Polarity in filamentous fungi: establishment, maintenance and new axes

Michelle Momany

Germ tube emergence in filamentous fungi appears to be similar to bud emergence in yeast. Several key proteins (e.g. Cdc42, septins, Bni1 formin, Rho1 and Rho3) play common roles in polarity establishment and early polarity maintenance in both processes. Although germ tube extension, which can be thought of as extreme polarity maintenance, uses some of the same genes, they are likely to be regulated differently. Mutations in polarity maintenance genes often lead to a split tip in filamentous fungi, a phenotype without an analogue in yeast. Cell cycle regulation differs between tip splitting and subapical branching, but in both processes filamentous fungi maintain several axes of polar growth simultaneously.

Addresses
Department of Plant Biology, University of Georgia, Athens, GA 30602, USA; e-mail: momany@botany.uga.edu

Current Opinion in Microbiology 2002, **5**:580–585

1369-5274/02/$ – see front matter

Published online 30 October 2002

Abbreviations
GFP green fluorescent protein
IPC inositol phosphorylceramide

Introduction

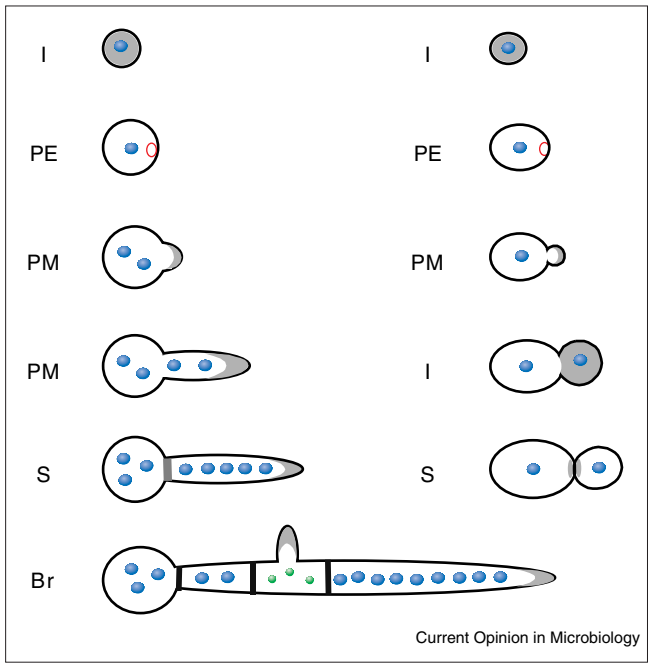
All organisms use polar — or asymmetric — growth; but few take it to the extreme that filamentous fungi do. After a brief period of isotropic — or symmetric — expansion, filamentous fungi switch permanently to apical extension (Figure 1) [1,2]. In this highly polar growth mode, a germ tube emerges. New material is added exclusively to the apex, resulting in an extending tubular cell, or hypha. Branches also emerge from the main hypha, establishing additional axes of polarity.

The budding yeast *Saccharomyces cerevisiae*, a close cousin to many filamentous fungi, also uses polar growth. Following a period of isotropic expansion as an unbudded cell, yeast switches to polar growth, and a bud emerges (Figure 1) [3]. But, yeast soon reverts to isotropic growth and the bud expands. Polar and isotropic growth alternate throughout the budding cycle.

The conceptual framework for fungal morphogenesis is deceptively simple. First, the quiescent spore breaks dormancy and expands isotropically. During isotropic expansion, the materials and machinery needed for making new plasma membrane and cell wall are dispersed throughout the cortex. Second, a spot is chosen for germ tube (or bud) emergence, in a process called 'polarity establishment'. Third, materials and machinery for making new plasma membrane and cell wall are redirected to the chosen spot, and the germ tube (or bud) emerges, in a process called 'polarity maintenance'. Fourth, in filamentous fungi, materials and machinery continue to be directed to the chosen spot and the germ tube extends apically. In budding yeast, materials and machinery are once more dispersed, and the bud enlarges. Finally, crosswalls, or septa, partition cells. In filamentous fungi, however, adjacent cells remain attached and a new spot is chosen, this time for branch emergence. Polar growth continues along the main hypha and begins in the branch. In yeast, mother and daughter cells separate and isotropic growth begins in both cells.

The proteins involved in breaking dormancy and isotropic growth vary among fungi and will not be discussed here (see [4,5•,6]). From studies in *Saccharomyces cerevisiae* we know that the number of proteins needed to establish and maintain polarity is staggering [3,7]. Choosing the right spot for polar growth is dependent on cortical cues, and timing of nuclear division must be coupled to bud formation [8]. Getting the materials and machinery for plasma

Figure 1



Morphogenesis in filamentous fungi and budding yeast. Gray shading shows areas of growth. I, isotropic expansion; PE, polarity establishment; PM, polarity maintenance; S, septation; Br, branching. Blue ovals represent interphase nuclei; green dots, mitotic nuclei; small open red circles, cortical markers.

Table 1

Polarity genes recently cloned from filamentous fungi.

Step*	Protein/fungus	Comments	References
PE	AgCdc42/ *A. gossypii*	Cdc42 Rho-GTPase, complements *S. cerevisiae cdc42*; Δ*Agcdc42*†: isotropic, delocalized actin patches, no GTE	[11••]
PE	AgCdc24/ *A. gossypii*	Cdc24 GEF necessary for activity of Cdc42, complements *S. cerevisiae cdc24*; Δ*Agcdc24*‡: identical to Δ*Agcdc42*	[11••]
PE/PM	CflA/ *P. marneffei*	Cdc42 Rho-GTPase; OE: no phenotype; dominant-negative: slow growth, decreased rate GTE, curled hyphae, swollen cells; dominant-activated: slow growth, increased rate of GTE, short, swollen cells	[12•]
PE/PM	Cdc3, -10, -11, -12/ *C. albicans*	Septins; septin–GFPs localize in ring at neck of yeast and pseudohypha, in cap at site of hyphal GTE and diffuse collar at base of germ tube; Δ*cdc10* and Δ*cdc11*: curved hyphae and aberrant GTE sites	[16••]
PE/PM	SwoF/ *A. nidulans*	*N*-myristoyl transferase, increases affinity of target proteins for the membrane; *swoF* temperature-sensitive mutant: no GTE, some elongated cells	[17]
PE/PM	AurA/ *A. nidulans*	Plasma membrane sphingolipid (IPC) synthase; Δ*aurA*‡: no GTE, cell cycle arrest; inhibition of AurA activity after GTE: tip splitting, actin cap disorganization	[18•]
PE	ArtA/ *A. nidulans*	Homologue of 14-3-3 scaffold protein, implicated in vesicle transport in other systems; OE: delayed GTE	[32]
PM/Br	SepA/ *A. nidulans*	Formin; Δ*sepA*: split tips and wide hyphae; SepA–GFP localizes to septa and hyphal tips simultaneously, with small, bright spot just behind tip, localizes to site of GTE and BrE	[24••]
PM	DigA/ *A. nidulans*	Homologue of Pep3 (Vps18) involved in vesicle sorting to vacuole; *digA* mutant: split tips, actin cap asymmetrical, clustered mitochondria and nuclei	[27]
PM	HogA/ *A. nidulans*	Homologue of HOG1 salt stress mitogen-activated protein kinase; Δ*hogA* : reduced hyphal extension and split tips in high salt	[28]
PM	AgRho1/ *A. gossypii*	GTPase important in cell wall integrity; Δ*Agrho1*: slow growth, high rate of cell lysis, enlarged, irregular hyphae	[11••]
PM	AgRho3/ *A. gossypii*	GTPase involved in coordinating actin and secretory apparatus; Δ*Agrho3*: slow growth, swelling with delocalized actin at hyphal tips	[11••]
Br	AspB/ *A. nidulans*	Septin, AspB localizes to BrE site pre-mitotically; *aspB* temperature-sensitive mutant shows increase in subapical branching	[31••]
Br	Pah1/ *P. anserina*	Homeobox protein; Δ*pah1*: reduced growth and subapical hyperbranching; *pah1* OE: swelling and twisting along hypha	[33]

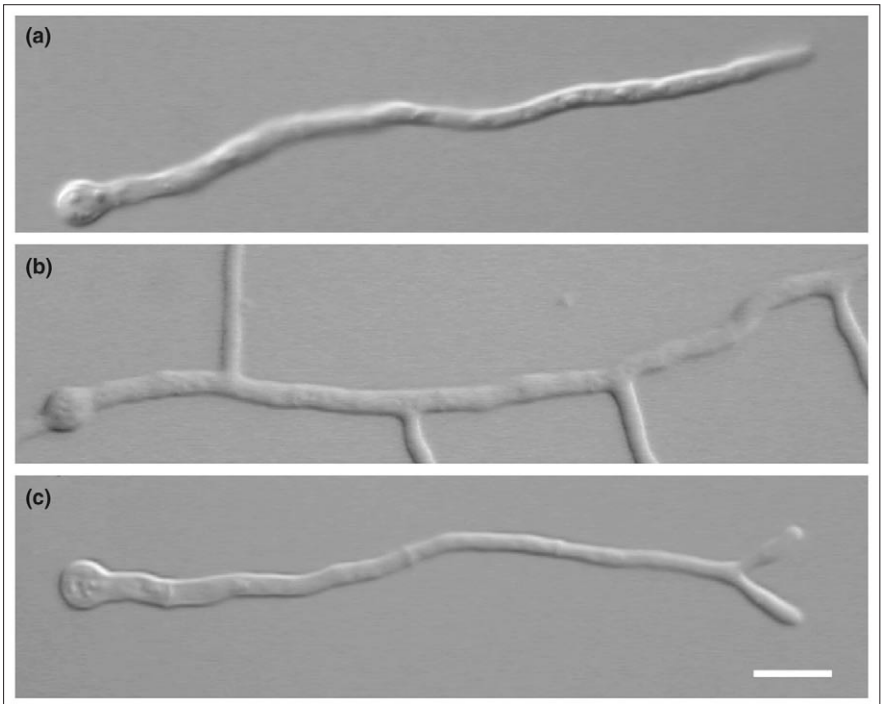
Only polarity-related genes from the recent literature (2001–2002) are included. In cases of pleiotropic phenotypes, only those related to polarity are listed. *Boundaries between steps not always clear (see text). Br, branching; BrE, branch emergence; GTE, germ tube emergence; PE, polarity establishment; PM, polarity maintenance. †Deletion is lethal. Early growth phenotype was examined in conidia from heterokaryon. ‡Deletion is lethal. Early growth phenotype was examined with conditional promoter. GEF, guanine nucleotide exchange factor; OE, overexpression.

membrane and cell wall synthesis to the right place on the cortex requires the secretory system and the actin cytoskeleton, and the existing wall must be kept intact as new wall is added.

Several recent papers (see Table 1) show that the central proteins in polarity establishment and maintenance in filamentous fungi are the same as those in budding yeast. Although few details are currently known, branching is also likely to have establishment and maintenance steps and to involve at least some of the same proteins.

In this review, I attempt to put polarity in filamentous fungi into the context of the simple morphogenetic framework outlined in Figure 1. Three important caveats should be kept in mind. First, the boundary between polarity establishment (marking the spot) and polarity maintenance (extending the germ tube) is often blurred. Although they are genetically separable [9], some gene products are involved in both steps. As germ tube emergence is an easily scored marker, many researchers have used it as a proxy for polarity establishment and even for the much earlier event of breaking dormancy. Further confusing the issue is the fact that some researchers use the term 'germination' to include not only breaking dormancy but also all of the events up to and including germ tube emergence. Second, because filamentous fungi always grow in a polar manner, mutations in 'housekeeping' genes may give phenotypes identical to mutations in polarity genes [10]. Hence, some of the recently identified 'polarity' genes may turn out to be related to polar growth only indirectly; however, it is also possible that metabolic inputs might regulate polar growth. Third, polarity establishment in filamentous fungi is studied in recently dormant spores, while in *S. cerevisiae* it is studied in actively budding cells. This difference in developmental context is likely to lead to differences in regulation. Despite these problems, classifying proteins as important for polarity establishment, polarity maintenance

Figure 2



Polarity in *A. nidulans*. **(a)** Germ tube elongation. **(b)** Subapical branching. **(c)** Tip splitting. Scale bar = 10 μm. Figure courtesy of Brian D Shaw.

or branching is a useful — if somewhat artificial — device for discussion.

Polarity establishment: choosing and marking the spot

In *S. cerevisiae*, the location of the Rho-GTPase Cdc42 drives polarity [3]. Cdc42 activates proteins that regulate and organize the actin cytoskeleton, which is required for directed plasma membrane and cell wall construction. Cdc42 is found exclusively in areas of growth. It is dispersed during isotropic expansion and restricted to the bud during polar extension.

Cdc42 also appears to drive polarity in filamentous fungi. In the filamentous ascomycete *Ashbya gossypii*, deletion of *AgCDC42* is lethal; however, by examining early growth of cells derived from heterokaryons, Wendland and Phillipsen [11••] showed that *Agcdc42*-null alleles resulted in isotropically expanding cells and delocalized actin. In *Penicillium marneffei*, overexpression of a dominant-negative allele of the *CDC42* homologue *cflA* resulted in delayed germ tube emergence, whereas overexpression of a dominant-activated allele caused accelerated germ tube emergence [12•].

In *S. cerevisiae*, Cdc42 is recruited to a specific region of the plasma membrane by proteins that interact with the Bud protein cortical markers laid down during the preceding round of budding [3,13]. One of the consequences of Cdc42 recruitment and activation is the assembly of a septin ring at the future site of bud emergence [14]. The septins eventually form a scaffold that spans the mother-bud neck and organizes the division plane [15]. Bud proteins localize to the septin scaffold and several are left behind after cytokinesis. These Bud proteins serve as cortical markers, guiding the recruitment of Cdc42 in the next round of budding. Recent work in *Candida albicans* showed that septin knockouts had defects in selecting sites for germ tube emergence and that septin–GFP fusions formed collars at bases of emerging germ tubes [16••]. Although the nature of cortical markers for germ tube site selection is completely unknown, this result suggests that germ tube site selection might be similar to bud site selection.

Two recent reports in *Aspergillus nidulans* [17,18•] suggest connections between polarity establishment and the plasma membrane. In the first, the *swoF* gene, identified as a mutant defective in polarity establishment, was complemented by an *N*-myristoyl transferase [17]. *N*-myristoylation is thought to increase the affinity of its target protein for the plasma membrane, and several *N*-myristoylated proteins in *S. cerevisiae* are important in the secretory pathway [19]. Presumably, a target modified by SwoF is needed for polarity establishment. Cdc42 requires a similar lipid modification — geranylgeranylation — for polarity in *S. cerevisiae* [20,21]. The second paper connecting polarity of filamentous fungi to membranes examines the synthesis of inositol phosphorylceramide (IPC), a sphingolipid found in eukaryotic membranes [18•]. Inactivation of the *A. nidulans* IPC synthase AurA blocked germ tube emergence. Cheng *et al.* [18•]

suggest that IPC synthesis might be important in forming lipid rafts, sphingolipid-rich plasma membrane domains thought to act by selective inclusion of specific membrane-bound proteins [22]. Cheng *et al.* [18•] further suggest that lipid rafts in the tips of fungal hyphae might sequester polarity proteins. Although there is no experimental evidence, this is an attractive speculation for a link between the plasma membrane and the polarity apparatus.

Polarity maintenance: germ tube emergence and extension

Once the correct spot for germ tube emergence has been chosen and marked, the cell must polarize actin to bring materials to the site. Formin proteins act as adaptors, linking signals from Rho-GTPases to the actin cytoskeleton. Recent work in *S. cerevisiae* shows that the formin protein Bni1, a member of the tip-localized polarisome complex, is needed for actin-cable formation and that its overexpression causes extra actin-cable-like filaments to form ([23]; see also Schott *et al.*, this issue). SepA, the *A. nidulans* homologue of Bni1, localizes to sites of nascent germ tube emergence and co-localizes with actin at sites of septation and polar growth simultaneously [24••]. In contrast with results in *S. cerevisiae*, SepA is not required for actin-cable localization at hyphal tips, although actin is required for the localization of SepA. Interestingly, a bright spot of SepA–GFP is seen just behind a cap of SepA–GFP at the tip of growing hyphae [24••]. This is the same position occupied by the Spitzenkörper, a dense collection of vesicles, actin and microtubules thought to organize vesicles bound for fusion with the membrane at the hyphal tip [25,26]. Deletion of *sepA* results in hyphae that do not form septa and that split at the tips.

This splitting at the tip, often called 'dichotomous branching', is also seen in several other polarity-maintenance mutants (Figure 2c; Table 1). The *digA* mutant grows slowly and splits at the tip. First identified in a screen for nuclear movement mutants in *A. nidulans*, DigA is homologous to Pep3/Vps18, a *S. cerevisiae* protein involved in sorting vesicles bound for the vacuole [27]. The *digA* actin cap is asymmetric, unlike the usual uniform actin cap found at the tips of wild-type hyphae. Similarly, disrupting the activity of the IPC synthase AurA after polarity has been established results in disorganization of the apical actin cap and splitting of the tip cell [18•]. Deletion of the *A. nidulans hogA* salt stress gene also causes tip splitting in high-solute medium [28]. Han and Prade [28] suggest that this tip splitting may result from the inability of *hogA* mutants to maintain the positive turgor pressure needed for apical extension. Similarly, it has been suggested that hyperbranching might result whenever production of new cell wall material outpaces the capacity of the existing tip to organize it [29]. Defects in polarity maintenance would be expected to cause such an imbalance between synthesis and tip capacity.

During polarity maintenance in *S. cerevisiae*, the GTPase Rho1 stimulates cell wall synthesis to maintain cellular integrity, and Rho3 is involved in targeting secretory vesicles. Deletion of the homologous genes in *A. gossypii* suggests that they play a similar role in filamentous fungi [11••]. Deletion of *AgRHO3* results in swollen hyphal tips, and deletion of *AgRHO1* results in mis-shapen cells that eventually lyse (Table 1).

Many of the gene products involved in establishing polarity are also involved in maintaining it. The *P. marneffei cflA* (Cdc42) dominant alleles that alter germ tube emergence also show swollen hyphal cells. The *cflA* dominant-negative allele also shows unusually curved hyphae. Similar curved hyphae are seen in *C. albicans* septin mutants undergoing hyphal growth and septin–GFP fusions localize to the tips of emerging germ tubes as well as forming collars at their bases [16••].

Polarity along new axes: branching

After a period of tip growth, filamentous fungi establish new subapical axes of polarity by sending out branches from the main hypha (Figure 2b). Although the distinction is often not recognized, subapical branching probably differs substantially from the tip splitting described above for *sepA*, *digA*, *aurA* and *hogA*. In *A. nidulans*, only the apical compartment of the hypha remains mitotically active [30]. Subapical compartments are arrested in interphase unless a branch forms, at which time mitosis is reactivated and nuclei populate the new branch. Since subapical compartments are arrested in interphase and tips are not, subapical branching and tip splitting must differ, at least in terms of cell cycle regulation.

To date, only two markers of subapical branch formation are known in filamentous fungi. The *A. nidulans* formin SepA localizes to the sites of germ tubes and branches just before they emerge [24••]. The septin AspB also localizes to the branch site before branch emergence [31••]. However, AspB does not localize to sites of germ tube emergence, suggesting that there are differences between the two processes. In *S. cerevisiae*, septins form part of the morphogenesis checkpoint, coordinating the activation of mitosis after bud emergence [8]. Perhaps the septin AspB has an analogous role in reactivating mitosis in subapical compartments in *A. nidulans*? Genetic interactions have been reported between septins and Bni1 in *S. cerevisiae*, leading Sharpless and Harris [24••] to suggest that septins in *A. nidulans* might coordinate localization and activation of the SepA formin.

Conclusions

In its early stages, germ tube emergence appears to be similar to bud emergence. Several key proteins (e.g. Cdc42, septins, Bni1 formin, Rho1 and Rho3) play common roles in polarity establishment and early polarity maintenance in filamentous fungi and yeast. There are no data on cortical markers within the spores of filamentous fungi, although such markers seem likely to exist and to function in a manner analogous to Bud proteins.

The later stage of polarity maintenance (germ tube elongation) probably differs substantially from bud emergence. Defects in polarity maintenance in filamentous fungi frequently lead to splitting of the tip cell, a phenotype with no analogue in budding yeast. It is not clear if the position of tip splitting is directed by cortical markers or if it is the result of disruption of the tip polarity apparatus. Perhaps filamentous fungi are more tolerant of disturbance of the tip polarity machinery than are budding yeast.

Subapical branching presumably also has polarity establishment and maintenance steps and is likely to use some of the proteins involved in germ tube emergence. In *A. nidulans*, emergence of the first subapical branch occurs in a predictable position (BD Shaw and M Momany, unpublished data) and so is likely to be directed by as yet unknown cortical markers. It will be of great interest to see how the polarity establishment and maintenance machinery differ among germ tube emergence, tip splitting and subapical branching. It is not clear which cortical markers determine the germ tube, split tip or branch position; nor is it clear if nuclear division is coordinated between individual branches and the main hypha. Understanding the differences among germ tube emergence, tip splitting and subapical branching may allow us to understand how filamentous fungi coordinate multiple axes of polar growth simultaneously.

Update

The *swoA* mutant of *A. nidulans* has been previously shown to be defective in polarity maintenance [9]. Large isotropic cells developed when *swoA* was incubated at a restrictive temperature, and upon a shift to a permissive temperature, multiple germ tubes rapidly emerged from one hemisphere of cells. Recent work has shown that the *swoA* gene encodes a protein *O*-mannosyltransferase (PMT) [34]. PMTs are responsible for the first step in *O*-glycosylation, the co-translational addition of mannose to specific serine or threonine residues of target proteins. In *S. cerevisiae*, *O*-glycosylated proteins are either secreted or cell wall localized. It is likely that a protein needed for polarity maintenance must be *O*-glycosylated by SwoA for proper function or localization.

Acknowledgements

I thank Sarah Lea McGuire, Claiborne Glover, Greg May, Steve Harris and members of the Momany laboratory for comments on the manuscript. Work in my laboratory on polarity and septins is supported by DOE Biosciences (grant DE-FG02-97ER20275) and the National Science Foundation (grant MCB9904629).

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