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The Spitzenkörper: A signalling hub for the control of fungal development?

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Abstract

In *Aspergillus nidulans*, asexual development culminates in the formation of a multicellular conidiophore that bears spores known as conidia. Although several factors required for conidiophore formation have been characterized, the mechanisms underlying the transition from vegetative growth to development remain obscure. However, the recent characterization of two of these factors, FlbB and FlbE, provides important new insight. Notably, these studies suggest that the Spitzenkörper (i.e. apical body), which is known to regulate hyphal morphogenesis, might also serve as a signalling hub that co-ordinates development transitions.

The life cycle of most filamentous fungi can typically be divided into two phases. The first phase consists of vegetative growth, whereby a branched network of hyphae explores and colonizes the local environment. The unique tubular shape and impressive extension rates of hyphae are achieved by confining cell surface expansion and cell wall deposition to the extreme tip. A key component in this process is the Spitzenkörper, which is a phase-dark structure found at the tips of extending hyphae that among other functions appears to serve as a vesicle trafficking centre (Virag and Harris, 2006) (Figure 1A). Once nutrients are locally depleted and, in most cases, appropriate signals are received, fungi initiate the second phase of their life cycle: reproductive development. This phase results in the formation of reproductive structures that bear large numbers of spores of either asexual or sexual origin. Upon dissemination to the right environment, spore germination marks the return to the vegetative phase of the life cycle. The signals that co-ordinate the transitions between vegetative growth and reproductive development in filamentous fungi remain poorly understood. However, a recent set of articles published in *Molecular Microbiology* has provided significant new insight into these processes using the filamentous fungus *Aspergillus nidulans* (Etxebeste *et al*., 2009; Garzia *et al*., 2009).

Aspergillus nidulans is a homothallic ascomycete capable of producing both asexual and sexual spores within the same colony. Asexual development normally precedes sexual development, and requires exposure to air as well as the acquisition of developmental competence (i.e. typically 15–18 h of vegetative growth) (Timberlake, 1991; Kües and Fischer, 2006). This developmental pathway results in the formation of uninucleate spores (i.e. conidia) borne on multicellular conidiophores that project above the colony (Figure 1A). The first step in this pathway is the appearance of specialized hyphal compartments known as foot cells, from which an aerial branch emerges and grows away from the growth plane to a height of ~100 μm. Once this height is reached, apical extension ceases and the tip swells to produce a globose vesicle. Because nuclear division continues unabated during this time, the vesicle is multinucleate. Eventually, each nucleus migrates to a position just below the vesicle surface, and a synchronous round of budding followed by nuclear division leads to the formation of a tier of uninucleate metulae that lie atop the vesicle. Each metula then divides to produce a second tier of cells known as phialides, which are sporogenous cells that bud repeatedly to generate a chain of conidia, with the oldest spore found at the tip of the chain.

Extensive genetic and molecular analyses have resulted in the characterization of a core transcriptional pathway that underlies conidiophore development (Adams *et al*., 1998; Yu *et al*., 2006) (Figure 1B). In this pathway, the transcription factor BrlA activates expression of another transcription factor, AbaA, which in turn activates production of the DNA-binding protein WetA. AbaA also controls activation of BrlA, thereby constituting a feedback loop that reinforces commitment to development. BrlA, AbaA and WetA act in a sequential

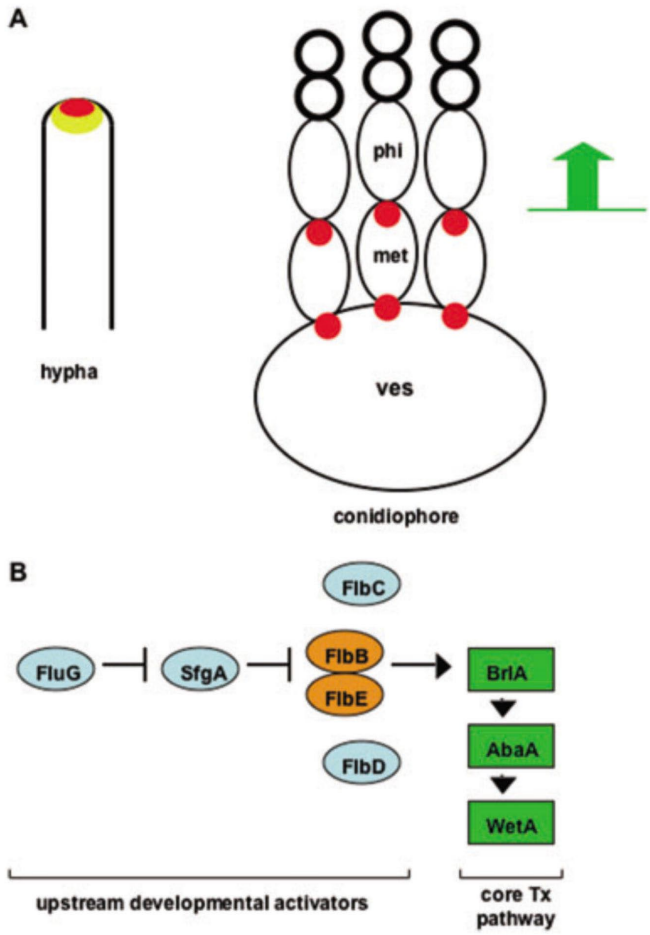

Figure 1. A. Schematic depiction of a vegetative hypha and reproductive conidiophore. The hypha features a prominent Spitzenkörper at the tip (yellow). FlbB localization (red) coincides with the Spitzenkörper; FlbB also accumulates in nuclei (not shown). Conidiophores feature a vesicle (ves), metulae (met) and phialides (phi). Phialides undergo repeated budding to generate a chain of conidia. FlbB localizes to bud sites in the vesicle and to the tips of metulae. Note that no Spitzenkörper has been found in these cell types. The transition from metulae to phialides appears to mark the point of irreversible commitment to sporulation (green arrow).

B. Two key pathways that regulate conidiophore development. The upstream developmental activators regulate expression of BrlA, which in turn activates the core transcriptional pathway. Based on results from Garzia *et al.* (2009), FlbB and FlbE are shown as an interacting pair.

manner to drive expression of a large number of genes required for different stages of conidiophore morphogenesis. For example, BrlA and AbaA act in concert to promote expression of genes required for phialide function, whereas WetA controls expression of genes involved in maintaining spore dormancy.

A key issue in understanding the transition from vegetative growth to conidiophore development is the regulation of BrlA. In particular, the characterization of BrlA activators should reveal the nature of the signals that govern this transition. Towards this end, a surprisingly complex network of upstream developmental activators (UDAs) required for BrlA expression was identified as a result of several genetic screens (Yu *et al.*, 2006) (Figure 1B). Key components of this network include: (i) FluG, which appears to control the synthesis of an extracellular factor required for conidiophore development, (ii) SfgA, a repressor of development whose activity is countered by FluG, and (iii) the Flb proteins (i.e. FlbB, C, D and E), which are apparent targets of SfgA. Results from epistasis analysis suggest that FlbB, FlbD and FlbE comprise a linear pathway, with FlbB serving as the terminal factor that activates BrlA. FlbD is a Myb-like DNA-binding protein, whereas the functions of FlbB and FlbE have remained poorly understood until now.

In their initial characterization of FlbB, Etxebeste *et al.* (2008) found that it is a predicted bZIP-type transcription factor that binds DNA. The absence of FlbB prevents the normal accumulation of *brlA* transcripts and, accordingly, abolishes conidiophore development (colonies undergo autolysis instead). Surprisingly, over-production of FlbB has the same effect, which led to the suggestion that FlbB might interact with a partner in a stoichiometric manner. Perhaps the most interesting observation is that FlbB exhibits a dynamic localization pattern at hyphal tips. It initially accumulates at hyphal tips, where it is found in the cytoplasm, before it subsequently shifts to nuclei, where it is predominantly found in the nucleus closest to the tip. Notably, the timing of this shift appears to coincide with the acquisition of developmental competence.

In a more recent publication, Garzia *et al.* (2009) identified FlbE as the FlbB-binding partner, and showed that the interacting pair colocalize at the hyphal tip (although FlbE is apparently not found in nuclei). Proper localization of each partner depends on the presence of the other, and also requires an intact actin cytoskeleton. Genetic analyses revealed that the absence of FlbE causes the same developmental defects as noted in flbB mutants, and epistasis tests demonstrated that the two proteins function at the same step upstream of BrlA. FlbE itself is a protein unique to filamentous fungi that possesses apparent domains of unknown function. Collectively, the initial characterization of FlbB and FlbE suggests that they form a complex at the hyphal tip that has an important role in the transition to conidiophore development.

In this issue of *Molecular Microbiology,* Etxebeste *et al.* (2009) further extend their characterization of FlbB. The tips of growing *A. nidulans* hyphae possess an "exocytic zone" at the extreme apex that is flanked by a subapical "endocytic" zone (Araujo-Bazan *et al.*, 2008; Taheri-Talesh *et al.*, 2008). Co-

localization experiments using a marker for the endocytic machinery reveal that FlbB localizes to the "exocytic zone," which is where the Spitzenkörper is also found. Thus, FlbB is likely to associate with the Spitzenkörper (Figure 1A), and this association might have functional implications, as *flbB* mutants exhibit a modest increase in hyphal branching. As might be expected, tip localization of FlbB persists during mitosis, whereas localization to the apical nucleus is lost. However, and quite remarkably, FlbB predominantly re-appears in the most apical of the two daughter nuclei upon completion of mitosis. This asymmetric segregation pattern might reflect the biased accumulation of an FlbB "retention factor" at one pole of the dividing apical nucleus. However, another intriguing possibility is the existence of a tip-high FlbB gradient, such that the vast majority of FlbB ends up in the daughter nucleus that is closest to the tip. The availability of nuclear distribution mutants (i.e. *nud* mutants) in *A. nidulans* provides one avenue to investigating these ideas.

Although FlbB is primarily confined to the apical nucleus in the hyphal tip compartment, Etxebeste *et al*. (2009) report that it localizes to all nuclei in older compartments found in the colony interior. This observation suggests that the acquisition of developmental competence somehow impacts FlbB nuclear localization (note that localization at the hyphal tip generally remains unaffected). A key intermediate in this response appears to be SfgA, as FlbB is found in all nuclei of the hyphal tip compartment in *sfgA* mutants. Accordingly, one effect of SfgA repression is to limit FlbB nuclear localization, and this must be overcome to enable the transition to asexual development.

Etxebeste *et al*. (2009) also examined FlbB localization in developing conidiophores. They found that FlbB de-localizes from the tips of conidiophore vesicles, which are presumably expanding in an isotropic fashion. However, it re-appears coincident with the budding of metulae from the vesicle, and remains at the tips of these cells as they elongate. Strikingly, the localization pattern of FlbB and the endocytic machinery in metulae is similar to that in hyphae, which implies that these cells retain hyphal-like features even though there is no evidence that they possess a Spitzenkörper (Figure 1A). Once phialides emerge, FlbB disperses throughout each cell of the conidiophore and is not found at all in conidia, which is not surprising, since *flbB* is not expressed in mature conidiophores. These results reinforce the notion that metulae and phialides are dramatically different cell types. Metulae resemble vegetative hyphae, and the observation that they can revert to hyphal growth demonstrates that they are not fully committed to asexual development (Sewall *et al*., 1990; Etxebeste *et al*., 2009). On the other hand, phialides are committed to the production of spores, which is reflected by the significant reprogramming of gene expression and the change in growth pattern (i.e. from acropetal to basopetal) observed in these cells. Thus, the emergence of phialides marks a critical juncture in the transition from vegetative growth to asexual development (Figure 1A).

In summary, the results reported by Etxebeste *et al*. (2009) have two important implications for understanding how the transition to asexual development is regulated in *A. nidulans* and other filamentous fungi. First, in addition to its known morphogenetic functions, the Spitzenkörper might also serve as a signalling hub that controls developmental transitions. This would seem to make sense, given the position of the Spitzenkörper at the tip of extending hyphae that are actively exploring the local environment. Second, the commitment to spore formation might occur very late in the process of asexual development, thereby potentially providing the fungus with a "way out" should conditions change. Several interesting questions remain with regards to FlbB. For example, what role does FlbE play in modulating the dynamics of FlbB localization at hyphal tips and in nuclei? Also, what are the mechanisms that limit FlbB localization to the apical nucleus? Finally, how is FlbB downregulated upon the transition from metulae to phialides. The answers to these and other questions should provide new and exciting insights into the regulation of fungal development.

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