of macroconidia to microconidia with decreased cell wall integrity. These *FvVE1*Δ mutants are unable to produce the deleterious mycotoxins fumonisin and fusarins (Myung *et al*., 2009). Similarly, *Ffvel1* of the plant pathogen and toxin producer *Fusarium fujikuroi* (*Gibberella fujikuroi*) acts as an activator of the mycotoxins fumonisins and fusarins, but in addition, as a repressor of the dark-colored pigment bikaverin. The development of microconidia is severely reduced in *Ffvel1*Δ mutants (Wiemann *et al*., 2010). The VeA homolog *PcvelA* of the industrial penicillin-producing fungus *Penicillium chrysogenum* plays an important role in penicillin biosynthesis and is required for conidiation (Hoff *et al*., 2010). Consistently, the deletion of

AcveA, the *veA* homolog of another biotechnologically relevant β-lactam antibiotic-producing fungus, *Acremonium chrysogenum*, results in a drastically reduced expression of the cephalosporin genes. The deletion triggers earlier fragmentation of hyphae than the wild type in fermentation media (Dreyer *et al.*, 2007). Knockouts of the *veA* counterpart *ve-1* of the fungal model organism *N. crassa* increase asexual sporulation and reduce carotenoid biosynthesis (Bayram *et al.*, 2008c; Olmedo *et al.*, 2010). *Aspergillus nidulans veA* can be exchanged by *N. crassa ve-1*. *Ncve-1* complements the deletion phenotype of *A. nidulans veA*Δ, resulting in sexual fruiting bodies in the dark, asexual conidia in the light and the appropriate secondary metabolism (Bayram *et al.*, 2008c). Similarly, the introduction of either *PcvelA* into the *Ffvel1* mutant of *F. fujikuroi* or *Ffvel1* into the corresponding *P. chrysogenum* mutant complements the corresponding developmental phenotypes (Hoff *et al.*, 2010; Wiemann *et al.*, 2010). [Correction added after online publication 5 September 2011: in the preceding sentence the word 'deletion' was changed to 'developmental'] These results corroborate that the molecular function of VeA and presumably molecular interactions with other proteins are at least conserved among different ascomycetes.

Other members of the VeA family

VeA represents only one of currently four members of the *velvet* family. VelB (velvet like B) had been described as a light-dependent regulator of fungal development and secondary metabolism of *A. nidulans*, which interacts with VeA. Deletion of the genes for both proteins results in similar, but not in identical impairments of development and secondary metabolism (Bayram *et al.*, 2008b). Similar to what is observed in *A. nidulans*, *Ffvel2* (*velB*) deletion strains of *F. fujikuroi* exhibit similar defects as *Ffvel1* (*veA*) mutants. Deletion of both genes also results in a substantially diminished virulence on the host plant (Wiemann *et al.*, 2010).

VosA (viability of spores A), which is another interaction partner of VelB, had been identified as a high-copy repressor of asexual development in *A. nidulans* (Ni & Yu, 2007; Sarikaya Bayram *et al.*, 2010). VosA is also required for trehalose accumulation in spores. *vosA*Δ mutants lose the long-term viability of spores due to the rapid depletion of cytoplasmic constituents as well as nucleic acids, which makes them vulnerable to heat and oxidative stress. There is no significant loss of secondary metabolite production in *vosA* mutants in the wild-type background with an intact VeA protein, indicating a primarily developmental function. However, the *vosA*Δ *veA1* double mutation leads to loss of ST production, underlining the involvement of *vosA* in mycotoxin production (Ni *et al.*, 2010).

VosA–VelB forms a dimeric complex that is present in vegetative hyphae and during development in the dark where sexual development is initiated. VosA–VelB provides trehalose to support the viability of asexual as well as sexual spores. Presumably, this function is fulfilled during vegetative growth, because VosA–VelB is only present during development in the dark when it has a second function: it supports the repression of asexual development (Sarikaya Bayram *et al.*, 2010). In light, when asexual development is favored, hardly any VosA or VelB is detectable. VosA- and VelB-related proteins of the VeA family can have different functions in the differentiation of other fungi and can also be linked to distinct environmental triggers. Whereas the VeA protein of *A. nidulans* responds to light, the counterparts of the velvet family proteins in the dimorphic fungal pathogen *Histoplasma capsulatum* respond to temperature. Ryp2 (VosA) and Ryp3 (VelB) proteins of this dimorphic human pathogen have been found in a genetic screen to be essential for the temperature-dependent transition from the saprophytic filamentous phase to the single-cell pathogenic yeast form (Webster & Sil, 2008). Wild-type *H. capsulatum* cells sense high temperature (37 °C) and switch to the yeast form, whereas *ryp2* and *ryp3* mutants are insensitive to an increase in temperature and produce constitutively filaments. Similar to the functions of the *A. nidulans* VosA–VelB heterodimer, *ryp2* and *ryp3* mutants show reduced spore viability. This suggests a conserved role for VosA–VelB for the viability of the spores within the fungal kingdom.

The role of VelC, the fourth member or the family, is still obscure and might be auxiliary. The deletion of *velC* in *A. nidulans* results in a slight increase in sexual fruiting body formation (Sarikaya Bayram *et al.*, 2010).

Velvet domains

The four members of the velvet family, VeA, VelB, VosA and VelC, share a common velvet domain that is conserved in the fungal kingdom (Fig. 2). This velvet domain comprises approximately 150 amino acids, where hardly any known common motifs are found. Some conserved stretches of amino acids including proline residues are present in the middle of the motif. VeA–VelB and VelB–VosA heterodimers as well as homodimers of velvet proteins have been identified (Sarikaya Bayram *et al.*, 2010). Therefore, it is tempting to speculate that the velvet domain represents a protein–protein interaction domain. Velvet domains are found in filamentous ascomycetes and basidiomycetes (Table 1). Exceptions are single-cell yeasts such as the hemiascomycete *S. cerevisiae*. Velvet domains are only found in some yeasts of the ascomycetes including *Yarrowia lipolytica* and, as described above, the dimorphic temperature-controlled *H. capsulatum*. Velvet domains are also conserved in *Zygomycetes* or *Chytridiomycetes* (Table 1). Whereas the frog pathogen chytrid *Batrachochytrium dendrobatidis* carries four genes for velvet domain proteins, another chytrid, *Spizellomyces punctatus*, possesses

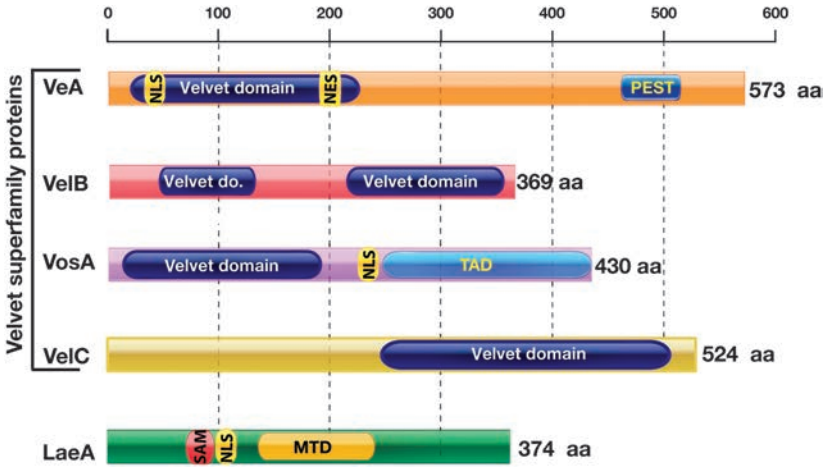

Fig. 2. Domain architecture of the velvet family proteins and LaeA in *Aspergillus nidulans*. SAM, *S*-adenosyl methionine-binding site; TAD, transcription activation domain; PEST, proline (P) glutamic acid (E) serine (S) and threonine (T) rich sequence.

even more velvet domain-encoding genes (Ruiz-Trillo *et al.*, 2007). This suggests an ancient origin of the genes for the velvet domain protein family.

The VeA protein of *A. nidulans* comprises 573 amino acids and includes a bipartite nuclear localization signal (NLS) in the N-terminal half. Furthermore, a nuclear export signal (NES) is also located in its N-terminal part. A proline-rich PEST region, which is typical for an unstable protein, is present in the C-terminal end of the protein. The N-terminally truncated *A. nidulans* VeA1 mutant protein, which shows constitutively reduced nuclear import, supports that the N-terminus of VeA is important for nuclear entry. The NLS sequence of *A. nidulans* VeA interacts with the α-importin KapA in the yeast two-hybrid system (Stinnett *et al.*, 2007). Moreover, KapA and VeA physically interact in the dark *in vivo* (Bayram *et al.*, 2008b). The VeA N-terminus is also necessary for the interaction of VeA with VelB, which is strongly reduced for the interaction of truncated VeA1 with VelB. There is currently no information on the functionality of the NES regions of VeA. The amount of *A. nidulans* VeA protein is reduced in light in comparison with the dark, but it is currently unknown whether protein stability and especially the PEST region are part of this control.

VelB is the shortest member of the velvet family, with a size of 369 amino acids in *A. nidulans*. VelB contains neither a canonical NLS nor NES signals. Efficient nuclear import of VelB requires VeA (Bayram *et al.*, 2008b). Interestingly, VelB is the only member of the family carrying two velvet domains located in the N- and C-terminal half, respectively. VelB homologs of other ascomycete or basidiomycete members also include two velvet domains, suggesting a common ancestor of *velB* before the separation of both groups during evolution. This might be the result of a genome rearrangement, which either resulted in the recombination of two ancestor velvet domain genes or the duplication of the corresponding exon within an original gene with a single velvet domain. Recombinations in the past might be the reason why both *velB* and *veA* are located on chromosome VIII and *vosA* and *velC* on chromosome VII, respectively. VelB interacts with VeA and VosA and is also able to form a homodimer (Bayram *et al.*, 2008b; Sarikaya Bayram *et al.*, 2010). It will be interesting to explore which of the two velvet domains is required for which function.

The *A. nidulans* VosA as the third member of the velvet family includes 430 amino acids and carries as VeA the velvet domain in the N-terminal half. VosA carries an NLS sequence that provides nuclear localization, but does not support VelB nuclear transport (Sarikaya Bayram *et al.*, 2010). VosA has been suggested to act as a transcription factor, which is supported by a transcription activation domain present at the C-terminus (Ni & Yu, 2007). The long C-terminal velvet domain of VelC as the fourth member of this family has not been analyzed as yet.

Interplay between the methyltransferase-domain protein LaeA and the VeA family proteins

LaeA and secondary metabolism

In 2004, the *laeA* (loss of *aflR* expression A) gene was identified genetically. The *A. nidulans laeA* mutant is unable to express the AflR transcriptional activator that controls the secondary metabolite cluster for the aflatoxin precursor sterigmatocystin. The deletion of *laeA* revealed that LaeA is not only required for sterigmatocystin and penicillin biosynthesis, but also for lovastatin used in health care to lower cholesterol to prevent cardiovascular diseases (Bok & Keller, 2004). Similarly, the *laeA* homologs of other fungi are involved in the control of secondary metabolism. It is required for penicillin biosynthesis and gibberellin production in the industrially used *P. chrysogenum* and the phytopathogenic *F. fujikuroi*, respectively (Hoff *et al.*, 2010; Wiemann *et al.*, 2010). [Correction added after online

Table 1. Presence and studies of the velvet family proteins and LaeA in the fungal kingdom

Fungal groups	Species	VeA	VelB	VosA	VelC	LaeA	References
Basidiomycota	*Coprinopsis cinerea*	+	+	+	+	+	Not studied
	Laccaria bicolor	+	+	+	+	+	Not studied
	Cryptococcus neoformans	+	+	+	+	−	Not studied
	Ustilago maydis	+	+	+	+	−	Not studied
Ascomycota	*Aspergillus nidulans*	+	+	+	+	+	Kim *et al*. (2002), Kato *et al*. (2003), Ni & Yu (2007), Stinnett *et al*. (2007), Bayram *et al*. (2008b), Sarikaya Bayram *et al*. (2010)
	Aspergillus fumigatus	+	+	+	+	+	Bok *et al*. (2005), Krappmann *et al*. (2005)
	Aspergillus flavus	+	+	+	+	+	Duran *et al*. (2007), Amaike & Keller (2009)
	Aspergillus parasiticus	+	+	+	+	+	Calvo *et al*. (2004)
	Aspergillus oryzae	+	+	+	+	+	Not published
	Neurospora crassa	+	+	+	+	+	Bayram *et al*. (2008c)
	Sordaria macrospora	+	+	+	+	+	Not studied
	Penicillium chrysogenum	+	+	+	+	+	Hoff *et al*. (2010)
	Acremonium chrysogenum	+	+	+	+	+	Dreyer *et al*. (2007)
	Trichoderma reesei	+	+	+	+	+	Not studied
	Fusarium verticillioides	+	+	+	−	+	Li *et al*. (2006)
	Fusarium fujikuroi	+	+	+	−	+	Wiemann *et al*. (2010)
	Histoplasma capsulatum	+	+	+	+	+	Webster & Sil (2008)
Hemiascomycota	*Saccharomyces cerevisiae*	−	−	−	−	−	Not studied
	Candida albicans	−	−	−	−	−	Not studied
	Yarrowia lipolytica	+	+	+	+	−	Not studied
Zygomycota	*Rhizopus oryzae*	+	+	+	+	+	Not studied
	Mucor circinelloides	+	+	+	+	+	Not studied
	Phycomyces blakesleeanus	+	+	+	+	+	Not studied
Chytridiomycota	*Batrachochytrium dendrobatidis*	+	+	+	+	−	Not studied
	Spizellomyces punctatus	+	+	+	+	−	Not studied
	Allomyces macrogynus	+	+	+	+	−	Not studied

(+) protein is present in the corresponding group; (−) protein is not found in the corresponding group.
[Correction added after online publication 5 September 2011: in column VelC of species *Fusarium verticillioides* and *Fusarium fujikuroi*, the '+' were changed to '−']

publication 5 September 2011: in the preceding sentence 'toxin production' was changed to 'gibberellin production'] The LaeA protein represents a global regulator of secondary metabolism. The protein does not possess any velvet domain. LaeA is primarily located in the nucleus, and a classical NLS region is located at the N-terminus of the deduced primary sequence. In the center of the protein, there is a putative *S*-adenosylmethionine-dependent (SAM) methyltransferase domain (MTD) that shows some similarity to arginine methyltransferases. Truncation experiments showed that the SAM-binding domain at the N-terminus of LaeA is required for function (Fig. 2). LaeA controls a region with distinct borders of 70 kb of the sterigmatocystin cluster. Artificial introduction of additional genes in this region results in a LaeA-dependent expression pattern, which suggests that LaeA exhibits an epigenetic control function (Bok *et al*., 2006). LaeA has been proposed to counteract to H3K9 methylation in the sterigmatocystin gene cluster (Reyes-Dominguez *et al*., 2010). The *laeA* gene is conserved in numerous filamentous fungi, except the yeast-like fungi similar to the velvet protein-encoding genes. In contrast to the velvet proteins, a *laeA* homolog cannot be found in *Chytridiomycetes*.

LaeA and light control

LaeA does not only control secondary metabolism, but also has key functions in development. Deletion of the *laeA* gene causes an impairment in conidiation in *P. chrysogenum* (Hoff *et al*., 2010). LaeA also plays an important regulatory role in *A. nidulans* morphology, where it is required for the light-dependent support of asexual development. This function requires the presence of an intact VeA protein. A *laeA*Δ mutant is unable to repress sexual development by light. This event results in reverse phenotypes for the deletions of *veA* and *laeA*: the *veA*Δ mutation constitutively performs the asexual program and produces no sexual fruiting bodies when LaeA is present. In contrast, the *laeA*Δ mutant almost exclusively forms cleistothecia and asexual spores are drastically reduced when VeA is present. Therefore, fungal

responses of *veA*Δ and *laeA*Δ are antagonistic and correspond in the wild type to permanent illumination and permanent darkness, respectively. This light control function is suppressed when the N-terminal part of VeA is lacking as in the *veA1* mutant strains that are studied in many laboratories (Sarikaya Bayram *et al.*, 2010).

The trimeric VelB–VeA–LaeA (velvet) complex

In 2008, it was found that LaeA and the two velvet family proteins VeA and VelB are part of a trimeric complex that is essential to coordinate secondary metabolism and development in darkness (Bayram *et al.*, 2008b) (Fig. 3). Therefore, the two antagonists of light control, VeA being required for sexual development, and LaeA being required for asexual development, do interact physically. The amount of the trimeric velvet complex is reduced in the presence of light when fruiting body and sterigmatocystin production are repressed. VeA forms the light-responsive bridging factor that links VelB to LaeA. Light results in less VeA protein either due to reduced biosynthesis or decreased stability or both and an additional impaired import of VeA into the nucleus. For this reason, illumination results in a limited interaction of VeA with VelB, whereas complexes with LaeA are no more detectable. The VelB–VeA dimer is formed in the cytoplasm, because VelB requires VeA for nuclear transport and the trimeric complex is only formed in the nucleus. The nuclear trimeric complex is not evenly distributed throughout the nucleus, but is concentrated at specific nuclear locations. It will be interesting to explore whether these localization sites correspond to secondary metabolite clusters. VelB and VeA are essential for fruiting body formation, whereas LaeA is essential to form sexual Hülle cells and to support asexual spore formation in an appropriate environment such as light. LaeA is also a global regulator for secondary metabolism, which is presumably modulated in its activity by the velvet family proteins VelB and VeA during development in the dark (Bayram *et al.*, 2008b; Sarikaya Bayram *et al.*, 2010). A recent multicopy suppressor screen revealed the additional regulator *rsmA* (remediation of secondary metabolism), whose overexpression remediates the lack of sterigmatocystin in *veA* and *laeA*Δ strains (Shaaban *et al.*, 2010). However, developmental dysfunctions of the *veA* and *laeA* strains cannot be rescued by the *rsmA* gene overexpression. Therefore, the molecular mode of RsmA function in the velvet complex remains unclear.

VelB has a second function. VelB is not only part of the trimeric complex, but also of the VosA–VelB heterodimer. VosA–VelB represses asexual differentiation during vegetative growth in a liquid culture or during development in the absence of light. Expression of VosA–VelB in light, therefore, inhibits artificial asexual development. LaeA has the opposite function and is required in light to allow asexual development. In addition, vegetative VosA–VelB activates the genes for trehalose biogenesis for spores to protect them against various types of stress. Light represses the protein levels of VelB and VosA drastically

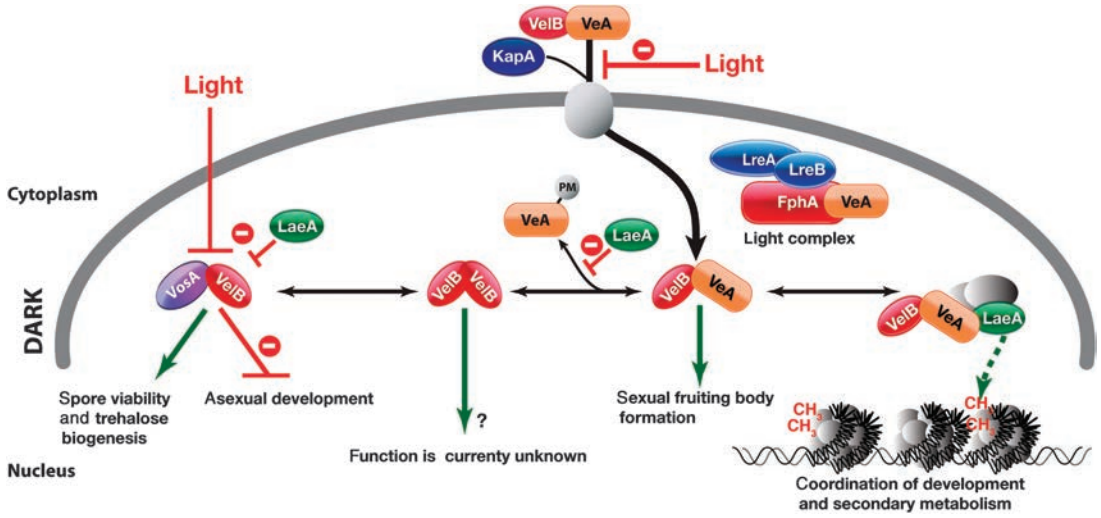

Fig. 3. Interactions between velvet family proteins and LaeA in *Aspergillus nidulans*. The α-importin KapA supports the entry of the VeA–VelB dimer into the nucleus in the dark. In the nucleus, the VelB–VeA dimer can interact with LaeA, forming the heterotrimeric velvet complex that regulates secondary metabolism and development. VelB can also form homodimers and is part of another heterodimer, VosA–VelB. VosA–VelB represses asexual development and is required for the viability of spores by activating trehalose biogenesis. In a submerged culture during vegetative growth, VosA–VelB represses differentiation and initiates trehalose biogenesis. Light decreases the cellular levels of VosA and VelB (red lines) and allows asexual sporulation, whereas the initiated trehalose biogenesis during vegetative growth still occurs. In the dark, VosA–VelB is present and negatively regulates asexual conidiation and still supports trehalose biosynthesis. VeA interacts with the red light receptor FphA that is associated with the blue light receptors LreA and LreB. PM, Post-translational modification.

during development, which correlates with increased asexual conidiation. In contrast to VeA, VosA does not seem to be able to transport VelB into the nucleus. This suggests that there might either be a competition for VelB between VeA and VosA or alternative nuclear carriers for VelB. VelB is also able to form a VelB–VelB homodimer, whose function is currently unknown (Fig. 3) (Sarikaya Bayram *et al.*, 2010).

LaeA control of velvet family proteins

Deletion strains in the genes for LaeA and the velvet family proteins show opposing phenotypes. This corresponds on the molecular level to a LaeA-mediated control of the levels of other velvet family proteins. During light response, LaeA reduces the levels of VelB and VosA and, thus, the VosA–VelB heterodimer is hardly detectable. This releases the repression of asexual development that takes place during development when it is dark. Without LaeA, the protein levels of VelB and VosA are significantly elevated and accordingly repression of asexual development is increased. Furthermore, LaeA protects VeA against significant post-translational modifications. VeA undergoes, in the absence of LaeA, a molecular size increase of approximately 10 kDa. The nature of this modification is currently unknown (Sarikaya Bayram *et al.*, 2010). The lack of LaeA results in hyperphosphorylation of VeA, which is known to be a phosphoprotein (Purschwitz *et al.*, 2009). This also reflects on a molecular level that LaeA is far more than a global secondary metabolism regulator and plays a key role in light control of development in *A. nidulans* by controlling the amount and modification of velvet family protein.

Light regulators and the trimeric complex

The trimeric velvet complex might directly perceive the light signal to coordinate secondary metabolism and development from light sensors. VeA can physically interact in the nucleus with the red light phytochrome receptor protein FphA. FphA is further associated with the blue light receptors LreA and LreB (Purschwitz *et al.*, 2008; Bayram *et al.*, 2010), which are the white-collar homologs of *A. nidulans*. The WC-1/WC-2 complex acts as a photoreceptor complex and regulates the circadian rhythm in the fungus *N. crassa* (Chen *et al.*, 2010). VeA interacts with FphA through its histidine kinase (HK) domain, but phosphorylation of VeA by FphA or LreA/LreB could not be found. Because the interaction of VeA with FphA is restricted to the nuclei of the fungal mycelia, VeA might serve as a scaffold protein recruiting additional regulators to modulate gene expression. The UV-light receptor CryA affects VeA protein levels by influencing the transcription of the *veA* mRNAs and is described in the next paragraph in more detail (Bayram *et al.*, 2008a).

LaeA and VeA control the formation of specific developmental cell types (Hülle cells) with a peculiar physiology

Hülle cells and the transition from filamentous to single-cell growth

Hülle cells (or described earlier as chlamydospores) are produced by different species of the *Aspergillus* genus, including *A. nidulans* and *Aspergillus heterothallicus*, where they are associated with cleistothecia, whereas in species such as *Aspergillus protuberus* and *Aspergillus ustus*, Hülle cells are formed in masses, but not in contact with the cleistothecia (Muntanjola Cvetkovic & Vukic, 1972; Ellis *et al.*, 1973; Carvalho *et al.*, 2002). These globular cells that surround the cleistothecium vary in shape between the more elongated and the globular type in different species (Carvalho *et al.*, 2002). An average Hülle cell is around 10–15 μm in size and possesses a thick cell wall resembling a banana under the light microscope (Fig. 4). Hülle cells have germination capability that can result in mature hyphae. These cells are multinuclear and nuclei can fuse and form a macronucleus. DNA contents per Hülle cell have been determined as being 17 times greater than the amount of DNA present in asexual conidia (Carvalho *et al.*, 2002). Already in the 1970s, it was described that the number of Hülle cells was increased in the dark and reduced in light in different Hülle cell-forming species of *Aspergillus* (Muntanjola Cvetkovic & Vukic, 1972).

The formation of round Hülle cells from filamentous hyphae requires a major rearrangement of the cellular architecture, including the intracellular membrane system. The *A. nidulans* striatin StrA localizes in the endoplasmic reticulum (ER). Striatins are scaffolding proteins that have been identified in animals and fungi. Striatins could play a role in restructuring the intracellular membrane system for Hülle cell formation because overexpression of the *strA* gene induces the artificial production of Hülle cells in a submerged culture, whereas wild-type *A. nidulans* cultures growing in a submerged culture form neither Hülle cells, cleistothecia nor conidiophores. Overexpression of *strA* enhances the early stages of fruiting body development, but is critical for later stages, resulting only in abnormally small cleistothecia that are defective in ascosporogenesis. In addition, *strA* overexpression impairs another filament to single-cell transition, which is asexual spore formation during conidiation and conidium germination. The additional production of a diffusible red pigment of the overexpression strain represents another link between secondary metabolism and development (Poggeler & Kuck, 2004; Busch & Braus, 2007; Wang *et al.*, 2010).

The ImeB (inducer of meiosis) protein kinase also acts at the transition of filamentous growth to Hülle cells, light

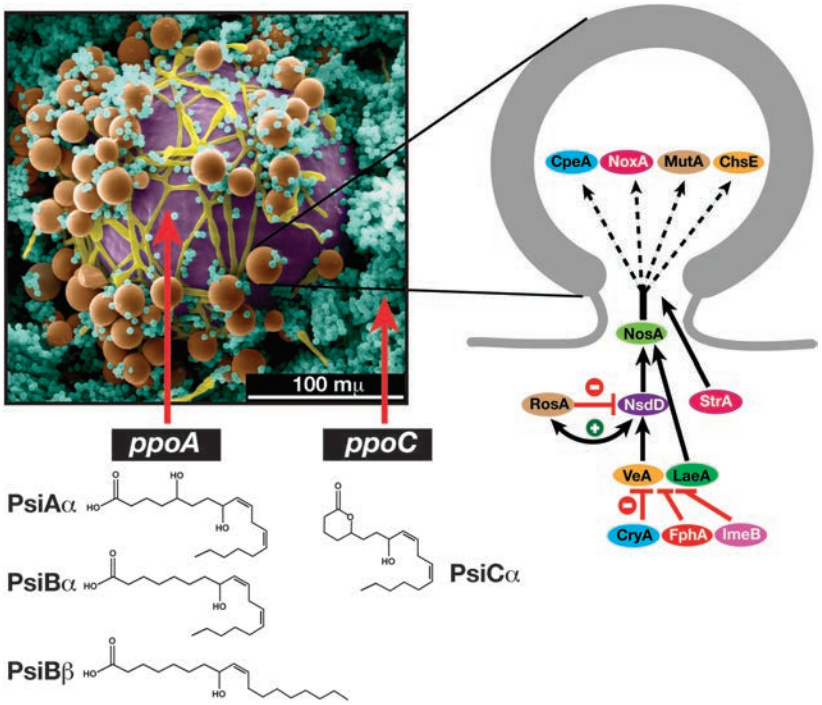

Fig. 4. Control of Hülle cell formation and hormonal control of sexual fruiting body development. A colored scanning electron microscopy picture of a mature cleistothecium of *Aspergillus nidulans* is shown. The genes *ppoA*- and *ppoC*-encoding dioxygenases are required for sexual and asexual development, respectively. PpoA is required for the indicated oxylipins. [Correction added after online publication 5 September 2011: in the preceding sentence the text '*psiB* [hydroxyl group at C-8, resulting in (8R)-hydroxy-(9,12)-octadecadienoic [= linoleic] acid] and *psiC* (hydroxyl group in C-8 and d-lactone ring) and PpoC for *psiA* [hydroxyl groups at C-5 and C-8 resulting in (5S, 8R)-dihydroxy-(9,12)-octadecadienoic acid]' has been deleted]. Sexual and asexual development are characterized by different ratios between the products of ppoA and ppoC. [Correction added after online publication 5 September 2011: in the preceding sentence '(8R)-hydroxy-(9)-octadecanoic [= oleic] acid (OHOA) and *psiB*. This ratio shifts from 1 : 8 in dark (sexual) to 1 : 3 in light (asexual).' was replaced with 'the products of ppoA and ppoC']. On the right, the signaling cascade for Hülle cell formation is depicted; the details are described in the text.

control and secondary metabolism. ImeB shares similarities to mitogen-activated protein (MAP) kinases and cyclin-dependent kinases and is widely conserved among eukaryotes. ImeB is a serine threonine kinase that is related to the yeast Ime2 (inducer of meiosis 2) kinase, which is unstable and can be stabilized by C-terminal truncation (Bolte *et al.*, 2002). The deletion of yeast *ime2* prevents pseudohyphal growth in the presence of unpreferred nonfermentable carbon sources (Strudwick *et al.*, 2010), corroborating a function in the transition between filamentous and single-cell growth. Ime2 has additional functions and is also involved in meiosis. Crk1, the ImeB homolog of the plant pathogen *U. maydis*, is necessary for sexual mating and virulence (Garrido & Perez-Martin, 2003; Garrido *et al.*, 2004). *Neurospora crassa* and *Cryptococcus neoformans* ImeB homologs have been shown to regulate sexual development negatively (Hutchison & Glass, 2010; Liu & Shen, 2010).

Knockout strains of *imeB* in *A. nidulans* result in artificial Hülle cell formation similar to overexpression of the striatin-encoding gene *strA*. *imeB*Δ strains form normal cleistothecia with viable ascospores, but are unable to turn off sexual fruiting body formation in light similar to a *laeA*Δ mutant. ImeB is also located at the interface between development and secondary metabolite formation, because ImeB is essential for the formation of the aflatoxin precursor sterigmatocystin. The lack of ImeB in combination with the phytochrome red light sensor FphA in the same cell results in a completely blind strain that is not responsive to any kind of light source. This indicates that ImeB acts as an additional and may be a parallel repressor in the light-dependent repression of cleistothecia formation (Bayram *et al.*, 2009). It is currently unknown whether ImeB directly acts on the trimeric velvet complex or on its subunits as LaeA or specific members of the velvet protein family.

Transcriptional regulators of Hülle cells

A number of Hülle cell-controlling genes have been described in *A. nidulans*. Besides the kinase ImeB, they include the light sensor *cryA* and the transcriptional repressor *rosA*, all of which inhibit Hülle cell formation. In contrast to *cryA* and *rosA*, overexpression of the genes for the velvet protein *veA* and the transcriptional activator *nsdD* artificially trigger Hülle cell formation similar to overexpression of the ER scaffold protein *strA*.

The UV-light sensor and Hülle cell repressor CryA represents the only fungal cryptochrome of *A. nidulans* and is conserved from chytrids to basidiomycete fungi. The *cryA*Δ strain forms significantly more cleistothecia in UV or blue light than the wild type (Bayram *et al.*, 2008a). The transcripts for the corresponding *N. crassa* cryptochrome CRY-1 are strongly induced upon blue light exposure. CRY-1 does not possess any DNA repair activity, but is involved in the circadian rhythm (Froehlich *et al.*, 2010). *Aspergillus nidulans* CryA has a dual function because it also possesses photolyase activity, which requires a direct interaction with

DNA. It is tempting to assume that there is an additional transcriptional activity that has not yet been analyzed. Deletion of the *cryA* gene causes abnormal formation of Hülle cells in a submerged culture (Bayram *et al.*, 2008a). The *rosA*Δ strain shows a similar derepression of sexual tissue characterized by abnormal Hülle cell production in the liquid submerged media. The zinc finger transcription factor RosA, which is related to *S. macrospora* Pro1 (Masloff *et al.*, 1999; Vienken *et al.*, 2005) and represents a transcriptional repressor of Hülle cells, is also involved in repression of development when the carbon source is depleted.

The lack of CryA results in a complex misregulation in the regulatory circuit of sexual development. As mentioned above, *veA* transcripts are increased in *cryA*Δ, which affects the trimeric velvet complex. Consistently, overexpression of the *veA* gene also induces the production of Hülle cells in a submerged culture (Kim *et al.*, 2002). The mechanism by which VeA activates the transcription of sexual genes and secondary metabolism is unknown. VeA triggers expression of the *nsdD* gene for a developmental transcriptional activator. *nsdD* overexpression also results in Hülle cell formation in a liquid medium (Han *et al.*, 2001). *rosA* transcripts increase simultaneously, which leads to a decline in the transcript levels of *nsdD* at a later stage. This represses further sexual development beyond Hülle cells in a liquid medium. Further sexual development for fruiting body formation requires the Zn(II)(2)2Cys(6) transcription factor NosA (number of sexual spores) transcriptional activator (Vienken & Fischer, 2006). NosA is another conserved protein that is related to its ortholog Pro1 from *S. macrospora* (Masloff *et al.*, 1999). NosA is also a paralog of the RosA repressor and both proteins share more than 40% identical amino acids. The deletion of *nosA* results in small fruiting bodies of 30 μm with little Hülle cells (Vienken & Fischer, 2006). The two related factors RosA and NosA play their molecular roles at different time points. RosA plays a role in very early developmental decisions and turns off the activator gene *nsdD* (Bayram *et al.*, 2008a), whereas NosA activity is required later during development for Hülle cell formation at the transition from primordia and microcleistothecia to mature cleistothecia (Vienken & Fischer, 2006).

Physiology of Hülle cells

Induction of Hülle cells by these regulators results in a fungal tissue with a peculiar physiology. Hülle cells develop strong phenol oxidase activity due to the accumulation of laccase type II enzyme, whereas laccase I is required for asexual spore formation (Hermann *et al.*, 1983). Hülle cells of fungi, which produce unpigmented cleistothecia, do not possess laccase II activity, suggesting that pigmentation of cleistothecia is a Hülle cell-driven process presumably for protection. The oxidation reactions of phenolic compounds in Hülle cells lead to the formation of multiple reactive oxygen species (ROS). Hülle cells, therefore, produce increased catalase, peroxidases or superoxide dismutase activities to cope with increased ROS formation. Consistently, the *A. nidulans* catalase peroxidase gene *cpeA* is expressed at the beginning of sexual development when Hülle cells start to be formed and the CpeA protein is enriched in Hülle cells (Scherer *et al.*, 2002). The *noxA* gene encoding NADPH oxidase of *A. nidulans* produces ROS and is essential for cleistothecia formation, but not for conidiation or hyphal growth (Lara-Ortiz *et al.*, 2003). Expression of *noxA* gene is also induced at the start of sexual development and inhibitors of Nox enzymes result not only in a decrease in superoxide species, but also in an accumulation of Hülle cells and primordia as cleistothecia precursors that are unable to proceed in development. Therefore, ROS are not only toxic to cells, but also seem to represent an essential endogenous signal that is required for further development of cleistothecia.

Hülle cells are centers for a number of additional enzyme activities, which are required for the degradation of cell wall components. The *mutA* gene responsible for mutanase, which possesses α-1,3-glucanase activity, and a *chsE* (chitin synthase E) gene product is localized in Hülle cells and expressed at the onset of sexual development (Wei *et al.*, 2001; Lee *et al.*, 2005). Cell wall material presumably serves as an energy source for the developing fruiting body and Hülle cells help to mobilize this energy by functioning as enzyme storage units. Thus, Hülle cells provide nutrients to the developing fruiting bodies and are also required for the mechanical protection of the fruiting bodies.

The methyltransferase-domain protein LaeA controls Hülle cell formation

LaeA plays a special role in Hülle cell morphogenesis, in addition to its requirement for secondary metabolism and its role to allow asexual spore formation in light. In the absence of LaeA, hardly any Hülle cells are formed. Consistently, the constitutively formed cleistothecia of a *laeA*Δ mutant are significantly smaller and similar to the small cleistothecia of the *nosA*Δ strain. Whereas wild-type fungi produce cleistothecia with a diameter of 200 μm, *laeA*Δ mutants only give rise to microcleistothecia sized between 20 and 40 μm. *laeA*Δ strains produce only three to five Hülle cells per fruiting body, whereas wild-type cleistothecia are surrounded by approximately 80–200 cells. The remaining Hülle cells produced by the *laeA* mutant strain lack *mutA* gene expression, suggesting that they are physiologically inactive. Therefore, LaeA is crucial for the nursing function of Hülle cells during fruiting body development. These cleistothecia cannot reach their normal dimensions due to the lack of the nursing function of Hülle cells.

The Hülle cell function of LaeA does not require an intact VeA protein and can also be fulfilled in the presence of truncated VeA1 (Sarikaya Bayram *et al.*, 2010). The role of LaeA in Hülle cells differs from the other described regulators, except the transcriptional activator NosA, because only LaeA and NosA affect the number and activity of Hülle cells. In contrast to NosA, which acts directly on DNA, LaeA might serve an epigenetic control function as it is suggested for the control of secondary metabolite clusters. *nosA* transcripts are almost absent in the *laeA*Δ mutant strain. *nosA* overproduction in *laeA*Δ increases the size of the cleistothecia, further supporting that NosA acts downstream of LaeA. Therefore, LaeA might represent an epigenetic pathway acting in parallel to the transcriptional control CryA–VeA–RosA pathway on NosA (Fig. 4) to express Hülle-specific structural genes. It is unknown whether the specific role of LaeA in Hülle cell's morphogenesis is also reflected in an increased secondary metabolite production of this cell type. Increased LaeA-mediated induction of secondary metabolite clusters could serve as a protection for Hülle cells and for the maturing cleistothecia against competitors in the soil.

Putative interactors of velvet proteins and LaeA in the control of sexual development and secondary metabolism

Transcription factor SteA is required for fruiting body formation, but not for Hülle cells

NsdD is required early for both Hülle cell formation and cleistotheica formation, whereas the LaeA-controlled NosA acts later at the transition between small- and normal-sized cleistothecia and leads to Hülle cell formation. SteA of *A. nidulans* represents another transcriptional activator that is indispensable for cleistothecia formation, but not for the formation of Hülle cells. Accordingly, a *steA*Δ mutant strain can only produce Hülle cells, but cannot form sexual fruiting bodies. These mutants are blocked at the early stages of cleistotheica development and cannot form heterokaryons (Vallim *et al.*, 2000). SteA represents a homeodomain transcription factor with more than 60% amino acid identities to yeast Ste12p, the transcription factor, which represents the final target of the yeast pheromone pathway required for mating (Schwartz & Madhani, 2004).

The chromatin-associated repressor RcoA (yeast Tup1p) is required for secondary metabolite production and development

The dimers VosA–VelB and VelB–VeA as well as the trimeric VelB–VeA–LaeA complex act within the nucleus. LaeA might act epigenetically and it remains to be shown whether velvet proteins act as transcription factors. RcoA represents, besides RosA, another important transcriptional repression function involved in fungal development. The exact relationship of RcoA with LaeA or the velvet proteins is currently unknown. RcoA of *A. nidulans* is conserved up to human and is similar to the Tup1 protein of *S. cerevisiae*, which represents, together with Ssn6, a general repressor domain. The Tup1–Ssn6 heterodimer cannot interact with DNA, but binds to other transcription factors and deacetylated histones, preventing RNA polymerase II from accessing its target promoters. Tup1–Ssn6 is associated with histone deacetylases. The C-terminus of Tup1 and its homologs bear seven WD (Trp–Asp) repeats for protein–protein interactions to various regulators.

Neurospora crassa RCO-1 (Tup1) participates in the control of asexual and sexual sporulation (Yamashiro *et al.*, 1996) and is, together with RCM-1 (Ssn6p), involved in photoadaptation (Olmedo *et al.*, 2010). The Ssn6 encoding homolog of *A. nidulans* is presumably essential for growth (Garcia *et al.*, 2008). The deletion of *rcoA* in *A. nidulans* leads to drastically reduced gene expression for the sterigmatocystin cluster transcription factor AflR and loss of production of this aflatoxin precursor. *rcoA* mutants also have reduced transcription of the asexual regulator *brlA*, resulting in reduced asexual conidiation (Hicks *et al.*, 2001). *rcoA*Δ cannot develop sexual fruiting bodies because they are self-sterile (Todd *et al.*, 2006). Overexpression of *veA* in an *rcoA* mutant background cannot rescue the mutant phenotype of *rcoA*, suggesting that RcoA might act downstream of VeA in a mechanism that remains to be resolved.

Control factors at the interface between primary metabolism, secondary metabolism and development

A number of parameters have to be taken into account in coordinating secondary metabolism and development. Changing nutritional conditions can overrule the light signal input of *A. nidulans*. Increasing the glucose concentration is one possibility to overcome light repression of sterigmatocystin production. Cultures grown with 1% glucose and exposed to light produce less sterigmatocystin than cultures grown in the dark. Increased supply of 2% glucose results in enhanced sterigmatocystin production. Glucose abundance also affects the blue-light-sensing proteins LreA and LreB and the light-dependent subcellular localization of the VeA protein. LreA, LreB and FphA, do not only modulate sterigmatocystin, but also penicillin biosynthesis (Atoui *et al.*, 2010). Similarly, a number of nutritional conditions, including amino acid supply, nitrogen source, trace elements like metal ions or the pH, can have an important impact on secondary metabolism and development that is connected to

additional control proteins for development and/or secondary metabolism.

Amino acid regulator Gcn4p/CpcA/CPC-1

The formation of fruiting bodies necessitates the synthesis of numerous additional proteins to differentiate into the various necessary cell types. This requires amino acids as precursors of translation. Amino acids are also required for secondary metabolism, especially for the nonribosomal peptide synthases (NRP). *Aspergillus nidulans* possesses genes for 27 putative NRPs, where the corresponding secondary metabolite is mostly unknown (von Dohren, 2009). Amino acid limitation in fungi results in the induction of a genetic network that induces genes for enzymes of multiple amino acid biosynthetic pathways as well as for aminocyl-tRNA synthases. This genetic system is named cross-pathway control in filamentous fungi or general control in yeast (Braus, 1991; Braus *et al*., 2004; Hinnebusch, 2005). Other biosynthetic pathways such as the purine biosynthesis are also induced (Mösch *et al*., 1991). The key transcription factor CpcA or CPC-1 activates this network in numerous filamentous fungi and is related to yeast Gcn4 and human ATF4 (Ameri & Harris, 2008). This Gcn4 family of transcription factors carries a DNA-binding multiple activation domain besides a characteristic bZIP domain for dimerization (Drysdale *et al*., 1995).

The supply of amino acids controls fungal development. *Saccharomyces cerevisiae* Gcn4 does not only provide a metabolic, but also a developmental response. Activation of Gcn4 by amino acid limitation activates the transition from single-cell yeast growth to filamentous growth (Herzog *et al*., 2011). Gcn4 of the dimorphic human pathogen *C. albicans* is part of the switch control from the unicellular yeast to the multicellular hyphal form (Sanchez-Martinez & Perez-Martin, 2001). CpcA of the opportunistic human pathogen *A. fumigatus* controls amino acid supply in combination with an important role in virulence (Krappmann *et al*., 2004). Similarly, the Gcn4 homolog *VlCpcA* of the fungal plant pathogen *Verticillium longisporum* is induced in infected plants (Singh *et al*., 2010).

Amino acid limitation impairs cleistothecia formation in *A. nidulans* (Serlupi-Crescenzi *et al*., 1983; Eckert *et al*., 1999). *Aspergillus nidulans* CpcA is responsible for amino acid supply during starvation and induces an arrest in fruiting body formation when amino acids are lacking. Accordingly, overexpression of *cpcA* leads to small cleistothecia that only contain hyphae, but no more meiotically formed ascospores. This block can be released by adding amino acids and represents a connection between the control of primary metabolism and fungal development (Hoffmann *et al*., 2000, 2001). The molecular targets of CpcA that mediates the blocking fruiting body formation are currently unknown.

Nitrogen source, pH, iron supply and secondary metabolism and fungal development

Growth on a nitrate medium of *A. nidulans* increases the production of the aflatoxin precursor sterigmatocystin combined with an increased sexual development. In contrast, growth on ammonium represses sterigmatocystin production and simultaneously increases asexual conidiation. In other aspergilli, there is a different link between nitrogen source and secondary metabolism. Nitrate serves as an inhibitor of aflatoxin biosynthesis of *A. parasiticus*, whereas ammonium activates the expression of aflatoxin genes (Feng & Leonard, 1998). The transcription factor AreA, which possesses a C-terminal GATA Cys(2)Cys(2) zinc finger DNA-binding domain, is the *Aspergillus* homolog of *S. cerevisiae* Gln3 (Krappmann *et al*., 2004) and is highly conserved in filamentous fungi. AreA controls nitrogen source-utilizing genes. AreA homologs of *F. fujikuroi* regulate the gibberellin biosynthesis by activating responsible genes (Mihlan *et al*., 2003). The AreA protein of *A. parasiticus* binds to the promoters of some of the aflatoxin-regulatory genes *aflR* and *aflJ* (Cary *et al*., 2007). *areA*Δ mutants of *A. nidulans* can only grow on an ammonium or a glutamine medium and are impaired in asexual sporulation and spore germination (Kudla *et al*., 1990).

In addition, acidic pH is required for the production of sterigmatocystin, whereas penicillin production is induced under alkaline conditions. pH regulation, therefore, acts reciprocal to the production of these two secondary metabolites. PacC is the major regulator protein for pH sensing in *A. nidulans* and is conserved in the fungal kingdom. *pacC* mutants of pathogenic fungi often lose their virulence or have an impaired invasion rate in their respective hosts (Penalva *et al*., 2008). PacC also controls *A. nidulans* development, because *pacC* deletion strains exhibit impaired conidiation (Tilburn *et al*., 1995). PacC protein binds and activates promoters of alkaline pH-dependent genes as well as penicillin biosynthetic genes (Then Bergh & Brakhage, 1998). PacC has also been proposed to act directly as a repressor on sterigmatocystin cluster genes under alkaline pH conditions (Keller *et al*., 1997; Ehrlich *et al*., 1999). The interplay between LaeA and AreA, AflR and PacC in controlling secondary metabolism remains to be elucidated.

The tetrameric HapB–HapC–HapE–HapX complex (AnCF) is also well conserved throughout the fungal kingdom and beyond from yeast to humans. This tetrameric transcription factor complex binds to the CCAAT motif in the promoter of penicillin biosynthetic genes and is essential for the expression of penicillin (Steidl *et al*., 1999). Penicillin biosynthesis can also be repressed by the basic helix–loop–helix (bHLH) protein AnBH1 that is encoded by an essential gene (Caruso *et al*., 2002). Mutants of the *hapB*, *hapC* and *hapE* genes do not only impair penicillin

biosynthesis, but also asexual conidiation. The HapX component is controlled by iron availability and HapX binds to the HapB/C/E complex under iron-deficient conditions (Hortschansky *et al.*, 2007). Therefore, there has to be a link between penicillin secondary metabolite production, iron-dependent gene expression and asexual fungal development.

Control of protein levels during development

VosA–VelB, VelB–VeA and the trimeric VelB–VeA–LaeA complex represent a small proportion of the considerable number of positively or negatively acting proteins controlling fungal development and secondary metabolism. Besides protein synthesis, stability control and degradation of proteins represent additional measures to control the amount of regulators during distinct steps of development. In order to proceed in development, certain proteins also including regulators might have to be degraded. Autophagosomes and vacuoles are major compartments of degradation. Nuclear factors such as the velvet domain proteins are primarily degraded by the 26S proteasome after the conjugation of several ubiquitin moieties to a lysine residue of a target protein. Cullin-based multisubunit ubiquitin ligases represent a major class of ubiquitin ligases where a specific F-box protein subunit recognizes the protein substrates to be marked for degradation (Feldman *et al.*, 1997; Skowyra *et al.*, 1997). F-box proteins, which often recognize only phosphorylated substrates, have to be exchanged from a cullin complex if other substrate proteins should be degraded. Hence, cullin complexes have to be assembled and subsequently disassembled. Assembled cullin complexes are activated by neddylation, which is the attachment of the ubiquitin-like Nedd8 to a lysine residue of the cullin scaffold proteins (Kawakami *et al.*, 2001; Sakata *et al.*, 2007). Disassembly of cullin complexes requires the detachment of Nedd8 by the conserved COP9 signalosome multisubunit complex (Cope & Deshaies, 2003; Busch *et al.*, 2007). Unneddylated cullin interacts to Cand1 before another round of assembly and re-entry into the neddylation cycle for another active cullin ubiquitin ligase (Pan *et al.*, 2004).

COP9 signalosome, Cand1 and fungal development and secondary metabolism

Defects in the genes controlling the fungal protein degradation machinery impair the fungal clock in *N. crassa* (He *et al.*, 2005) or *A. nidulans* fruiting body formation (Busch *et al.*, 2003). Defects in the gene *csnE* for the COP9 signalosome deneddylase block fungal fruiting body formation in the early primordia state. Hülle cell formation is still possible, suggesting that this kind of protein degradation control is not required for this transition from hyphae to single cells. However, the *csn* mutants are highly sensitive against oxidative stress and might be blocked at the primordia state because they are unable to cope with the internal Nox-mediated ROS signal, which is supposedly triggering the further development from primordia to mature hyphae. In addition, *csnE*Δ mutants are strongly impaired in secondary metabolism and are blind and unable to respond to light appropriately. They do not only form primordia in the dark, but constitutively even if the fungus is illuminated (Nahlik *et al.*, 2010). An even more pronounced phenotype characterizes the two genes required for Cand1 formation. Deletion of the genes for CandA-N and CandA-C corresponding to the N- or the C-terminal part of human Cand1 results in an even earlier developmental block. Hülle cells are still formed, but sexual development produces only nests and cannot proceed to primordia. The *candA*Δ colonies are even more pigmented than *csnE*Δ colonies, which reflects an even more misregulated secondary metabolism (Helmstaedt *et al.*, 2011). These data suggest that the sophisticated control of assembly and disassembly of cullin ubiquitin ligases is not crucial for filamentous growth or the formation of single-cell conidia or Hülle cells, but for the transition from hyphae to unitary fruiting bodies.

Fungal F-box proteins

The function and the substrates of most of the fungal F-box proteins are unknown. The F-box is an approximately 50 amino acid protein motif found mostly at the N-terminus of the protein and comprises specific proline and leucine compositions required for the binding of the F-box to Skp1, another subunit of cullin complexes. The C-terminal part of the F-box protein, which binds the substrate proteins, can contain various conserved patterns such as WD (Trp, Asp repeats) or LLR (leucine-rich repeats) required for the interaction with the substrate (Schmidt *et al.*, 2009). *Neurospora crassa* F-box proteins such as FWD1 serve in the regulation of the circadian clock and are crucial for the degradation of the clock regulator protein FRQ1 (He *et al.*, 2005). The role of most of the approximately 70 F-box protein-encoding genes of *A. nidulans* (Draht *et al.*, 2007) remains to be explored. The GrrA protein is one of the *A. nidulans* F-box proteins involved in development. *grrA* mutants of *A. nidulans* produce sexual cleistothecia without viable spores (Krappmann *et al.*, 2006). Similarly, the deletion of the gene for the Grr1 homolog in the plant pathogen *Gibberella zeae* is impaired in sexual development and virulence (Han *et al.*, 2007). GrrA corresponds to *S. cerevisiae* Grr1, which has multiple functions, including the degradation of the protein kinase Ime2, the counterpart of *A. nidulans* ImeB that is described above (Purnapatre *et al.*, 2005). The connection between F-box proteins and the stability of velvet family proteins or LaeA has not yet been

identified. The PEST region of VeA makes it a putative target of the protein degradation machinery, and it will be interesting to find the suitable F-box protein.

Signal transduction pathways for fungal development and secondary metabolism

The plethora of control factors that act in concert with the velvet domain family and LaeA have to be correctly synthesized, localized, activated and degraded in response to external and internal cues to fulfill their function in fungal development and secondary metabolism. This requires various signal transduction pathways (Fig. 5).

SteA and the MAP kinase pheromone pathway

The pheromone MAP kinase pathway is best studied in *S. cerevisiae*, where it regulates pheromone signaling of the two different sexes representing a or α cells during mating. Most mutations of genes for this pathway result in a sterile (*ste*) phenotype characterized by the lack of response to pheromones that are small modified peptides in yeast (Schwartz & Madhani, 2004). Components of this MAP kinase cascade are also involved in the transition from the yeast growth mode to pseudohyphal growth (Kohler *et al*., 2002). This MAP kinase module contains the MAP kinase Fus3 required for cell fusion (*fus*) that finally phosphorylates nuclear transcription factors such as Ste12, which is the yeast counterpart of *A. nidulans* SteA. Fus3 MAP kinase receives the signal from the MAP kinase kinase (MAPKK) Ste7 that is phosphorylated by the MAP kinase kinase kinase (MAPK3) Ste11. The three yeast kinases are assembled on the scaffold protein Ste5. Many components of MAP kinase modules have been identified and deleted in the fungal kingdom, where they are involved in fungal development or pathogenicity (Muller *et al*., 1999; Kawasaki *et al*., 2002; Garrido & Perez-Martin, 2003; Kraus *et al*., 2003; Reyes *et al*., 2006; Park *et al*., 2008; Valiante *et al*., 2009). The functional homologs of the yeast pheromone pathway are present and expressed in *A. nidulans* during sexual development (Paoletti *et al*., 2007). MpkB is the homolog to the yeast Fus3p MAP kinase. The genome of *A. nidulans* contains, besides MpkB, three other MAP kinase-encoding genes: *sakA*/*hogA*, *mpkA* and *mpkC* (May *et al*., 2005). *sakA* encodes a protein that is a homolog to the yeast Hog1 MAP kinase responsible for osmoregulation. SakA is not only activated in the response to osmotic and oxidative stress, but is also required for the repression of sexual development. However, the regulator that is controlled by the SakA MAP kinase in sexual development is unknown (Kawasaki *et al*., 2002). The deletion of *A. nidulans mpkB* (corresponding to *FUS3*) controlling transcription factor SteA leads to a similar phenotype as the *steA* deletion. *mpkB*Δ strains are blocked in the formation of sexual fruiting bodies without losing the potential to form Hülle cells and are impaired in asexual sporulation. Furthermore, the levels of the aflatoxin precursor sterigmatocystin are decreased in *mpkB*Δ deletions due to the reduced expression of the *aflR* regulator gene as well as structural genes of the sterigmatocystin cluster. Similarly, genes for penicillin and for terrequinone biosynthesis are decreased in the *mpkB*Δ strain. The MpkB-mediated signal transduction pathway does not affect LaeA control (Atoui *et al*., 2008). The deletion of *A. nidulans steC* (encoding the Ste11 MAPK3 counterpart) located upstream of the transcription factor SteA and the MAP kinase MpkB also affects sexual and asexual development (Wei *et al*., 2003). *steC*Δ mutants form Hülle cells, but cannot complete the sexual cycle and produce only initial nests that are blocked for further development. These mutants cannot form stable heterokaryons.

Oxylipin hormones and fruiting body formation in *A. nidulans*

Activation of the yeast pheromone Fus3 MAP kinase pathway requires two different sexual hormones, a-factor and α-factor, which are 10–12 amino acid peptides produced by the different sexes and that are recognized by the pheromone receptors Ste2 or Ste3 of the opposite sex. Binding of pheromones triggers the Fus3 pheromone pathway and results in mating. Peptide pheromones are not known in aspergilli.

A different class of pheromones are the *psi* (precocious sexual inducer) factors that are oxylipins derived from lipid acids. *psi* factors represent a mixture of different hydroxylinoleic acid moieties, which can induce sexual development and repress asexual conidiation pathway (Champe *et al*., 1987; Champe & el-Zayat, 1989). They are related to prostaglandin hormones secreted by various mammalian tissues. Receptors for oxylipins have not yet been identified in aspergilli. *psi* factors are produced by dioxygenases like the enzymes encoded by *ppoA* and *ppoB*. The proteins PpoA and PpoB are similar to mammalian prostaglandin synthetases and act as antagonists. The ratio between the formation of the three described *psi* factors *psiA*, *psiB* and *psiC* determines the balance between asexual and sexual development. PpoA is required for *psiB* (10-hydroxy-9,12-octadecadienoic acid) and *psiC* (8-hydroxy-9,12-octadecadienoic acid) biosynthesis, which are prerequisites for sexual development. *psiC* seems to be the most potent *psi* pheromone. The deletion of *ppoA* gene results in increased asexual development. PpoC produces *psiA* (8-hydroxy-9,12-octadecanoic acid), which is required for asexual sporulation (Fig. 4). The *ppoC* deletion increases sexual development and the same phenotype results from overexpression of *ppoA* (Tsitsigiannis *et al*., 2004a, b). The *psi* factors do not only regulate fungal development, but also secondary

metabolite production. *ppoA*/*ppoC* double mutants cannot produce sterigmatocystin, whereas the lack of *ppoB* increases sterigmatocystin levels (Tsitsigiannis *et al*., 2005; Yu & Keller, 2005).

The receptors, the signal transduction pathways and the transcriptional regulators that are required for *psi* hormone-mediated support of fungal development are currently unknown. The *psi* pathway is impaired if the neddylation/deneddylation-mediated control of protein stabilities is no more functional. Deletion of the gene for the fungal COP9 signalosome deneddylase alters secondary metabolism by inducing the orsellinic acid biosynthetic cluster or by accumulating intermediates of sterigmatocystin biosynthesis. In addition, defects in the COP9 signalosome also alter the overall *ppoC* expression levels during development and even earlier in the vegetative phase and this expression pattern is combined with an increased expression of *ppoA*. This results in a similar effect of increased sexual development. A *ppoC* deletion or overexpression of *ppoA* might be one of the reasons why this mutant strain is unable to repress sexual development in light (Nahlik *et al*., 2010). The targets of the COP9 signalosome in the *psi* pathway are unknown.

The protein kinase A (PKA) pathway

The PKA pathway is also well studied in yeast, where it can cross-talk to MAP kinase signal transduction pathways (Mösch *et al*., 1999; Lengeler *et al*., 2000). PKA catalytic subunits phosphorylate target proteins in the cytoplasm as well as in the nucleus. Target proteins of PKA for fungal development or secondary metabolism are currently unknown, but it is known that this pathway is important for fungal development and secondary metabolism. PKA activation requires binding of cyclic AMP, which releases the catalytic subunits from the regulatory subunits of PKA. The adenylate cyclase acts upstream of PKA and elevates cellular cAMP levels. The PKA pathway activates adenylate cyclase in response to nutritional conditions, including glucose supply as the carbon source that is provided by the environment. Glucose supply can affect fungal development as described above and the PKA pathway is also involved in fungal pathogenicity (D'Souza & Heitman, 2001; Shimizu *et al*., 2003; Banno *et al*., 2005; Ni *et al*., 2005; Huang *et al*., 2007). *Aspergillus nidulans* possesses two genes for catalytic subunits of PKA. *pkaA* is the major player acting in a manner opposite to *pkaB* as a minor player. *pkaA*/*pkaB* double-deletion strains are not viable. *pkaA* is required for vegetative growth and germination of conidia. Overexpression of *pkaA* results in a repression of asexual spore formation and a loss of sterigmatocystin production, suggesting a second function in supporting secondary metabolism (Ni *et al*., 2005).

The diacylglycerol-dependent protein kinase C (IP3-DAG-PKC or PKC) pathway

Another signaling pathway that is important for fungal development is the cell integrity or the PKC pathway (Heinisch, 2005). The key component PKC phosphorylates target regulatory proteins. PKC is activated by diacylglycerol and Ca^{++} ions. Phospholipase C acts upstream of PKC and can respond to damages in the cell wall and breaks phosphoinositol phosphate into inositol-3-phosphate (IP3) and diacylglycerol. The *A. nidulans* genome encodes the two PKC-encoding genes *pkcA* and *pkcB*, which are both essential. PkcA shows higher degrees of amino acid sequence similarities to the corresponding genes of fungi other than PkcB. *pkcA* antisense expression supports a link to fungal development because the formation of asexual conidia is reduced. *pkcA* also inhibits secondary metabolism by controlling AnBH1 penicillin regulatory protein, which represses penicillin biosynthesis. Reducing the *pkcA* leads to an increase in penicillin production by sequestering AnBH1 to the cytoplasmic fraction and therefore relieving the expression of penicillin biosynthetic genes (Caruso *et al*., 2002; Herrmann *et al*., 2006).

G-protein-coupled receptors (GPCRs)

Numerous signals for MAP kinase, PKA or PKC pathways are received by membrane-bound GPCR proteins (Fig. 5). A transmembrane protein consisting of seven transmembrane domains senses the external signal. Signals are received by the signal perception domains of GPCR located outside of the cytoplasmic membrane and result in a conformational change in the domain found inside the cell. This domain is in contact with the heterotrimeric G-proteins that transmit the signal further downwards elements (Lengeler *et al*., 2000; Yu & Keller, 2005; Yu, 2006).

The *A. nidulans* genome encodes 16 GPCR proteins named GprA-P and NopA, respectively (Han *et al*., 2004b; Lafon *et al*., 2006; Yu, 2010). PreB (GprA) and PreA (GprB) are closely related to the yeast pheromone receptors (Ste2 and Ste3) and are presumably the sensors for the *A. nidulans* MpkB/Fus3 MAP kinase pathway. GprA and GprB are involved in homothallism, which is the self-fertility that allows *A. nidulans* to produce the fruiting bodies in the absence of an additional partner (Seo *et al*., 2004). The *gprD* gene is involved in the control of sexual development. *gprD* mutants are delayed in growth and spore germination, and sexual development takes place irrespective of illumination (Han *et al*., 2004a, b). *nopA* encodes an opsin homolog protein similar to NOP-1 of *N. crassa* (Bieszke *et al*., 1999), but *nopA* mutants do not manifest any phenotype under tested conditions (Rodriguez-Romero *et al*., 2010).

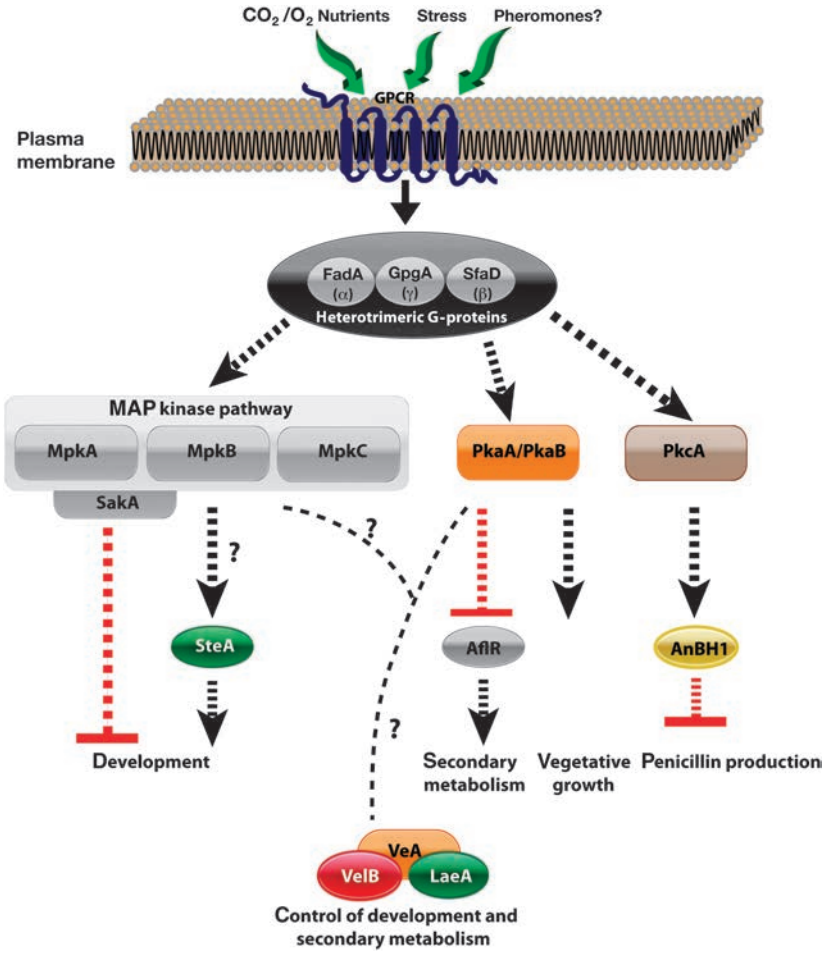

Fig. 5. Signal transduction pathways involved in the coordination of secondary metabolism and development in filamentous ascomycetes like *Aspergillus nidulans*. External signals are perceived, for example by GPCRs, transmitted to heterotrimeric G-proteins such as FadA/GpgA/SfaD and transferred within the fungal cells by various signal transduction pathways (details in text) that activate or inhibit fungal regulators such as SteA, AflR, AnBH1 or the trimeric complex VelB–VeA–LaeA "?" Question mark means that "the functional connection is unknown".

Heterotrimeric G-proteins (αβγ) are located downstream of GPCRs. Mutants in heterotrimeric G-protein subunits have been isolated and characterized in *A. nidulans* many years ago as *fluffy* mutants (Wieser *et al.*, 1997). The *fluffy* colonies are produced as a result of profusions of aerial hyphae. *fadA* (fluffy autolytic dominant A) encodes the major Gα-subunit of *A. nidulans*. *fadA* mutants do not only show a fluffy phenotype, but are also unable to produce sterigmatocystin (Yu *et al.*, 1996; Wieser *et al.*, 1997). The active form of FadA (FadA-GTP) supports vegetative growth and represses differentiation and mycotoxin production. FlbA is the regulator of G-protein signaling, which increases the GTPase activity of FadA. The lack of *flbA* causes symptoms similar to those observed in *fadA* mutants, which are characterized by a fluffy phenotype and lack of sterigmatocystin biosynthesis (Yu *et al.*, 1999; Yu & Keller, 2005). *ganA* and *ganB* represent two additional Gα-encoding genes (Chang *et al.*, 2004). *ganB* is responsible for cAMP levels in response to carbon source availability during germination of spores and, therefore, important for the PKA pathway.

In addition to Gα-subunits, several Gβ-units have been investigated including *sfaD*. SfaD (suppressor of *flbA*) inhibits inappropriate sporulation (Rosen *et al.*, 1999; Seo & Yu, 2006). *sfaD* mutants still produce Hülle cells, but sexual development is impaired. These developmental defects are associated with the loss of sterigmatocystin production. Phosducin proteins modify Gαβγ functions. Deletion of the phosducin-encoding gene *phnA* results in similar defects in development and sterigmatocystin production as *sfaD* mutants (Seo & Yu, 2006). A single Gγ-subunit encoded by the *gpgA* gene is necessary for sexual development and mycotoxin production. Therefore, heterotrimeric G-protein signaling as well as modifiers of this pathway play major roles in fungal development and secondary metabolism.

Concluding remarks and future perspectives

Aspergillus nidulans represents an amenable model system to study the fungal control of secondary metabolism and

development. The insights gained from these studies might be important for fungi relevant to economy and human health. The velvet domain family and LaeA are central players in this coordination process and increasingly more pieces of the puzzle are being assembled. This improves our understanding of the interplay between regulators, the control of their protein synthesis, stability, localization and activity. The exact molecular connection between these regulators and the sensed signals like light, CO_2/O_2 levels, glucose, amino acids, trace elements, pH or ROS remains to be explored. There are many questions that remain to be answered. What type of signal is received by each receptor? What is the target of each specific signal? Which protein complexes are the targets of the PKA, MAP kinase or the PKC signaling pathways? Which target proteins are activated or inactivated? This includes the characterization of the real signals, their sensors and their regulation. The exact mechanism of *psi* hormone function is still elusive. Furthermore, the interplay between the various regulatory G-proteins has to be explored. Similarly, the cross-talk between the different signal transduction pathways has to be determined as well as the interplay between the final players that regulate transcription, chromatin and degradation of regulators.

VeA can serve as a scaffold to recruit more proteins involved in coordination of development, and this role should be explored in other less related fungal taxa. Is a connection to a nonvelvet LaeA-like protein conserved? Is the LaeA control of velvet domain protein levels and their modification conserved? What are the other interactors that mediate signals other than the FphA–LreB–LreA, which provides a light response? Are LaeA proteins of other fungi also involved in the formation of specific cells such as the Hülle cells of *A. nidulans*? What is the exact role of the velvet domain and what is the structure? How does LaeA activate gene clusters or heterochromatin in general? The answers to all of these questions will not only improve our understanding of fungal development and secondary metabolism, but might help to control and manipulate food-related or biotechnologically relevant fungi.

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