

Growth control and polarization

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Filamentous fungi and yeasts both undergo polar growth. In *Saccharomyces cerevisiae*, where the mechanisms for polar growth are well-understood, polarity requires three steps: establishment of cortical markers specifying the site of bud emergence; relaying the bud site information via the Cdc42 Rho GTPase module; and recruitment of the morphogenetic machinery needed to remodel the cell surface to the specified site. Comparison of the genomes of *Aspergillus fumigatus*, *A. nidulans* and *A. oryzae* with that of *S. cerevisiae* show that the cortical markers are absent or poorly conserved, while the RhoGTPase signaling module and the morphogenetic machinery are highly conserved in the aspergilli. Genetic approaches to polarity using *A. nidulans* polarity mutants with defects in germ tube emergence (*swo* mutants) or branching (*ahb* mutants) will also be discussed.

Keywords branching, polarity

Introduction

While the conidia of *Aspergillus fumigatus* are ubiquitous in the environment and frequently inhaled, a competent immune system in most individuals generally clears them before they cause disease. Studies have not addressed early growth of *A. fumigatus* inside the immunocompromised host, but *in vitro* investigations have established the landmarks of early development [1]. After a conidium breaks dormancy, the reactivated *A. fumigatus* cell undergoes a brief period of isotropic expansion before a germ tube emerges. As is true for all filamentous fungi, later growth is highly polar, occurring exclusively at the tips of hyphae and branches. In invasive aspergillosis this highly polar tip growth allows *A. fumigatus* to invade blood vessels and tissue where it continues to grow, eventually causing hemorrhage and necrosis.

Polarity in *Saccharomyces cerevisiae* and aspergilli

Much has been learned about polar growth in the budding yeast *Saccharomyces cerevisiae*. In this fungus, cortical markers specify the site of bud emergence and

the Cdc42 Rho GTPase relays this information to the morphogenetic machinery, including actin and components of the secretory system. Genome comparisons reveal differences and similarities in polarity between *S. cerevisiae* and *A. fumigatus*, *A. nidulans* and *A. oryzae* [2]. The suite of cortical markers that specify the site of bud emergence in yeast (including Bud3p, Bud4p, Bud8p, Bud9p, Axl12p and Rax2p) are either absent or very poorly conserved in the aspergilli. However the signal relay consisting of the Cdc42p Rho GTPase and its associated GEF (Cdc24p), GAPs (Rga1p, Bem2p and Bem3p) and downstream effectors (Ste20p and Cla4p) are highly conserved. Of particular interest is the fact that the Cdc42p orthologues are essential in *Candida albicans* and *Ashbya gossypii*, both relatives of *S. cerevisiae*. However, they are nonessential in the more distantly related filamentous fungi *A. nidulans*, *Penicillium marneffeii* and *Magnaporthe grisea*. These filamentous fungi also contain the Rac GTPase, which is absent in yeasts. Deletion of Rac in *A. niger* and in *P. marneffeii* results in increased branching [2]. Though there is no experimental evidence yet, it seems possible that Rac might be at least partially redundant with Cdc42 and that this redundancy might explain why deletion of Cdc42 is not lethal in these fungi.

The positional signal that is relayed through the Rho GTPase modules ultimately affects the morphogenetic machinery that remodels the cell surface. Many

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components of the morphogenetic machinery are conserved between *S. cerevisiae* and the aspergilli. Not surprisingly, cytoskeletal elements such as actin and tubulins are highly conserved. Components of the polarisome, the complex responsible for organizing actin assembly in polar growth are also conserved, as are components of the exocyst, a protein complex important in secretion.

Filamentous fungi are not just tall yeasts

It is increasingly clear that germ tube emergence in filamentous fungi shares certain key features with bud emergence in *S. cerevisiae*, but important differences exist [3,4]. In filamentous fungi polar growth is persistent, while in yeast it is sporadic. In filamentous fungi polarity signals must be coordinated over an extended multicellular hypha, while in yeast those signals need only reach a single small cell. In filamentous fungi multiple axes of polarity are established simultaneously through branching, while in yeast a single axis of polarity is established through budding. Furthermore, in filamentous fungi the cell cycles of multiple nuclei must be coordinated with each other and with germ tube and branch emergence. Some of these key differences suggest that the basic polarity machinery is regulated differently in filamentous fungi.

Genetic approaches to polarity: *swo* mutants

Classical genetics is a powerful tool for dissecting cellular processes. In *A. nidulans* we have taken a genetic approach to polarity, generating mutants with defects in germ tube emergence and branching. The temperature-sensitive *swo* (swollen) mutants do not properly extend a germ tube at the restrictive temperature of 42°C [5]. Based on temperature-shift experiments, we have classified the eight *swo* mutants into groups with defects in polarity establishment (marking the spot for germ tube emergence) and those with defects in polarity maintenance (transducing signals or recruiting the morphogenetic machinery so that the germ tube extends). Here we will focus on two of these mutants, *swoF* and *swoA*. Previous work has shown that *swoF* is required for both polarity establishment and maintenance while *swoA* is required for polarity maintenance. At restrictive temperature *swoF* cells swell slightly but do not extend a germ tube, even though they continue nuclear division. Sequencing of the gene that complemented the *swoF* mutant showed that it encodes an N-myristoyl transferase (NMT) [6]. NMTs co-translationally add a fatty acid group to the N-terminus of target proteins. This myristoylation is

thought to increase the affinity of the target protein for the plasma membrane. Our working hypothesis is that modification by *swoF* is needed for either proper localization or function of a protein or proteins required for appropriate polar growth. In *S. cerevisiae*, the targets of NMTs include proteins involved in vesicle assembly, which is consistent with a role in polarity.

The conidium of a *swoA* mutant does not extend a germ tube, but swells dramatically, becoming as much as ten times wider than a wild-type conidium [5]. These mutants continue nuclear division filling up with 60 or more nuclei. Sequencing of the gene that complemented the *swoA* mutant showed that it encoded a protein mannosyl transferase (PMT) [7]. PMTs add mannose residues to serine or threonine of target proteins post-translationally in the endoplasmic reticulum. Our working hypothesis is that a protein or proteins needed for appropriate polar growth must be modified by *swoA* for proper localization or function. Known targets of PMTs in *S. cerevisiae* are cell wall or secreted proteins. Phenotypes of both the *swoF* and *swoA* mutants are consistent with defects in the morphogenetic machinery that is needed to remodel the cell surface.

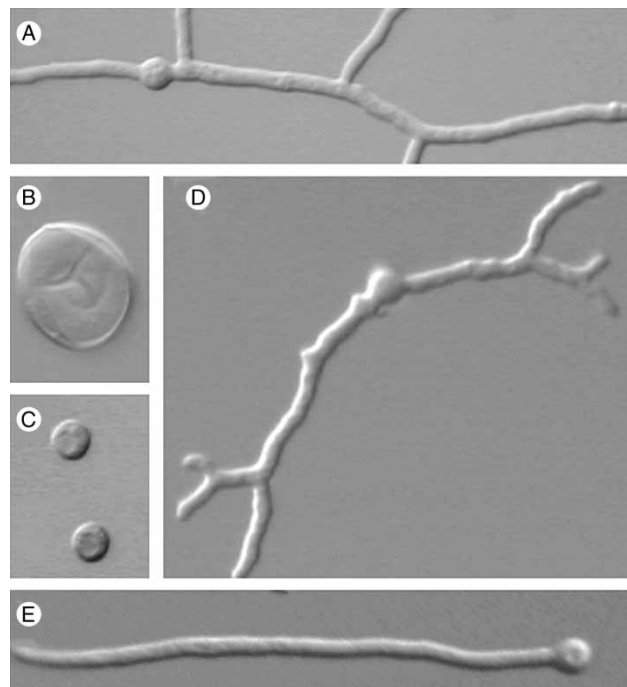


Fig. 1 *Aspergillus nidulans* mutant phenotypes at restrictive temperature. (A) wild-type; (B) *swoA*; (C) *swoF*; (D) *ahbA*; (E) *ahbB*. Panel A from *Euk Cell* 1:242; B from *Fungal Genet and Biol* 37:266; D, E from *Fungal Genet and Biol* 41:1001.

Genetic approaches to polarity: *ahb* mutants

In addition to the polar growth of the emerging germ tube, filamentous fungi show polar growth with the extension of branches. We have identified two temperature-sensitive abnormal hyphal branch (*ahb*) mutants [8]. The *ahbA* mutant makes few or no branches, while the *ahbB* mutant makes more branches than wild type. The nuclear number is also unusual in these mutants with *ahbA* showing fewer nuclei and *ahbB* showing more nuclei than wild type. Both mutants make abnormal conidiophores that appear to lack some layers, but form viable conidia. Sequencing of the gene that complements *ahbA* showed that it encodes NimX, the *A. nidulans* orthologue of the Cdc2 cyclin dependent kinase (Cdc28p in *S. cerevisiae*). Sequencing of the mutant allele showed that *ahbA1* was identical to a *nimX* allele previously identified by Osmani *et al.* [9]. This study did not include a description of the branching phenotype, most likely due to the fact that it focused on germlings too young to branch. That a cell cycle regulator would be involved in branching underlines the need to coordinate the cycles of multiple nuclei with polar growth in filamentous fungi.

Sequencing of the gene that complemented the *ahbB* mutant showed that it encodes a novel protein. Database searches revealed *ahbB* orthologues in other filamentous fungi, but none in yeast or other organisms. The only discernable homology was a small region shared with the heme-binding domain in a human P450 involved in sterol biosynthesis. We tested the *ahbB* mutants for sensitivity to the ergosterol biosynthesis inhibitor imidazole. While *ahbA* showed no increased sensitivity to the drug, *ahbB* was more sensitive than wild type consistent with some role in the synthesis of membranes.

The fact that *ahbB* has no apparent orthologues in branching illustrates that comparative genomics alone is unlikely to reveal all of the gene products needed for polar growth of filamentous fungi. Comparative approaches should be combined with other approaches such as genetics.

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