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Review

Polarity in filamentous fungi: moving beyond the yeast paradigm

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Abstract

Filamentous fungi grow by the polar extension of hyphae. This polar growth requires the specification of sites of germ tube or branch emergence, followed by the recruitment of the morphogenetic machinery to those sites for localized cell wall deposition. Researchers attempting to understand hyphal morphogenesis have relied upon the powerful paradigm of bud emergence in the yeast *Saccharomyces cerevisiae*. The yeast paradigm has provided a useful framework, however several features of hyphal morphogenesis, such as the ability to maintain multiple axes of polarity and an extremely rapid extension rate, cannot be explained by simple extrapolation from yeast models. We discuss recent polarity research from filamentous fungi focusing on the position of germ tube emergence, the relaying of positional information via RhoGTPase modules, and the recruitment of morphogenetic machinery components including cytoskeleton, polarisome and ARP2/3 complexes, and the vesicle trafficking system.
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1. Introduction

The formation of polarized hyphae is the defining feature of filamentous fungi, allowing them to efficiently colonize and exploit new substrates. Polarity establishment occurs at two distinct points in the vegetative growth of most filamentous fungi: primary germ tube emergence from the spore and branch emergence from the hypha. Polarity establishment presumably requires the specification of sites, followed by the recruitment of the morphogenetic machinery for localized cell wall deposition. Once established, polarity must be maintained during hyphal extension. Polarity maintenance is dependent upon the sustained localization of the morphogenetic machinery at the tips of extending hyphae. For many years, researchers attempting to understand hyphal morphogenesis have relied upon the powerful paradigm of bud emergence in the yeast *Saccharomyces cerevisiae*. While this paradigm provides a useful framework, several features of hyphal morphogenesis cannot be explained by simple extrapolation from yeast models. Unique features of hyphal organization and growth, such as the ability to maintain multiple axes of polarity and an extremely rapid extension rate, imply the existence of novel regulatory modes. In addition, recent genetic screens in *Aspergillus nidulans* and *Neurospora crassa* have uncovered several "pioneer" polarity proteins along with others whose importance in polarity would not have been predicted based on characterization of their yeast orthologs. Accordingly, this is an appropriate time to summarize our current understanding of how filamentous fungi establish and maintain hyphal polarity. Besides providing a context for future studies, our goal is to highlight important differences between filamentous fungi and the well-known yeast models.

1.1. The yeast paradigm

The mechanisms underlying polarized morphogenesis have been extensively characterized in the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*. A summary of these mechanisms highlights several important features that are likely to apply to filamentous fungi as well.

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In *S. cerevisiae*, cortical markers working with a Ras-related GTPase module specify bud site position (Pruyne and Bretscher, 2000b). Mating site position is specified by an occupied mating receptor complexed with a heterotrimeric G-protein. In both cases, the conserved Cdc42 GTPase module transduces positional information to several effectors that promote localized organization of the morphogenetic machinery. Similarly, in *S. pombe*, cell ends are thought to be specified by cortical markers working with a Ras GTPase, and positional information is transduced through the Cdc42 GTPase module to the morphogenetic machinery (Chang and Peter, 2003). In both *S. cerevisiae* and *S. pombe*, the morphogenetic machinery includes the actin cytoskeleton and the secretory apparatus; however, in *S. pombe*, microtubules also play an essential role in transporting cortical markers to cell ends (Snaith and Sawin, 2003). Finally, although there are distinct differences in the mechanisms that specify the septation site, both budding and fission yeast cells re-localize the morphogenetic apparatus from the poles to this site in order to undergo cytokinesis.

Several relevant concepts have emerged from the yeast models. First, unique cortical markers specify sites of polarized morphogenesis. The uniqueness of the markers is underscored by the apparent absence of the *S. cerevisiae* bud site markers Bud3p, Bud8p, and Bud9p in the proteome of *S. pombe*. Second, the conserved Cdc42p signaling module relays positional information to the morphogenetic machinery. Third, scaffolding proteins, such as formins and septins, play an important role in organizing the morphogenetic machinery at sites of polarized growth.

2. Positional information: choosing the spot

In *S. cerevisiae*, the cortical landmark proteins that generate the positional signal are well characterized (Pringle et al., 1995). These include markers that define the axial budding pattern (Bud3p, Bud4p, and Axl2p) or the bipolar budding pattern (Bud8p, Bud9p, and Rax2p). Several of these markers (Axl2p, Bud8p, and Bud9p) are cell wall proteins whose delivery to the cell surface is tightly coordinated with cell cycle progression and changes in the pattern of localized secretion (Lord et al., 2000; Schenkman et al., 2002). Strikingly, the recent release of several complete fungal genome sequences shows that these markers are poorly conserved in *A. nidulans* and other filamentous fungi (Axl2p, Bud4p, and Rax2p) or completely absent (Bud3p, Bud8p, and Bud9p) (Table 1). One exception is the recent identification of a Bud3p homologue in the filamentous ascomycete *A. gossypii* (Wendland, 2003). However, it should be noted that *A. gossypii* is thought to have recently diverged from *S. cerevisiae*, and the two fungi share large stretches of chromosomal synteny (Brachat et al., 2003). The lack of strong positional marker homologues might indicate that filamentous fungi do not use landmark proteins, however several observations suggest that germ tube and branch emergence are subject to spatial regulation. In both *A. nidulans* and *A. gossypii*, spore germination occurs in a bipolar fashion (Harris, 1999; Wendland and Philippsen, 2001) that, at least in *A. nidulans*, depends on the integrity of the cytoskeleton. Temperature shift experiments with the *A. nidulans swoA1* mutant suggest that only certain regions of the spore surface are competent for germ tube emergence (Momany et al., 1999). The selection of branch sites is clearly non-random in *A. gossypii* (Knechtle et al., 2003), and perhaps also in other filamentous fungi (Walther and Wendland, 2003). How, then, might filamentous fungi generate positional information? Below, we consider three models for specifying the sites of polarized growth: polarization directed by cortical landmarks, activated receptors, or stochastic fluctuations.

2.1. Model 1: cortical landmarks

Filamentous fungi might simply use a unique set of cortical markers to specify positional information in a

Table 1
Homologues of *S. cerevisiae* positional landmarks in *A. nidulans*

Landmark protein[a]	*A. nidulans* homologue[b]	Comments
Bud3p	No hit	
Bud4p	AN6150.1	Homology confined to C-terminal Pleckstrin homology (PH) domain
Axl2p	AN1359.1	Homology confined to N-terminal CADG domains, which are reported to bind Ca^{2+}
Bud8p	No hit	
Bud9p	No hit	
Rax2p	AN6658.1	Homology distributed across entire protein; homologues present in other filamentous fungi

[a] Query sequences are from *S. cerevisiae* and were obtained from *Saccharomyces* Genome Database (http://www.yeastgenome.org) using the Global Gene Hunter search option.

[b] BLASTp searches were performed against the *A. nidulans* database (http://www-genome.wi.mit.edu/annotation/fungi/aspergillus/index.html) using default parameters. The top hit with an *e* value lower than $1e^{-3}$ is indicated.

system analogous to that of yeast. Spore production potentially furnishes asymmetric marks that could be exploited for this purpose. For example, conidiation in *A. nidulans* produces chains of spores from a single phialide by budding (Timberlake, 1980). Accordingly, each spore possesses a birth scar capable of marking the subsequent polarization site. Moreover, as recently described in *A. gossypii* (Knechtle et al., 2003), markers originally deposited at the hyphal tip could ultimately specify sites of branch emergence. In molecular terms, what could serve as a cortical landmark in filamentous fungi? Remnants of the morphogenetic machinery, such as the septins or plasma membrane microdomains, could serve this function, as was suggested for *A. gossypii* Bud3p (Knechtle et al., 2003). Alternatively, a species-specific cell wall protein could mark polarization sites as Bud8p and Bud9p do in *S. cerevisiae*.

2.2. Model 2: activated receptors

Yeast cells mate via a polarized projection formed in response to a pheromone gradient secreted from a partner of opposite mating type (Chang and Peter, 2003). Pheromone receptors cluster at the point on the cell surface experiencing the highest level of pheromone, and trigger activation of an associated heterotrimeric G protein that provides a positional landmark for the recruitment of the Cdc42 signal transduction module (Jackson et al., 1991). A necessary prerequisite for this chemotropic response is the ability to override the positional signals from landmark proteins, thereby allowing activated receptors to commandeer the morphogenetic machinery. In *S. cerevisiae*, pheromone-dependent stabilization of an adaptor protein that interacts with the free Gβ subunit and the Cdc42 GTPase module appears to override the cortical bud site markers (Shimada et al., 2000).

In filamentous fungi, it is possible that sites of germ tube and branch emergence could be specified by positional signals emanating from occupied receptors. Filamentous fungi are clearly capable of responding to pheromones during mating (Coppin and Debuchy, 2000). Further, genomic analyses suggest that much of the machinery needed for the pheromone response in mating yeast cells is present in filamentous fungi (Dyer et al., 2003). Filamentous fungi might also mark polarization sites by receptor clustering in response to signals other than pheromones. For example, saprophytic fungi might use nutrient receptors to specify the polarization site, while plant pathogens might use receptors that bind to specific plant surface components.

2.3. Model 3: spontaneous polarization

Many cell types are capable of establishing an axis of polarity in the complete absence of any cortical landmark (Wedlich-Soldner and Li, 2003). *Saccharomyces cerevisiae* cells lacking cortical landmarks and mating pheromone gradients still undergo polarized morphogenesis (Wedlich-Soldner et al., 2003). Mathematical models predict that such spontaneous polarization could be achieved if a local positive feedback loop amplifies a stochastic fluctuation in Cdc42p signaling and a long-range inhibitor blocks signaling from adjacent sites. Positive feedback requires the assembly of actin cables, which transport additional Cdc42 module components to the polarization site and so reinforce the original signal. Long-range inhibition of the signal appears to involve endocytic retrieval of signaling components from regions flanking the polarization site. As suggested by Wedlich-Soldner et al. (2003), the role of cortical landmarks may be simply to impose a bias on a polarity axis that was initially established in response to random signal variation.

In filamentous fungi, stochastic signal fluctuation might specify the site of first germ tube emergence from the spore. This random polarization event might then trigger spatial regulatory mechanisms that direct the second germ tube emergence from a site on the opposite side of the spore, thereby generating the observed bipolar pattern (Harris, 1999; Wendland, 2001; Wendland and Philippsen, 2000). Similarly, sites for branch emergence could be chosen by random variation in signal intensity. For both germ tube and branch emergence, amplification of the initial stochastic GTPase signal might require the localized assembly of actin cables, as is true for bud emergence in yeast. However, microtubules have a critical role in regulating the direction of hyphal extension (Riquelme et al., 1998), suggesting that they might also be involved in generating the positive feedback loop.

3. Rho GTPase modules: relaying the signal

In *S. cerevisiae*, once positional information is established, it is transduced via the Cdc42 Rho GTPase module to the morphogenetic machinery (Pruyne and Bretscher, 2000b). Like other Rho GTPases, Cdc42p functions as a molecular on/off switch, relaying signals in its active GTP-bound form, but not in its inactive GDP-bound form. Consistent with this central role in transducing polarity information, Cdc42p accumulates at the plasma membrane in areas of polar growth (Richman et al., 2002; Ziman et al., 1993), and specific mutations of *CDC42* prevent polarization and bud emergence (Adams et al., 1990), while other mutations cause the emergence of multiple buds (Richman and Johnson, 2000). The guanine-nucleotide exchange factor (GEF) Cdc24p promotes the exchange of GDP for GTP, thus stimulating the formation of active Cdc42-GTP. It is the Cdc24p GEF that is first recruited to the

site of polar growth specified by cortical markers (Bender, 1993; Zheng et al., 1995). Once in position, Cdc24p recruits Cdc42-GDP and promotes the exchange of GDP for GTP. Active Cdc42-GTP in turn recruits the Bem1p adaptor protein that appears to stabilize Cdc24p at the polarization site (Butty et al., 2002; Gulli et al., 2000; Sohrmann and Peter, 2003). The GTPase activating proteins (GAPs) Rga1p, Bem2p, and Bem3p stimulate the hydrolysis of GTP to GDP, thus inactivating Cdc42p and ending polar growth. Because yeast cells with mutated GAPs do not efficiently shut down Cdc42p, they form dramatically elongated, hyperpolarized buds (Smith et al., 2002; Ziman et al., 1991). Cdc42-GTP transduces signals to multiple downstream effectors that in turn signal to the actin cytoskeleton. Among the best-characterized effectors are the p21-activated kinases (PAKs) Ste20p and Cla4p, which amongst other functions may control actin cytoskeletal organization by regulating the polarisome (Goehring et al., 2003).

Unlike the positional landmarks, Cdc42 module proteins are highly conserved in *A. nidulans* (Table 2) and other filamentous fungi. Recently, homologues of Cdc42p and Cdc24p have been cloned from several dimorphic and filamentous fungi (Table 3). In *A. gossypii*, deletion of *CDC42* or *CDC24* is lethal, however it is possible to observe the early growth phenotype of *ΔAgcdc42* and *ΔAgcdc24* conidia from heterokaryons (Wendland and Philippsen, 2001). In both cases null mutants show only isotropic growth and no germ tube emergence. Similarly *CDC42* and *CDC24* are essential in *Candida albicans*, but in null mutants with either *CDC42* or *CDC24* expressed behind inducible promoters, yeast cells cannot transition to hyphae and are avirulent in mice (Bassilana et al., 2003; Ushinsky et al., 2002). In contrast, strains possessing a dominant negative allele of the *Penicillium marneffei CDC42* homologue *cflA* are viable (Boyce et al., 2001). Similarly, deletion of *CDC42* in the polymorphic pathogen *Wangiella dermatitidis* is not lethal, and *ΔWdcdc42* strains make hyphae and yeast with only slight morphological abnormalities (Ye and Szaniszlo, 2000). In the rice pathogen *Magnaporthe grisea*, mutants deleted for *CDC42* are viable and display no obvious defects in hyphal growth (S. Wu, personal comunication; Zhao et al., 2001). In the maize pathogen *Ustilago maydis* deletion of the *CDC24* GEF *don3* causes no defect in filamentous growth and only subtle defects in yeast growth (Weinzierl et al., 2002).

Table 2
Homologues of *S. cerevisiae* Cdc42 module proteins in *A. nidulans*

Protein[a]	*A. nidulans* homologue[b]
Cdc42p	AN7487.1
YlRac1	AN4743.1
Cdc24p	AN5592.1
Rga1p	AN1025.1
Bem2p	AN4745.1
Bem3p	AN5787.1
Ste20p	AN2067.1
Cla4p	AN8836.1

[a] Query sequences are from *S. cerevisiae* and were obtained from *Saccharomyces* Genome Database (http://www.yeastgenome.org) using the Global Gene Hunter search option except for Rac1, where the query sequence is *Y. lipolytica* Rac1p obtained from GenBank.

[b] BLASTp searches were performed against the *A. nidulans* database (http://www-genome.wi.mit.edu/annotation/fungi/aspergillus/index.html) using default parameters. The top hit with an *e* value lower than $1e^{-24}$ is indicated.

P. marneffei, *W. dermatitidis*, and *M. grisea* possess a second Rho GTPase, Rac. Rac appears to be especially important in filamentous fungi; there are no Racp homologues in the yeasts *S. cerevisiae* or *S. pombe* and Rac clearly plays a role in hyphal growth that Cdc42 does not. *P. marneffei* strains deleted for the Rac homologue *cflB* make conidiophores and hyphae that do not polarize properly (Boyce et al., 2003) and strains of the dimorphic yeast *Yarrowia lipolytica* deleted for *RAC1* lose the ability to make true hyphae (Hurtado et al., 2000).

The only Cdc42 GAP studied thus far from a dimorphic or filamentous fungus is *Agbem2* from *A. gossypii* (Wendland and Philippsen, 2000). Because GAPs stimulate GTP hydrolysis and thus stop signal transduction by the Cdc42 module, they are thought to be especially important in regulating the timing and position of polar growth. In the *ΔAgbem2* mutant extra germ tube initials emerge from the germ cell and dichotomous tip branching increases consistent with a role for the AgBem2 GAP in temporal and spatial regulation of polarization. The only downstream effector of Cdc42 that has been characterized in filamentous or dimorphic fungi is the PAK Ste20. Deletion of both copies of the *C. albicans STE20*, *cst20*, causes a reduction in hyphal formation on some media and reduced virulence (Kohler and Fink, 1996; Leberer et al., 1996). On the other hand, deletion of the *U. maydis STE20*, *don3*, does not affect filamentous growth of the dikaryon, but does cause cell separation defects in the yeast (Weinzierl et al., 2002).

Based on this limited data, a few trends are emerging. The Cdc42 GTPase module is needed for germ tube emergence and is essential in those dimorphic and filamentous fungi most closely related to *S. cerevisiae* (*A. gossyippi* and *C. albicans*). However, in more distantly related fungi (*A. nidulans*, *M. grisea*, *P. marneffei*, *U. maydis*, and *W. dermatitidis*), *CDC42* does not appear to be essential. The Rac GTPase, which is nonessential in all filamentous fungi so far examined, might partially compensate for loss of Cdc42 in these cases, though there is no experimental evidence to support redundancy between Cdc42 and Rac in filamentous fungi. The phenotypes displayed by *rac* mutants and its notable absence from monomorphic yeasts argue that Rac's major role is in hyphal growth.

Table 3
Homologues of Cdc42 module proteins from filamentous and dimorphic fungi

Fungus Gene name	Comments[a]	Reference
Rho GTPase: CDC42		
Ashbya gossypii AgCDC42	Complements *S. cerevisiae cdc42*; *Δ*Agcdc42:[b] isotropic, delocalized actin patches, no GTE	(Wendland and Philippsen, 2001)
Aspergillus nidulans modA	OE dom hyperactive *modA*: delayed GTE, swollen hyphae, no conidia	(Harris and Lee, unpublished)
Candida albicans CaCDC42	ΔΔ*CaCDC42* + inducible *CaCDC42* in repressing conditions. Yeast: arrest large, round, unbudded, and no transition to hyphae; OE: no effect; OE Dom hyperactive: multibud; Hyphae: GTE, but short; Dom mutants: tip branching	(Ushinsky et al., 2002)
	Δ*CaCDC42*/pMet*CDC4:* no invasive hyphae, yeast normal; avirulent in mice	(Bassilana et al., 2003)
Magnaporthe grisea MgCDC42	Δ*MgCDC42* viable; conidial shape abnormal; saprophytic filamentous hyphae normal, infection hyphae bulbous, less virulent	(Zhao et al., 2001; S.Wu, pers. comm.)
Penicillium marneffei cflA	OE: no phenotype; Dom-negative: slow growth, decreased rate GTE, curled, swollen hyphae; Dom-active: slow growth, increased GTE, short, swollen cells. Needed for polarization of yeast and hyphae	(Boyce et al., 2001)
Suillus bovines SbCDC42	RNA and protein expressed in vegetative and ectomycorrhiza-forming hyphae; co-localized with actin at hyphal tips	(Gorfer et al., 2001)
Wangiella dermatitidis WdCDC42	Complements *S. cerevisiae cdc42-1*; Δ*WdCDC42*: viable, yeast slim, hyphal microcolonies; Dom active: induces isotropic growth, represses hyphae	(Ye and Szaniszlo, 2000)
Rho GTPase: RAC		
Aspergillus niger racA	Δ*racA:* viable, accelerated 2°GTE and increased apical branching, compact, reduced conidiation, actin localization normal	(Ram et al., 2001; A.F.Ram pers. comm)
P. marneffei cflB	Δ*cflB:* yeast cells normal, hyphae and conidiophores defective polarization, hyperbranch and tip branch. GFP::CflB co-localized with actin at septa and hyphal tips	(Boyce et al., 2003)
S. bovines SbRAC1	RNA expressed in vegetative and ectomycorrhiza-forming hyphae	(Gorfer et al., 2001)
Yarrowia lipolytica YlRAC1	Δ*Ylrac1*: yeast round, pseudohyphae, no true hyphae; actin polarized; *YlRAC1* RNA increases in yeast to hyphae transition	(Hurtado et al., 2000)
GEF: CDC24		
A. gossypii AgCDC24	Complements *S. cerevisiae cdc24*; *ΔAgcdc24*[b] identical to *ΔAgcdc42*	(Wendland and Philippsen, 2000)
C. albicans CaCDC24	*ΔCaCDC24*/ pMet*CDC24* cannot form invasive hyphae, yeast normal; avirulent in mice	(Bassilana et al., 2003)
Ustilago maydis don1	*don1* mutant and *Δdon1*: yeast separation and bud site selection defects; normal filamentous growth of dikaryon; interacts with *UmCDC42* in 2 hybrid	(Weinzierl et al., 2002)
GAP		
A. gossypii AgBEM2	*ΔAgbem2:* swollen germ cells, possible extra GTE from germ cell, increased tip branching	(Wendland and Philippsen, 2000)
PAK: STE20		
C. albicans CST20	*ΔΔcst20* reduced hyphae on some media; reduced virulence.	(Kohler and Fink, 1996; Leberer et al., 1996)
U. maydis don3	*don3* mutant and *Δdon3:* yeast separation defect; normal filamentous growth of dikaryon; Interacts with *UmCDC42* in 2 hybrid	(Weinzierl et al., 2002)

[a] *Abbreviations:* Dom, dominant allele; GTE, germ tube emergence; OE, Overexpression.
[b] Essential gene, deletion early phenotype was observed in conidia from heterokaryons.

4. The morphogenetic machinery: generating polarity

Positional information is relayed by the GTPase signaling modules to the morphogenetic machinery that remodels the cell surface. Undoubtedly, the bulk of the morphogenetic machinery involved in the establishment and maintenance of hyphal polarity awaits discovery. Nonetheless, those elements that have been described offer significant insight into the mechanics of polarized morphogenesis. These elements include the cytoskeleton and associated proteins, as well as the vesicle trafficking complexes that direct the formation, transport, and fusion of secretory vesicles with target membranes.

4.1. Actin

In yeast, actin is absolutely required for polarized morphogenesis. During bud growth, actin cables extend from the mother cell into the bud, and actin patches

localize to growth sites on the bud surface (Adams and Pringle, 1984). In almost all cases, mutations that disrupt organization of the actin cytoskeleton prevent the formation of buds and mating projections (Pruyne and Bretscher, 2000a). The use of actin-depolymerizing agents has shown that actin filaments are also required for the establishment and maintenance of hyphal polarity in filamentous fungi (Heath, 1994; Torralba et al., 1998). In addition, *A. nidulans* mutations that affect actin organization or its utilization as a "vesicle highway" display dramatic polarity defects (Harris, 1999; McGoldrick et al., 1995). Localization experiments have revealed the presence of actin patches and filaments at the presumptive site of germ tube emergence from spores and at hyphal tips, as well as actin rings at forming septa (Harris et al., 1994; Momany and Hamer, 1997). The most likely role for actin filaments in polarized morphogenesis is to serve as tracks for the myosin-based transport of vesicles to polarization sites. Actin filaments presumably direct localized vesicle transport from the apical vesicle cluster (the Spitzenkörper) to the hyphal tip (Bourett and Howard, 1991; Roberson, 1991).

4.2. Polarisome and ARP2/3 complexes

In yeast, two distinct multi-protein complexes, the polarisome and the Arp2/3 complex, function downstream of the Cdc42 GTPase module to direct the localized assembly of actin filaments at polarization sites. The polarisome regulates the formation of linear, unbranched actin filaments (actin cables). The key component of the polarisome is the formin Bni1p, which binds to the barbed ends of actin filaments and nucleates microfilament assembly (Pruyne et al., 2002; Sagot et al., 2002a; Sagot et al., 2002b). The remaining polarisome components appear to regulate the timing and location of Bni1p activity (Sagot et al., 2002b). The Arp2/3 complex regulates the formation of branched actin filaments, which, unlike linear cables, form a fine meshwork that typically underlies the cell surface. In yeast, where this complex regulates actin patch formation, key components include the WASP homologue Las17p/Bee1p, the actin-related proteins Arp2 and Arp3, and the class I myosin Myo3p (Lechler et al., 2001).

The polarisome seems likely to direct the formation of actin cables at polar growth sites in filamentous fungi as well. Most polarisome components are conserved in *A. nidulans* (Table 3), and the formin SepA controls the assembly of actin cables at hyphal tips and septation sites (Sharpless and Harris, 2002). Similarly, the Arp2/3 components are conserved in *A. nidulans* (Table 3). Moreover, the *A. nidulans* class I myosin MyoA is required for polarity establishment and organization of the actin cytoskeleton (McGoldrick et al., 1995). Taken together, these observations suggest that signals emanating from Cdc42 GTPase modules trigger local organization of actin filaments via similar mechanisms in filamentous fungi and yeast.

In contrast, the role of actin patches in the establishment and maintenance of hyphal polarity is less clear. In yeast, actin patches are required for endocytosis, which promotes polar bud growth by retrieving and recycling positional landmarks and components of the morphogenetic machinery (Engqvist-Goldstein and Drubin, 2003). It seems likely that endocytosis also plays a crucial role in promoting polarized hyphal growth (Read and Kalkman, 2003; Torralba and Heath, 2002), though this view is not universal (Torralba and Heath, 2002). Indeed, the *A. nidulans swoC* polarity mutant possesses a defect in endocytosis (Lin and Momany, 2003).

4.3. Microtubules

Although microtubules have no apparent role in polarity establishment in *S. cerevisiae*, they are required to position cell end markers in *S. pombe* (Chang and Peter, 2003). Elegant genetic studies in *A. nidulans* and other filamentous fungi have established that the essential function of microtubules is to enable chromosome segregation during mitosis, as is also true in *S. cerevisiae* (Aist and Morris, 1999). However, several observations suggest that cytoplasmic microtubules also have a crucial, but non-essential role in polarized hyphal growth. Disruption of microtubule organization does not prevent polarity establishment, but does cause the formation of morphologically aberrant hyphae (Doshi et al., 1991; That et al., 1988). Live cell imaging studies have shown that cytoplasmic microtubules are required to maintain the position of the Spitzenkörper at hyphal tips (Riquelme et al., 1998). Mutations affecting the microtubule motors dynein and kinesin cause defects in long-range vesicle transport to and from the tip region (Seiler et al., 1999). Finally, a unique temperature sensitive allele of the *A. nidulans* γ-tubulin gene causes a complete defect in polarity establishment (Jung et al., 2001).

What are the potential roles of cytoplasmic microtubules in polarized morphogenesis? One obvious function is to facilitate vesicle transport to the tip from distal regions of hyphae. Accordingly, mutations affecting microtubules or their associated motor proteins would lead to distorted hyphal morphology because of failure to supply the growing tip with sufficient levels of precursors. However, cytoplasmic microtubules may have a previously unrecognized function in the spatial regulation of polarized morphogenesis. Intriguing results from *S. pombe* show that the plus ends of cytoplasmic microtubules transport polarity determinants to the cell tip (Mata and Nurse, 1997) where they subsequently appear to specify sites of actin filament assembly by recruiting components of the polarisome (Glynn

et al., 2001). By analogy, microtubules might locally modulate actin dynamics at germination or branch sites by delivering signaling proteins or polarisome components. In this manner, they would play a key role in specifying polarization sites in filamentous fungi.

4.4. Septins

Though not considered cytoskeletal elements in the strictest sense (Field and Kellogg, 1999), septins do have many features of cytoskeletal proteins—they form filaments in vitro, bind GTP and appear to organize regions of the cell's interior (Longtine and Bi, 2003; Longtine et al., 1996). In *S. cerevisiae* septins have many roles including organization of the division site. After cytokinesis, septins remain at the division site where they recruit the cortical landmarks that specify the bud emergence site in the next cell cycle. Though the mechanism is not yet clear, Cdc42-GTP has a direct role in recruiting and/or organizing septins at the site of bud emergence. In *cdc42* mutants, septins do not localize properly to the bud site (Gladfelter et al., 2001; Jeong et al., 2001; Pringle et al., 1995). Cdc42p and septins colocalize to a cap in schmoos and mutations in the Cdc42 GAP proteins perturb the timing of septin organization (Caviston et al., 2003).

Septins have been cloned from the filamentous fungus *A. nidulans* (Momany et al., 2001) and the dimorphic pathogen *C. albicans* (Sudbery, 2001; Warenda and Konopka, 2002). In both fungi, localization studies and mutant phenotypes support roles in polarity establishment of germ tubes and branches in addition to the expected role in septation. In *A. nidulans*, the septin AspB localizes to emerging secondary germ tubes, branches, and conidiophore layers. Strains carrying a temperature-sensitive mutation in *aspB* show hyperbranching as well as conidiophore defects (Westfall and Momany, 2002). In *C. albicans*, septins localize to the necks of yeast and the emerging germ tubes of developing pseudohyphae and true hyphae (Sudbery, 2001; Warenda and Konopka, 2002). Mutations in certain *C. albicans* septins are lethal while mutation of other septins gives rise to abnormally curved hyphae with perturbed hyphal site selection (Warenda and Konopka, 2002).

4.5. Vesicles

The rapid rate of hyphal extension—as high as 1 μm/s for *N. crassa* (Seiler and Plamann, 2003)—places extreme demands on the vesicle trafficking machinery that delivers precursors to the tip. It is not surprising that mutations affecting vesicle transport are turning up with increasing frequency in genetic screens for polarity mutants. A comprehensive screen for morphogenetic mutants in *N. crassa* identified homologues of several

Table 4
Homologues of morphogenetic machinery multi-protein complex members in *A. nidulans*

Protein[a]	*A. nidulans* homologue[b]	Comments
Polarisome		
Bni1p/Bnr1p (formin)	SepA	Required for septation and polarized morphogenesis (Sharpless and Harris, 2002)
Spa2p	SepG	Required for septation (Harris et al., 1994); role in polarized morphogenesis not yet tested
Bud6p	AN1324.1	
Pea2p	No hit	
Arp2/3 WASP complex		
Las17p (WASP)	AN8715.1	Weak homology to other WASPs, but does possess characteristic WH1 domain
Arp2p	AN0673.1	
Arp3p	AN0140.1	
Vrp1p	AN1120.1	Weak homology to Vrp1, but does possess consensus WH1-binding motif
Myo3p/Myo5p	MyoA	Required for polarized morphogenesis (McGoldrick et al., 1995)
Exocyst		
Sec3p	AN0462.1	
Sec5p	AN1002.1	
Sec6p	AN1988.1	
Sec8p	AN7730.1	
Sec10p	AN8879.1	
Sec15p	AN6493.1	
Exo70p	AN6210.1	
Exo84p	AN0560.1	

[a] Query sequences are from *S. cerevisiae* and were obtained from *Saccharomyces* Genome Database (http://www.yeastgenome.org) using the Global Gene Hunter search option.

[b] BLASTp searches were performed against the *Aspergillus nidulans* database (http://www-genome.wi.mit.edu/annotation/fungi/aspergillus/index.html) using default parameters. The top hit with an *e* value lower than $1e^{-3}$ is indicated.

yeast *SEC* genes, as well as a subunit of the coatomer complex that mediates vesicle formation (Seiler and Plamann, 2003). Similarly, the coatomer complex is required for polarity establishment in *A. nidulans* (Whittaker et al., 1999) as are components of two distinct protein complexes that regulate intra-Golgi vesicle transport, TRAPP and COG (Shi et al., 2003). These results distinguish filamentous fungi from yeast, where mutations affecting apparent orthologues of these same genes have no obvious morphogenetic defect (Seiler and Plamann, 2003). Similarly, the chemical brefeldin A disrupts polarized morphogenesis in filamentous fungi, largely through the inhibition of post-Golgi vesicle transport to the hyphal tip (Cole et al., 2000).

Despite the importance of vesicle trafficking for the establishment of hyphal polarity, several questions regarding the localization of secretory organelles and the regulation of trafficking remain unanswered. In filamentous fungi, the endomembrane system consists of the endoplasmic reticulum and Golgi equivalents. Unlike animal cells, fungi do not possess discrete Golgi stacks (Satiat-Jeunemaitre et al., 1996). The localization of Golgi equivalents relative to sites of germ tube emergence or branch formation has not yet been established. Do Golgi equivalents accumulate at polarization sites, as they do at budding sites in yeast (Preuss et al., 1992)? Also, do accumulated Golgi equivalents contribute to the subsequent formation of a Spitzenkörper at these sites?

The precise spatial regulation of vesicle exocytosis is undoubtedly crucial for polarized morphogenesis. Key regulators of exocytosis include SNAREs, conserved proteins that mediate fusion between vesicles and their target membranes. In *N. crassa* plasma membrane SNAREs localize in a tip-high gradient and are intimately involved in the establishment and maintenance of hyphal polarity (Gupta et al., 2003). Another important regulator is the multi-protein exocyst complex, which ensures that vesicles dock at the correct location on the cell surface in yeast and animal cells. As expected, the entire exocyst is conserved in *A. nidulans* (Table 4), though its role in polarized morphogenesis awaits investigation. Accordingly, the Rab GTPase that regulates the exocyst, Sec4, should be required for polarity establishment, as it is in yeast. However, as shown in *Aspergillus niger*, polarized morphogenesis is largely unaffected by disruption of the putative *SEC4* homologue (Punt et al., 2001). This result suggests that the regulation of post-Golgi vesicle trafficking is more complex in filamentous fungi than in yeast.

5. Future directions: moving beyond the paradigm

There are clearly crucial differences between the brief, sporadic polar growth of the monomorphic yeasts and the sustained, multi-axis polar growth of filamentous fungi. The yeast paradigm for establishing positional information, relaying the signal and organizing the morphogenetic machinery offers a good starting framework for understanding polarity in filamentous fungi, but many questions remain to be answered. Perhaps the most intriguing question is how is positional information generated at sites of germ tube and branch emergence. Answering this question is likely to require the identification of putative cortical landmarks using genetic screens for mutants with altered patterns of polarized morphogenesis and bioinformatic approaches. Other important questions include how extensive is the role of the Rac GTPase and is there crosstalk between the Rac and Cdc42 GTPase modules. To address these questions, regulators of Rac activity as well as downstream effectors of both modules must be identified and characterized. A final crucial question is how has the morphogenetic machinery been adapted to permit continuous polarized growth over long distances. These adaptations are likely to involve a greater role for microtubules along with other modifications. Future investigations answering these questions in filamentous fungi will yield fundamental insights into polarized morphogenesis of higher eukaryotes.

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