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MicroCommentary

How to build a fungal fruit body: from uniform cells to specialized tissue

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Summary

It is a challenge in biology to explore the molecular and cellular mechanisms necessary to form a complex three-dimensional structure composed of different cell types. Interesting models to study the underlying processes are fungi that can transform their wire-like hyphal filaments into complex and sometimes container-like fruit bodies. In the past, the role of developmental triggers and transcription factors was a major focus of research on fungal model organisms. In this issue of *Molecular Microbiology*, Nowrousian and collaborators report that fruit body development of the model organism *Sordaria macrospora* includes a novel player, a specific membrane protein of the endoplasmic reticulum that is not required for vegetative growth. This finding represents an important step towards connecting regulation of development with the co-ordinated changes in cellular compartments.

Sexual filamentous fungi: fast growth and protected meiospores

Fungi are an evolutionary highly successful group because they are able to explore and conquer new ecological niches rapidly. Their specific growth mode comprises filaments of elongated cells termed vegetative hyphae (mycelia) that expand at the apex of the tip cell. As reviewed recently (Sietsma and Wessels, 2006), this polarized growth requires a cell wall that is plastic at the apex but shaping at the older hyphal parts. Rigidification of the wall seems to occur through linkage of mainly chitin and glucans outside the cytoplasmic membrane. According to present knowledge, new wall components and proteins destined for secretion originate from the endoplasmic reticulum (ER) and are transported as part of a sophisticated transport and communication system in vesicles to the apex. Associated with local secretion of gluey or celluloytic compounds at the tip is the ability of filamentous fungi to adhere to and invade substrates. In undisturbed environments such as forest soil, filamentous fungal growth results in giant organisms that are assumed to be among the largest and oldest individuals on earth, demonstrating the evolutionary success of growing with hyphae composed of interconnected cell modules.

In addition to the unlimited growth of hyphae, sexually reproducing filamentous fungi can develop multicellular unitary structures of definite size: fruit bodies. These highly organized multicellular structures originate from vegetative hyphae [the fast progress in understanding fruit body development has been extensively reviewed recently (Poeggeler *et al*., 2006)]. They are composed of different specialized cell types and protect the meiotically derived spores. The molecular mechanisms underlying fruit body formation are barely understood. Therefore, the key molecules that are responsible for the transition from hyphal growth to fruit body formation have to be identified and their interplay has to be studied. To obtain a complete picture, the analyses should include a comparison of heterothallic (self-sterile) and homothallic (self-fertile) ascomycetes. In heterothallic representatives like *Neurospora crassa*, which require two sexually different fungal individuals to complete a sexual cycle, the mating types have been a major research focus. Homothallic fungi like *Aspergillus nidulans* or *Sordaria macrospora* can form fruit bodies in the absence or presence of a partner, and are therefore especially tractable for genetic approaches and the analyses of mutant strains.

Aspergillus nidulans has long been used as model organism to study asexual development. In recent years, it also proved suitable for studying sexual development, especially with regard to the balance of sexual and asexual reproduction. The self-fertile *Sordaria macrospora* lacks an asexual cycle and is therefore especially suited for studies on regulation of sexual development. *S. macrospora* is closely related to *N. crassa*, and genome sequence information as well as microarrays for this ascomycete can also be used as tools for

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S. macrospora. While *A. nidulans* forms closed fruit bodies called cleistothecia, fruit bodies of *S. macrospora* are bottle-like structures termed perithecia, with a pre-formed opening at the neck through which the spores are released. In both cases, fruit bodies consist of an outer envelope that protects the inner space, including the cells that finally differentiate into sexual spores (Fig. 1).

Trigger for development: environment and internal readiness

Growth by vegetative hyphae is often sufficient for survival, but the mycelium also can function as the starting point for differentiation into various cell types in response to the microenvironment the fungus encounters, as reviewed recently (Ugalde, 2006). Thus, fruit bodies are not formed as a basic necessity but only after perception of several environmental cues and endogenous auto-regulatory signals. External factors generally include a plethora of parameters including nutrient availability, light conditions, oxygen/CO_2 pressure, surface contact, osmolarity and pH. Cell density and partner availability often also play a critical role in deciding whether or not to initiate development. Endogenous factors include intermediates of metabolism with signalling action, like pheromones or hormone-like substances, and the cellular redox status. For example, the *A. nidulans*, fatty acid oxygenases (PpoA–C) involved in hormone production (Psi factor) are important in establishing the ratio of sexual and asexual development.

Perception of the various signals involved in initiating fruit body development has long been studied on a physiological level. For example, it has been known for many years that sexual development in *A. nidulans* is favoured in the absence of light under low oxygen. The mechanism of light perception in *A. nidulans* includes the red light receptor phythochrome (Blumenstein *et al*., 2005) as well as the velvet factor VeA (Kim *et al*., 2002). On a molecular level, the most important systems of signal perception are probably guanine nucleotide-binding (G) protein-coupled receptors (GPCRs) and their corresponding G proteins. Three of 16 proposed GPCRs of *A. nidulans*, the hormone receptors GprA and GprB as well as GprD, are involved in regulation of sexual development (Yu, 2006).

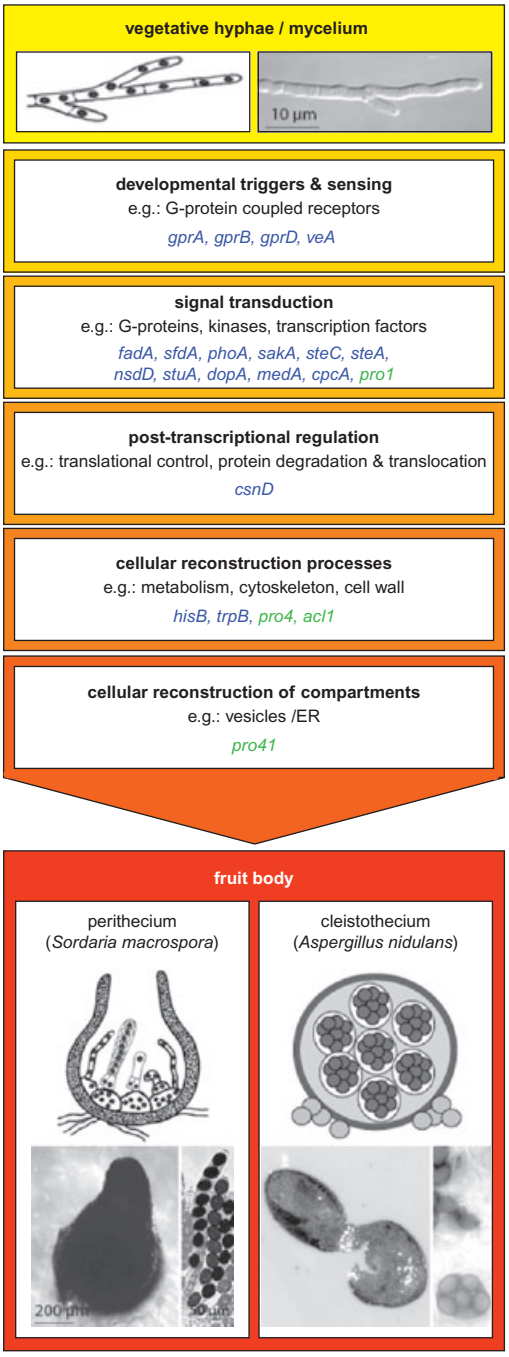

Fig. 1. Transition from vegetative hyphae to multicellular reproductive structures (fruit bodies) of homothallic filamentous fungi. Examples of genes involved in the regulation of fruit body formation in *A. nidulans* (blue print) and *S. macrospora* (green print) are given as reviewed before (Poeggeler *et al*., 2006).

Signal transduction: from signal perception to gene expression – and then?

Different pieces of the puzzle of the intracellular network of connections during development between receptors, G-proteins and the response of the nucleus have been identified in different fungi (Poeggeler *et al*., 2006). Besides the G proteins FadA, SfaD and GpgA of *A. nidulans*, the mitogen-activated protein kinase (MAPK) module containing SakA/HogA, and several transcription factors including SteA, NsdD, StuA, DopA, MedA and CpcA are involved in the regulation of fruit body formation. However, most of the target genes of these transcription factors are yet unknown. One gene whose expression is dependent on the transcription factor Pro1 of

S. macrospora (Masloff *et al*., 1999) encodes the novel ER-compound Pro41 identified by Nowrousian *et al*. (2007).

Expression of many developmental genes also involves regulation at the post-transcriptional level. CpeA of *A. nidulans* (Scherer *et al*., 2002) and APP of *S. macrospora* (Nowrousian *et al*., 2006) are both transcriptionally upregulated in early development but accumulation of the corresponding proteins is delayed. This might reflect translational control of protein synthesis or post-translational regulation of protein stability. The COP9 signalosome that regulates the cellular ubiquitin-dependent protein degradation machinery and adaptors for the E3 ubiquitin ligase like the F-box protein GrrA are all important for fruit body formation and ascosporogenesis (Busch *et al*., 2003; Krappmann *et al*., 2006). This suggests that fruit body formation might require a unique set of proteins in developing cells that are absent from hyphal cells. The change in the protein inventory presumably cannot be accomplished by the synthesis of novel proteins in combination with the regular turnover but might require the specific destruction of proteins specific for hyphal growth.

The discovery of a novel and specific conserved ER resident protein that is essential for fruit body formation of *S. macrospora* (Nowrousian *et al*., 2007) but is dispensable during vegetative growth suggests that fungal development might even require adaptations of specific compartments of the cellular architecture. Fungal hyphae consist of modules that are reiterated and have a tip-controlled polarity. The formation of the tissues, which comprise the perithecium, is based on different structural prerequisites that must result in the formation of the densely packed cells of the outer envelope as well as a number of specialized cell types inside the fruit body. The ER is the gateway for proteins that enter the secretory pathway. The ER is also crucial for protein quality control and protein glycosylation as well as the distribution of proteins to membrane-bound organelles. Specific changes in the ER membrane to allow development suggest that one or several of these processes have to be adapted for development. It will be interesting to find out whether a different ER is necessary to integrate different receptors into the fungal cell.

Fruit body formation: necessity of major cellular reconstructions

Reconstruction of rather uniform hyphae into the complex structure of a fruit body requires major changes in a variety of cellular systems (Poeggeler *et al*., 2006). The complex developmental programme requires more energy than simple vegetative growth, and the vegetative mycelium seems to accumulate nutrients that might later nurture the developing fruit bodies. Therefore, primary metabolism has to be adapted to use these sources. Major changes also have to occur in secondary metabolism, for example, in pigment production. Furthermore, cell wall biogenesis and degradation processes are especially affected by the reconstructions. Notably, β-1,3-glucan is especially stored during vegetative growth and mobilized as a carbon source for sexual development. Accordingly, genes like the chitin synthase gene *chsC* and the α-1,3 glucanase gene *mutA* involved in cell wall and carbohydrate metabolism are specifically regulated during development.

Rearrangement of vesicle transport might play a major role in cell wall remodelling. Transport within the hyphae growing exclusively at the apex requires two directions, the transport towards the tip and retrotransport. Most cargo will be transported to the apex that contains the Spitzenkörper composed of vesicle aggregates. In contrast, fruit body formation has to enable growth in different directions and this should be reflected in the vesicle system of the cell. This scenario predicts that the vesicle system of the fungal cell must be reorganized to allow the transition from hyphal growth to development. The first molecular indication that compartment reorganization requires different molecular players has been achieved by the discovery of Nowrousian and co-workers that the fungal ER membrane protein Pro41 is essential in fruit body formation.

Conclusion

With their relatively simple genome and formation of complex developmental structures, sexual filamentous fungi represent ideal model organisms that will help to understand eukaryotic differentiation. The field is at an exciting stage; several elements of the regulatory network controlling fruit body formation have been identified but the precise mechanisms of how cellular core components are finally used to give rise to different cell types is not known.

The paper by Nowrousian *et al.* adds to the background of fruit body-specific cell type differentiation the important aspect of a specific involvement of ER-related proteins. The ER is a central hub for membrane proteins and lipids for a number of organelles including vacuoles and some of peroxisomal components (Hoepfner *et al*., 2005). Thus, development-specific functions of the ER will most likely affect other organelles as well. In recent years, mitochondria, peroxisomes and vacuoles have all been shown to play a role in sexual development of filamentous fungi (Bowman *et al*., 2000; Contamine *et al*., 2004; Stumpferl *et al*., 2004; Bonnet *et al*., 2006). It will be interesting to see how development-specific functions of the ER can be integrated with developmental roles of other fungal organelles.

The novelty of recent findings is the accumulating evidence for subtle regulation on protein level including protein targeting or degradation during development. The multiple functions of the ER, ranging from protein maturation, glycosylation, quality control, sorting and vesicle formation, are an interesting challenge to dissect the mechanisms specifically required to allow the formation of complex three-dimensional structures.

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