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Building Hyphae in the Air

Despite many obvious morphological and physiological differences, some fungal and bacterial species share strikingly similar patterns of growth and development. Both the filamentous fungi *Aspergillus nidulans* (an ascomycete) and *Schizophyllum commune* (a basidiomycete) and the gram-positive soil bacterium *Streptomyces coelicolor* grow vegetatively in a filamentous structure called a mycelium that penetrates the substrate on which it grows. The mycelium grows by the addition of material to the tips of individual filaments, or hyphae. Under certain conditions, hyphae begin to grow into the air rather than along the substrate. Some or all of these hyphae then enter a pathway of morphological differentiation that culminates in the formation of spore-producing reproductive structures. Spore-producing hyphae must protrude into the air if spore dispersal is to be effective, so the formation of aerial hyphae is an essential component of morphogenesis.

In the sporulating bacterium Streptomyces, a fuzzy aerial mycelium forms when nutrient levels become low. Specialized aerial hyphae then develop into chains of cells that differentiate into spores (Chater, 1989). Aspergillus, by contrast, is induced to develop not by nutrient starvation but by contact with air (Timberlake, 1990). Hyphal stalks emerge from specialized cells on the surface of the induced colony, and once these stalks reach a certain height, they differentiate into asexual spore-producing structures called conidiophores. Spores, or conidia, then bud off from specialized conidiophore cells.

Unlike the spore-producing hyphae of Streptomyces and the conidiophores of Aspergillus, the reproductive structures of Schizophyllum are of sexual origin (Casselton and Economou, 1984). In sterile (monokaryotic) cultures, the mycelium grows vegetatively in the substrate until it reaches a certain size, at which point a mass of aerial hyphae begins to grow. Dikaryotic cultures formed by mating compatible monokaryons (which have different alleles at each of two mating-type loci) also grow in a vegetative mycelium from which aerial structures arise. Most of these structures differentiate into spore-producing fruiting bodies.

Although many of the details of the development of aerial and reproductive structures in Aspergillus, Schizophyllum, and Streptomyces are different, the remarkable morphological similarities in the process as a whole raise the question of whether there are similarities at the cellular and molecular levels as well. For example, do the three organisms use similar strategies to propel hyphae up off the substrate surface?

In an attempt to understand the mechanisms that underlie the development of reproductive structures, several laboratories have begun to isolate mRNAs or proteins that accumulate specifically in aerial hyphae and spore-bearing structures. Willey et al. (1991) recently characterized a small spore-associated protein, SapB, that is involved in aerial mycelium formation in Streptomyces, and Wessels et al. (1991a) and Stringer et al. (1991) have recently found that secreted hydrophobic proteins, or hydrophobins, are involved in the development of aerial structures in Schizophyllum and Aspergillus. Genetic and morphological evidence from all three studies suggest that the hydrophobins and SapB are morphogenetic proteins that allow—or cause—hyphae to emerge off the substrate and into the air.

The SapB protein was first identified as a component of the spore wall, from which it can be removed by detergent treatment (Guijarro et al., 1988). SapB appears when aerial hyphae begin to form. In their recent work, Willey et al. (1991) have further characterized SapB, finding that it is an 18-amino acid peptide composed of only about eight different amino acids. SapB is both associated with, and secreted from, colonies that are in the process of forming an aerial mycelium.

Several lines of evidence lead to the conclusion that SapB is involved in aerial mycelium formation. First, the timing and location of SapB accumulation are closely coupled to the timing and location of aerial mycelium formation. Second, mutations that eliminate aerial hyphae altogether (mutations in any of six *bald* genes) block the accumulation of SapB, whereas mutations that allow aerial hyphae to form but block spore formation (mutations in the *white* genes) have no effect on SapB accumulation. The loss of SapB can actually be used as an assay to isolate additional *bld* mutants. Third, and most important, when *bld221* mutant colonies are grown adjacent to *bld*$^+$ colonies—or even supplied with purified SapB protein—they regain the ability to form aerial hyphae. That is, exogenous SapB can complement the *bld221* defect. It is not yet known, however, whether exogenous SapB can complement mutations in other *bld* genes. Interestingly, one of the other *bld* genes encodes a tRNA for a leucine codon that is rarely used in Streptomyces (Lawlor et al., 1987). The SapB protein contains five leucines; perhaps this codon encodes one or more of these leucines.

Although the SapB protein may turn out to be a signaling molecule that induces aerial growth in hyphae that

receive it, it is required in such large amounts that it is more likely to be a structural molecule of some sort (Willey et al., 1991). If so, the ability of purified SapB to complement at least one *bld* mutant suggests that this small protein functions extracellularly, perhaps by providing an external scaffold on which aerial hyphae can grow or otherwise allowing hyphae to break surface tension (Willey et al., 1991).

Proteins with a different structure, but which may play a role in filamentous fungi similar to that of SapB in Streptomyces, have recently been found in Schizophyllum and Aspergillus. The Schizophyllum proteins were identified in screens for genes that are expressed specifically in the aerial hyphae of monokaryons or in dikaryotic fruiting bodies (Dons et al., 1984; Mulder and Wessels, 1986). Three of these genes, *Sc1*, *Sc3*, and *Sc4*, encode proteins that are very similar to one another (Schuren and Wessels, 1990). The encoded proteins are small (not much more than 100 amino acids), they contain N-terminal secretion signal sequences, they are hydrophobic, and they contain eight cysteine residues in conserved positions. Wessels et al. (1991a) have found that these proteins, called hydrophobins, are found in the cell wall of aerial structures, where they form insoluble complexes that appear to be held together by a combination of disulfide cross-links and hydrophobic interactions.

As with SapB, genetic evidence suggests that the hydrophobins are intimately associated with the emergence of aerial hyphae and fruiting bodies. The *thin* (*thn*) mutation, which blocks the formation of all aerial structures (Wessels et al., 1991b), also blocks the accumulation of the transcripts for all three hydrophobins. The *fruit body formation* (*fbf*) mutation, which prevents the development of fruiting bodies but not aerial hyphae (Springer and Wessels, 1989), blocks the accumulation of the *Sc1* and *Sc4* mRNAs but not the *Sc3* mRNA. These genetic observations are consistent with data showing that *Sc3* mRNA accumulates in all aerial structures but that *Sc1* and *Sc4* mRNA accumulate in fruit bodies specifically.

How might the hydrophobins contribute to the formation of aerial structures? One possibility is that they become incorporated into the cell walls of hyphae that excrete them, thereby making the hyphae walls hydrophobic and/or rigid enough to emerge off the aqueous substrate. Wessels et al. (1991a) have made the interesting observation that when Schizophyllum is grown on the underside of netting that is floating on liquid medium, the submerged hyphae secrete hydrophobins into the medium but do not incorporate hydrophobins into their walls. Perhaps the air-hyphal interface induces hydrophobins to become cross-linked to the cell wall; hydrophobins secreted by submerged hyphae may simply diffuse into the medium and be unavailable for cross-linking in the cell wall.

The importance of the Schizophyllum hydrophobins to fungal morphogenesis has been underscored by the recent finding of Stringer et al. (1991) that the *rodA* gene of the ascomycete Aspergillus, whose disruption makes spore walls less hydrophobic than normal, encodes a secreted, hydrophobic protein very similar to the hydrophobins. The *rodA* gene was initially identified not through genetic analysis but by the accumulation pattern of its cDNA. The CAN41 cDNA, as it is called, is one of a large number of cDNA clones that correspond to transcripts that accumulate early in conidiophore development (Boylan et al., 1987).

To investigate the function of CAN41, Stringer et al. (1991) used homologous recombination to replace wild-type genomic CAN41 sequences with an inactivated CAN41 gene. The resulting CAN41$^-$ cells display an intriguing mutant phenotype: the conidia are more wettable than those of the wild type, and the colonies become waterlogged. When examined by electron microscopy, the walls of the conidia and of some of the conidiophore cells are seen to lack an outer cell wall layer, the rodlet layer. Although a definite connection between the rodlet layer and the hydrophobins remains to be made, the rodlets probably consist at least partially of cross-linked, insoluble hydrophobin molecules. Like the Schizophyllum hydrophobins, the Aspergillus rodlet layer is restricted to aerial structures: conidia induced to form in liquid medium accumulate *rodA* transcripts (Adams et al., 1988) but lack the rodlet layer.

It is not yet possible to compare directly the *rodA* phenotype with the phenotype caused by the elimination of just one of the Schizophyllum hydrophobins because such mutations have not been isolated. Unlike the Schizophyllum *thn* mutation, which eliminates aerial structures and prevents the accumulation of at least three hydrophobins, and the *fbf* mutation, which prevents fruiting body formation and blocks the accumulation of two of the hydrophobins, the Aspergillus *rodA* mutation does not hinder the production of aerial structures per se—conidiophores, albeit somewhat abnormal ones, still form. This result may indicate that hydrophobins are not important in the emergence of aerial structures in Aspergillus, but more likely, it suggests that like Schizophyllum, Aspergillus contains several hydrophobin genes that are expressed at restricted times or locations. For example, a distinct *rod* gene may encode the hydrophobin that functions in the emergence of the conidiophore stalk.

Another major question that has yet to be answered concerns the relationship, if any, between SapB and the hydrophobins. Although it is tempting to speculate that the hydrophobins and

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SapB play similar roles in the morphogenesis of aerial structures in fungi and sporulating bacteria, there are important differences between the proteins. For one thing, their structures are completely different. The cysteine-rich hydrophobins are over five times the size of SapB, which lacks cysteines. It is not even known whether SapB is synthesized on ribosomes (most likely as a larger precursor polypeptide) or assembled enzymatically (Willey et al., 1991). More important, the hydrophobins do not share with SapB the ability to complement mutants defective in their synthesis. That is, neither *rodA* mutant Aspergillus nor Schizophyllum regulatory mutants that lack hydrophobins are rescued by adjacent wild-type colonies. This lack of extracellular complementation may point to a fundamental difference in the functions of the hydrophobins and SapB. Unlike Streptomyces, in which SapB is not incorporated into the cell wall but functions externally, the fungi may lack an extracellular scaffold as such. Rather, the hydrophobins would make the cell walls hydrophobic and rigid, providing the walls themselves with the properties that impel them off the substrate and into the air.

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REFERENCES

Adams, T.H., Boylan, M.T., and Timberlake, W.E. (1988). *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell **54**, 353-362.

Boylan, M.T., Mirabito, P.M., Willett, C.E., Zimmerman, C.R., and Timberlake, W.E. (1987). Isolation and physical characterization of three essential conidiation genes from *Aspergillus nidulans*. Mol. Cell. Biol. **7**, 3113-3118.

Casselton, L.A., and Economou, A. (1984). Dikaryon formation. In Developmental Biology of Higher Fungi, D. Moore, L.A. Casselton, D.A. Wood, and J.C. Frankland, eds (Cambridge, England: Cambridge University Press), pp. 213-229.

Chater, K.F. (1989). Sporulation in Streptomyces. In Regulation of Procaryotic Development, I. Smith, R. Slepecky, and P. Setlow, eds (Washington, DC: American Society for Microbiology), pp. 277-299.

Dons, J.J.M., Springer, J., de Vries, S.C., and Wessels, J.G.H. (1984). Molecular cloning of a gene abundantly expressed during fruiting body initiation in *Schizophyllum commune*. J. Bacteriol. **157**, 802-808.

Guijarro, J., Santamaria, R., Schauer, A., and Losick, R. (1988). Promoter determining the timing and spatial localization of transcription of a cloned *Streptomyces coelicolor* gene encoding a spore-associated polypeptide. J. Bacteriol. **170**, 1895-1901.

Lawlor, E.J., Baylis, H.A., and Chater, K.F. (1987). Pleiotropic morphological and antibiotic deficiencies result from mutations in a gene encoding a tRNA-like product in *Streptomyces coelicolor* A3(2). Genes Dev. **1**, 1305-1310.

Mulder, G.H., and Wessels, J.G.H. (1986). Molecular cloning of RNAs differentially expressed in monokaryons and dikaryons of *Schizophyllum commune* in relation to fruiting. Exp. Mycol. **10**, 214-227.

Schuren, F.H.J., and Wessels, J.G.H. (1990). Two genes specifically expressed in fruiting dikaryons of *Schizophyllum commune*: Homologies with a gene not regulated by mating type genes. Gene **90**, 199-205.

Springer, J., and Wessels, J.G.H. (1989). A frequently occurring mutation that blocks the expression of fruiting genes in *Schizophyllum commune*. Mol. Gen. Genet. **219**, 486-488.

Stringer, M.A., Dean, R.A., Sewall, T.C., and Timberlake, W.E. (1991). *Rodletless*, a new *Aspergillus* developmental mutant induced by directed gene inactivation. Genes Dev. **5**, 1161-1171.

Timberlake, W.E. (1990). Molecular genetics of *Aspergillus* development. Annu. Rev. Genet. **24**, 5-36.

Wessels, J.G.H., de Vries, O.M.H., Ásgeirsdóttir, S.A., and Schuren, F.H.J. (1991a). Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in Schizophyllum. Plant Cell **3**, 793-799.

Wessels, J.G.H., de Vries, O.M.H., Ásgeirsdóttir, S.A., and Springer, J. (1991b). The *thn* mutation of *Schizophyllum commune* which suppresses formation of aerial hyphae affects expression of the *Sc3* hydrophobin gene. J. Gen. Microbiol., in press.

Willey, J., Santamaria, R., Guijarro, J., Geistlich, M., and Losick, R. (1991). Extracellular complementation of a developmental mutation implicates a small sporulation protein in aerial mycelium formation by S. coelicolor. Cell **65**, 641-650.