Fungal Morphogenesis

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Morphogenesis in fungi is often induced by extracellular factors and executed by fungal genetic factors. Cell surface changes and alterations of the microenvironment often accompany morphogenetic changes in fungi. In this review, we will first discuss the general traits of yeast and hyphal morphotypes and how morphogenesis affects development and adaptation by fungi to their native niches, including host niches. Then we will focus on the molecular machinery responsible for the two most fundamental growth forms, yeast and hyphae. Last, we will describe how fungi incorporate exogenous environmental and host signals together with genetic factors to determine their morphotype and how morphogenesis, in turn, shapes the fungal microenvironment.

PROPERTIES OF MORPHOGENESIS IN FUNGAL CELLS

The defining morphogenetic characteristic of fungal cells is their polarity, whereby cell surface expansion and wall deposition are confined to discrete sites on the cell surface. Although hyphae and yeasts show obvious differences in their modes of growth (Fig. 1), they share three basic properties that enable polarized growth and the formation of a diverse array of cell shapes. The first is symmetry breaking, in which an initially isotropic cell generates an axis of polarized growth. The second is polarity maintenance, which refers to the stabilization of the polarity axis such that polar growth en-sues. The third is depolarization, in which polarity is lost in a controlled manner. The balance between polarity maintenance and depolarization generates the diversity of fungal cell shapes.

Fungal cells, typically, are not polarized during the earliest phases of development. They usually undergo an initial period of nonpolar isotropic expansion (e.g., yeast mother cells, spores). Ultimately, however, cellular symmetry must be broken and a polarity axis generated, either for bud site selection or the development of polar structures, such as hyphae. One form of symmetry breaking relies on preexisting spatial landmarks that direct the recruitment of the morphogenetic machinery to a discrete site on the cell surface. The best-known example is the
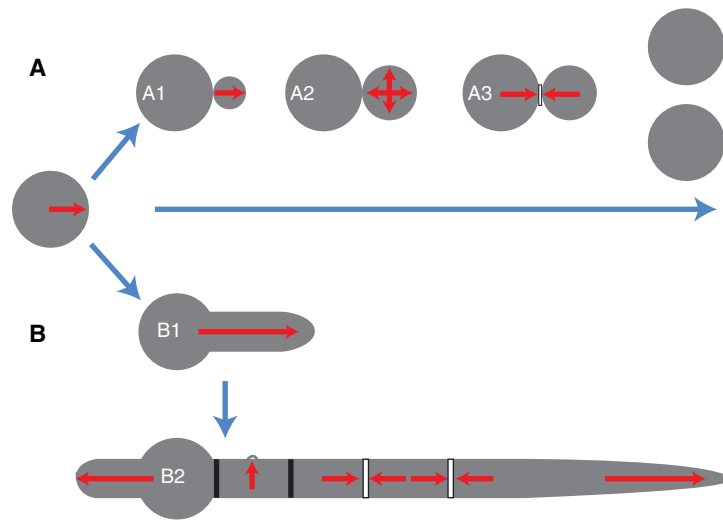
Figure 1. Distinct patterns of morphogenesis in yeast and hyphal cells. Yeasts and filamentous fungi typically initiate growth as nonpolarized cells or spores. Budding yeasts such as *Saccharomyces cerevisiae* or *Cryptococcus neoformans* (*A*) establish an axis of polarity that directs the emergence of a new bud (*A1*). Following a period of polarized growth, depolarization enables the formation of an ellipsoidal bud (*A2*). Following nuclear division, the construction and controlled degradation of a septum (*A3*) results in cell separation. Filamentous fungi such as *Aspergillus nidulans* and *Candida albicans* (*B*) establish a polarity axis that directs the emergence of a germ tube (*B1*). Unlike yeasts, sustained polar growth leads to the formation of a hypha that grows by apical extension. Moreover, hyphae are able to simultaneously support multiple polarity axes to allow the formation of septal cross-walls (bars) and lateral branches (*B2*). For many filamentous fungi, spores also generate secondary germ tubes once they are partitioned from the primary hypha by a septum.

bud site selection process in *Saccharomyces cerevisiae*, which uses a set of landmark proteins to communicate positional information to the morphogenetic machinery via sequential GTPase modules (Chant 1999). In contrast, the fission yeast *Schizosaccharomyces pombe* uses cell end markers coupled to a microtubule-based delivery system to demarcate polarization sites (Martin 2009). Another form of symmetry breaking refers to the formation of a polar axis without any detectable, preexisting landmark (Wedlich-Soldner et al. 2003). Although a full consensus has yet to be reached regarding the specific details of this form of symmetry breaking (Johnson et al. 2011), it does involve the simultaneous action of multiple feedback loops that direct the accumulation of the activated form of the Cdc42 GTPase at a discrete site on the cell surface (Freisinger et al. 2013). Such feedback loops can be regulated to permit formation of multiple polarity axes based on studies in *S. cerevisiae*

(Wu and Lew 2013). This might provide a basis for understanding how filamentous fungi can generate several hyphae from a single cell.

Once an axis of polarized growth has been generated, it must be stabilized to ensure that cell surface expansion and wall deposition are confined to a discrete site. The existence of a stable polarity axis is reflected by the resulting asymmetry of cellular organization (Riquelme 2013). Vesicle exocytosis is largely confined to that discrete site, which is flanked by endocytic zones in which membrane material and proteins are retrieved for recycling. Localized actin filaments support exocytosis, whereas actin patches mediate endocytosis. Arrays of cytoplasmic microtubules are organized in parallel to the polarity axis and, generally, mediate longer-range vesicle movements to and from the growth site. Localized changes in plasma membrane composition might be one broadly applied mechanism for maintaining polarity in

both yeasts and hyphae. This is consistent with the recent observation that polarity factors are spatially segregated into distinct clusters on the cell surface in both *S. pombe* and *S. cerevisiae* (Dodgson et al. 2013). Although some of these features vary across different growth forms (i.e., yeast cells vs. hyphae), they appear to be universally coordinated by Cdc42/Rho1-related GTPase modules and their numerous effectors.

Whereas a great deal of attention has focused on how polarity is established in fungal cells, little has been devoted to understanding how fungal cells depolarize. Nevertheless, careful regulation of depolarization presumably underlies much of the variation in cell shape that is observed in fungi. In *S. cerevisiae*, depolarization manifests as the "apical-to-isotropic" switch that enables the formation of an ellipsoidal yeast cell as opposed to more elongated pseudohyphae (Bi and Park 2012). In contrast, hyphae, by definition, undergo sustained polarized growth. During subsequent developmental phases, such as conidiation, however, regulated depolarization undoubtedly plays a vital role in dictating the diverse patterns of cell division that result in taxonomically informative characters, such as conidial ontogeny and spore shape (Cole and Samson 1979).

Hyphal Morphogenesis

Hyphae are multicellular filaments in which symmetry breaking leads to the formation of a new hypha referred to as a germ tube or branch initial (Fig. 1). To date, there is no evidence supporting the existence of spatial landmarks that designate sites of germ tube or branch emergence. It seems reasonable that these sites are selected stochastically (e.g., spontaneous polarization). On the other hand, there is evidence suggesting that secondary polarization events in germinating spores are biased toward the pole that is opposite to the original polarization site (i.e., a bipolar polarization pattern) (Fig. 1) (Harris 1999). The basis of this bias remains unknown.

The continued growth of germ tubes and branch initials results in their maturation into hyphae. This generally correlates with an in-crease in the rate of apical extension (Horio and Oakley 2005), as well as the appearance of a Spitzenkörper (apical body). The latter structure is typically located proximal to the hyphal tip (Girbardt 1957; Sudbery 2011a), largely composed of vesicles of different sizes, microfilaments, and ribosomes (Harris et al. 2005; Verdin et al. 2009). An extensive body of data support the view that Spitzenkörper dynamics determine the shape of the hyphal tip, dictate the orientation of hyphal extension (Bartnicki-Garcia et al. 1995; Riquelme 2013), and help maximize rates of apical extension (Kohli et al. 2008). Nevertheless, the presence of a Spitzenkörper is not an obligate requirement for the formation of polarized hyphae. It should be noted that the Spitzenkörper exists within the context of a larger assembly that has been referred to as the hyphal tip complex (Taheri-Talesh et al. 2008). Components of this complex include the polarisome and exocyst, which localize to the extreme hyphal apex in which they regulate exocytosis, as well as the subapical collar that consists of actin patches and the endocytic machinery (Sudbery 2011a). As characterized in *Aspergillus nidulans*, precise spatial organization of the hyphal tip complex does seem to be a strict requirement for polarized hyphal growth. Thus, the Spitzenkörper might be a highly structured hyphal tip complex required for efficient apical growth.

A single fungal spore is capable of generating a mycelium composed of an extensive network of branched hyphae. At the margins of the colony, individual hyphae show an avoidance response, whereas in the colony interior, hyphal fusion (anastomosis) permits the exchange of nutrients and perhaps growth signals (Rayner 1996; Simonin et al. 2012). An important concept that underlies mycelium formation is apical dominance, which refers to the suppression of secondary polarity axes in the vicinity of an actively extending hyphal tip (Rayner 1991). Apical dominance enables the precise regulation of lateral branching by minimizing the competition between hyphal tips for existing resources. Although not well understood, the enforcement of apical dominance in some filamentous fungi requires the accumulation of re-

active oxygen species at the hyphal tip (Tanaka et al. 2006; Semighini and Harris 2008). It seems likely that calcium gradients would also play a role in this process (Schmid and Harold 1988). Because of apical dominance, lateral branches often emerge from subapical hyphal compartments separated from the tip by a septum. In some filamentous fungi, septa conceivably could play a role as "branching landmarks" (Harris 2011a), although there appears to be no universal pattern of lateral branching. One distinct branching pattern observed in some hyphae is apical branching, whereby an existing hyphal tip "splits" into two distinct tips. This pattern of branching is shown by rapidly growing hyphae of *Ashbya gossypii* (Philippsen et al. 2005).

Yeast Morphogenesis in Ascomycetes

Yeasts generally divide either by budding or fission (Martin and Arkowitz 2014). Even in nearly spherical and symmetric yeast cells, cell polarity plays a major role in cell division and growth. The establishment of subcellular asymmetry is required in the context of the most polar events of yeast cell growth, for example, establishing the site of new bud emergence (Fig. 1). In *S. cerevisiae*, symmetry breaking leads to localized cell surface expansion and wall deposition at the incipient bud site. As a new daughter cell begins to form, the mother cell must direct the trafficking of new cellular material to this growing cell. Subsequently, the morphogenetic machinery then relocalizes to the mother–bud junction to enable septum formation and cytokinesis. With cytokinesis, there is a transition to the more symmetric phase of isotropic growth until the cells are ready for new budding events. Therefore, yeast cell division is dependent on a dynamic and ordered transition between cytoskeletal polarization and loss of polarization to complete its budding cell cycle (Fig. 1).

A fundamental feature that distinguishes yeasts from hyphae is the coupling of cell polarization to subsequent depolarization. The obstruction of polarized growth leads to a unicellular lifestyle. Moreover, nuclear division is tightly coordinated with cellular morphogenesis in yeast growth, such that the same cyclin-dependent protein kinase (CDK) complexes that drive cell-cycle progression also trigger the transitions between polar and nonpolar growth (Wang 2009; Howell and Lew 2012). Furthermore, morphogenetic checkpoints maintain the coordination of nuclear division with polar growth to prevent the formation of anucleate or binucleate cells (Howell and Lew 2012). In contrast, early morphogenetic events during germ tube emergence or branching initiation occur largely independent of nuclear division (Momany and Taylor 2000). It seems that coordination between septation and nuclear division and migration is dispensable in vegetative multinucleate hyphae. However, it is important to note that such coordination is required for the growth of heterokaryotic hyphae generated after mating events and critical for subsequent nuclear fusion and meiosis during sexual reproduction in filamentous fungi (both ascomycetes and basidiomycetes).

Yeast Morphogenesis in a Basidiomycete

Much of the work studying the cell division events for common pathogenic yeasts uses paradigms established in the ascomycete *S. cerevisiae*. However, basidiomycete yeasts show both conserved and distinct features of morphogenesis. By definition, a basidiomycete fungus (e.g., *Cryptococcus neoformans*) produces hyphal structures during sexual differentiation that distinguish it from ascomycetes (Kwon-Chung 1975, 1976). Spores produced by *Cryptococcus* hyphae are quite infectious and may be the primary particle inhaled during a natural infection (Giles et al. 2009; Velagapudi et al. 2009). Therefore, the transition to hyphal growth that supports sporulation is required for the wide dissemination of *Cryptococcus* in the environment, as well as for infection of its mammalian hosts.

Once inhaled in the lung, cryptococcal spores germinate to produce yeast cells. During the context of infection, this fungus grows within the human host almost exclusively in a budding, yeast-like form. Detailed histopathological studies have shown that hyphal forms are rarely encountered during human *C. neofor-*

mans infections, with elongated fungal morphologies observed only as rare variants among clinical strains (Baker and Haugen 1955; Shadomy and Utz 1966). In addition to the typical yeast-cell budding dynamics, other morphological events are required for specific aspects of *C. neoformans* pathogenesis. In the lungs, small ovoid cryptococcal yeasts can undergo a morphological transition to form "giant" or "titan" cells (Zaragoza et al. 2010; Zaragoza and Nielsen 2013). In contrast to typical yeast cells, which measure 5 μm in diameter, *C. neoformans* titan cells can enlarge to sizes ranging from 15 to 100 μm. These giant cells are highly resistant to phagocytosis and reactive oxygen/nitrogen species, thus providing a more resilient form for early infection. Titan cells have polyploid nuclei, and undergo nuclear reduction to generate haploid progeny by unknown mechanisms (Zaragoza and Nielsen 2013). The process of titan cell formation is dependent on many central signaling proteins that control many aspects of cellular differentiation and morphogenesis, including pheromone receptors, PKA, the Rim101 transcription factor, GTPase-activating proteins Gap1 and Cnc1560, G_1 cyclin Pcl103, and Rho104 GTPase (Okagaki et al. 2011).

The cellular events during *C. neoformans* yeast-cell morphogenesis have been well described. In general, cells proceed through a cycle of cell division with repeated asexual, clonal budding of a haploid yeast cell (Moore 2000). Several features distinguish the *C. neoformans* budding cycle from those of other pathogenic yeast cells. The single nucleus of the mother cell migrates into the daughter cell after initial budding begins. Mitosis then culminates in nuclear division with one of the new nuclei migrating back into the mother cell before final cell separation occurs. Also, in contrast to *S. cerevisiae* in which subsequent budding events occur adjacent to prior bud scars, *C. neoformans* seems to preferentially and repeatedly bud from the same site. This results in a feathering appearance of cell wall material accumulated at the site of a recent bud (Moore 2000; Adams 2004). For this reason, counting bud scars is unlikely to provide an accurate measure of cryptococcal cell age.

Evolution of Fungal Morphogenesis

An intriguing question in fungal morphogenesis is the evolutionary relationship between hyphae and yeasts. It seems likely that the cell morphology of ancestral fungi resembled protists (Jones et al. 2011), and hyphal growth emerged as the dominant growth form that facilitated the colonization of terrestrial habitats (Stajich et al. 2009). Yet, many filamentous fungi also show yeast-like patterns of cellular morphogenesis, particularly during development (Cole and Samson 1979). It seems reasonable that specific ecological niches favored the loss of hyphal growth in the subphylla Saccharomycotina and Taphrinamycotina (i.e., the clades containing *S. cerevisiae* and *S. pombe*, respectively), resulting in the evolution of clades in which yeast growth forms dominate (Harris 2011a,b). Nevertheless, many members of these clades retained the capacity to undergo hyphal (or pseudohyphal) growth in response to different environmental triggers. These so-called dimorphic fungi include many prominent human and plant pathogens whose morphotype transitions play critical roles in virulence.

THE MORPHOGENETIC MACHINERY

Vesicle Trafficking

Both yeasts and filamentous fungi possess exocytic vesicles of distinct classes based on their size and presumed content (Harsay and Bretscher 1995). In filamentous fungi, each class of exocytic vesicles appears to transit through the Spitzenkörper on its way to the apex of the hyphal tip. The localization of exocyst components to the apex suggests that this is the ultimate site in which vesicles fuse to the target plasma membrane (Taheri-Talesh et al. 2008). However, recent studies with *Aspergillus oryzae* point to the existence of additional secretion sites, including septa and subapical sites (Hayakawa et al. 2011). These observations imply that the trafficking of exocytic vesicles is far more complex than previously imagined.

The specificity of vesicle interactions with their target plasma membranes is likely governed by interactions between vesicle and tar-

get soluble NSF (*N*-ethylmaleimide-sensitive factor) attachment protein receptors (v- or t-SNAREs). Filamentous fungi possess a large complement of v-SNAREs and there appears to be some degree of redundancy in their function (Kuratsu et al. 2007). It should be noted that alternative routes for the delivery of contents to the cell surface might not rely on the classical secretion pathway (Rodrigues et al. 2011).

The Golgi complex serves a pivotal role in vesicle trafficking, as it is the source of exocytic vesicles and terminus of many endosomes. Thus, in the Golgi, different classes of vesicles must be sorted and matched to their correct cargoes. The importance of the Golgi to vesicle trafficking and morphogenesis has long been established in *S. cerevisiae* (Schekman and Novick 2004), but only recently has its importance for sustained polar growth been described for filamentous fungi (Pinar et al. 2013). Indeed, whereas the Spitzenkörper appears to be required primarily for rapid rates of hyphal extension, polarized growth fails completely in the absence of a functional Golgi complex.

The Cytoskeleton

The organization and function of both microfilaments and -tubules have been well characterized in yeasts and filamentous fungi. Formins assist nucleating microfilament formation at polarization sites (Evangelista et al. 2003; Pearson et al. 2004; Schmitz et al. 2006), but the formation of a stable axis of hyphal polarity can occur in their absence, as shown for *A. nidulans* (Sharpless and Harris 2002). On the other hand, regulators of actin patch formation and dynamics appear to be essential for the formation of a stable axis (Araujo-Bazan et al. 2008; Upadhyay and Shaw 2008; Hervas-Aguilar and Penalva 2010). These observations underscore the importance of endocytosis in polarized growth, but also imply that the localized delivery of cell wall material to polarization sites can occur in the absence of actin filaments. Limited evidence suggests that microtubules can potentially compensate for the absence of actin filaments (Virag et al. 2007). Normally, the key

function of microtubules in hyphae appears to be positioning of the Spitzenkörper to enable proper orientation of hyphal extension (Fischer et al. 2008). However, in the absence of actin filaments at the tip, microtubules likely ensure sufficient vesicle flux to support apical extension, albeit at less than optimal rates.

Septins are highly conserved throughout fungi and animals, wherein they play multiple roles in cellular morphogenesis (Hall et al. 2008). In *S. cerevisiae*, septins form scaffolds that are essential for normal polarized growth, septation, and cytokinesis (Oh and Bi 2011). In filamentous fungi, septins are needed for normal septum formation and appear to delimit growth sites (DeMay et al. 2009; Lindsey et al. 2010; Ryder et al. 2013). For example, the absence of septins in *A. nidulans* leads to the simultaneous emergence of multiple germ tubes and hyperbranching. However, further maturation of branch initials into secondary hyphae is blocked. In general, septins function as scaffolds that coordinate localized cell wall biosynthesis with the cytoskeleton and vesicle trafficking machinery. Thus, septins likely consolidate and stabilize polarity axes, thereby preventing the formation of spurious ones.

Positional Markers

The only spatial landmarks that have been characterized in filamentous fungi are those that also function in *S. cerevisiae* and *S. pombe*. In budding yeast, the cell surface protein Axl2 acts in conjunction with the septin-associated proteins Bud3 and Bud4 to specify the axial budding pattern of mating-type **a** or α cells, whereas a distinct set of cell surface proteins (i.e., Bud8, Bud9, Rax1, Rax2) specify the bipolar pattern observed in **a**/α cells (Chant 1999). In either case, the landmarks trigger local activation of Cdc42 via the Ras-like GTPase Bud1 (Bi and Park 2012). In fission yeast, the plasma membrane-anchored protein Mod5 provides a target for the delivery of Tea1 via the plus ends of cytoplasmic microtubules (Snaith and Sawin 2003). This serves to orient microtubules and allows the accumulation of a formin-containing complex that enables localized microfilament

formation (Martin 2009). The relationship between this pathway and Cdc42 in *S. pombe* is not clear, although some evidence suggests that they may act in parallel to promote cell polarity (Das et al. 2009).

Signal Transduction

Small GTPases, such as Ras, Rho, Cdc42, and Rac, play a fundamental role in the regulation of fungal morphogenesis. Typically, they act via multiple effectors to coordinate organization of the cytoskeleton and vesicle trafficking at polarization sites. Cdc42 effectors include p21-associated kinases such as Cla4, the Borg-related proteins Gic1 and Gic2, formins such as Bni1, Wiscott–Aldrich syndrome protein homologs such as Las17, and the exocyst (Park and Bi 2007). Rho GTPases have well-established roles in the regulation of β-glucan synthesis and cell wall integrity (Levin 2011). Less is known about potential effectors of Ras that might mediate polarized growth, particularly in filamentous fungi. It should be noted that there are examples of effectors with important roles in polarized hyphal growth that are not well conserved in *S. cerevisiae*. A particularly prominent example is NADPH oxidase, which appears to be an effector of Rac1 in filamentous fungi (Tanaka et al. 2006; Semighini and Harris 2008), in which it regulates seemingly diverse morphogenetic processes, such as lateral branching and infection-related morphogenesis (Tanaka et al. 2006; Egan et al. 2007). Small GTPases, such as Ras, Rho, and Cdc42, do not operate in isolation, but rather in a sequential manner as suggested by studies in *S. cerevisiae* and *S. pombe*. For example, considerable evidence shows that Ras GTPases function upstream of Cdc42 to regulate polarized growth (Chang et al. 1994), whereas Cdc42 and Rho1 may antagonize each other to spatially and temporally coordinate localized cell wall deposition (Gao et al. 2004). Collectively, these observations reinforce the central role of small GTPase modules as regulators of hyphal morphogenesis in fungi. Nevertheless, the extent to which individual GTPases contribute to specific aspects of morphogenesis appears to vary across fungal species.

The molecular events directing *C. neoformans* yeast cell polarity also possess conserved and unique features compared with ascomycetous yeasts. As in other fungi, the Ras1-Cdc24-signaling pathway appears to control many aspects of yeast-cell polarization and budding. Ras proteins control the activity of Cdc24, a guanine–nucleotide exchange factor, which, in turn, controls the activation of downstream effector proteins, such as Cdc42 (Zhao et al. 1995; Bassilana et al. 2003). In *C. neoformans*, mutation of either the *RAS1* or *CDC24* gene results in a mutant strain that cannot efficiently repolarize its actin cytoskeleton after exposure to stresses, such as elevated temperatures (37°C). This defect in cell polarity manifests as temperature sensitivity because of unchecked isotropic growth, with eventual arrest as a large, unbudded yeast (Alspaugh et al. 2000; Nichols et al. 2007). In addition to temperature elevations, the *ras1* and *cdc24* mutants are more susceptible to other physiologically relevant stresses, such as hypoxia (Nichols et al. 2007). Together, impaired resistance to conditions encountered in the host make these strains avirulent in animal models of cryptococcosis (Alspaugh et al. 2000; Nichols et al. 2007). Studies in these mutant strains, therefore, emphasize the role of proper cell morphogenesis and budding in survival within the host.

In *S. cerevisiae*, the Cdc42 protein is the major effector of Cdc24, as well as the primary determinant of cell polarity (Johnson 1999). In other fungi, especially those with more prominent hyphal morphological forms, the related Rac and Cdc42 proteins together direct cell polarity (Hurtado et al. 2000; Boyce et al. 2003; Chen and Dickman 2004; Vallim et al. 2005; Bassilana and Arkowitz 2006). *C. neoformans* is unique in that it possesses duplicated genes for Ras, Rac, and Cdc42 proteins (Ballou et al. 2013a). Recent studies have indicated that the Rac and Cdc42 proteins together serve as the downstream effectors of morphogenesis for *C. neoformans* Ras1 (Ballou et al. 2010, 2013a,b). For example, the CnRac proteins are required for the highly polar state of filamentous growth. Deletion of either the *RAC1* or *RAC2* gene results in the production of dysmorphic hyphae

during mating (Ballou et al. 2013b). Moreover, overexpression of *RAC* genes complements the *ras1* mutant defect in mating hyphal development (Ballou et al. 2013a). Subcellular localization of a Gfp–Rac2 fusion protein indicates enrichment at the site of early bud emergence and growing tip of the daughter cell, both sites of cell polarity (Fig. 2). Also, CnRac proteins are required for intracellular trafficking events, which are also important in the polarization process before cell division (Shen et al. 2012). The double *rac1*/*rac2* mutation appears to be synthetically lethal, suggesting a central role for Rac proteins in cell division and basic growth (Ballou et al. 2013b).

The duplicated Cdc42/Cdc420 proteins in *C. neoformans* are also important for cellular morphogenesis. These proteins direct the localization of septins to the site of cytokinesis
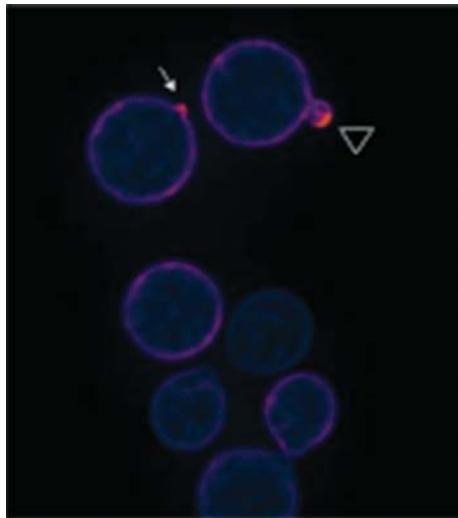
Figure 2. *C. neoformans* Rac2 localization suggests a role in cell polarity. A Gfp–Rac2 fusion protein was expressed in *C. neoformans* and visualized using an Olympus (Center Valley, PA) IX70 microscope. After image acquisition and deconvolution of serial images in the Z-coordinate using Deltavision (GE Healthcare, Issaquah, WA) software, pseudocolored, merged images were produced using Fiji (Madison, WI) software. The most intense fluorescent signal, representing enrichment of Rac2 localization, is present at the site of incipient bud emergence (arrow) and distal edge of a small daughter cell (arrowhead). (Image provided by S. Esher and K. Selvig, Duke University.)

(Ballou et al. 2013a). *C. neoformans* contains genes encoding homologs to the Cdc3, Cdc10, Cdc11, and Cdc12 septin proteins in *S. cerevisiae* (amino acid identity to *S. cerevisiae* proteins: 42%, 56%, 42%, and 49%, respectively). Unlike their *S. cerevisiae* orthologs, none of the *C. neoformans* septin genes is essential. However, the *cdc3*, *cdc11*, and *cdc12* mutants fail to grow at 37°C, and the *cdc10* mutant is modestly growth impaired at this higher temperature (Kozubowski and Heitman 2010). Similarly, mutation of either *C. neoformans* Ras or Cdc42 proteins results in mislocalized septins and failed cell separation under stress conditions. These strains are, therefore, poorly viable in animal models of cryptococcosis (Ballou et al. 2013a). Thus, Cdc42-mediated septin assembly is important for growth of *Cryptococcus* in vivo.

The complementary roles in morphogenesis for Cdc42 and Rac proteins have also been explored in *C. albicans*. Although the single Cdc42 ortholog is required for hyphal formation and pathogenesis, the *C. albicans* Rac protein is required only for polarized growth in a semisolid matrix. Moreover, despite high sequence conservation, overexpression of Rac or Cdc42-encoding genes does not cross-complement mutations of the other GTPase. Therefore, these two highly related proteins appear to serve distinct functions in the physiology of *C. albicans* (Bassilana and Arkowitz 2006).

MORPHOTYPE, NICHE ADAPTATION, AND FUNGAL VIRULENCE

Unlike in multicellular eukaryotes, in which most cells are typically embedded in a relatively constant environment, fungal cells face the challenge of unpredictable environmental fluctuations. Appropriate responses, including morphological changes, are often needed to survive, reproduce, and disperse. Cells in the hyphal form are inherently more effective in penetrating physical barriers and expanding colony growth three-dimensionally. Hyphae are also more likely to show gravitropism, thigmotropism, phototropism, aerotropism, and galvanotropism (Hoch et al. 1987; Crombie et al. 1990; Moore 1991; Moore et al. 1996; Aoki et al. 1998;

Idnurm and Heitman 2005; Brand et al. 2007). In contrast, yeasts or yeast-like cells (including conidia, endospores, etc.) are superior in amplification, stress tolerance, and dispersal in liquid or air. The ability to switch morphotypes helps fungi to survive or escape otherwise suppressive environments, contributing to the success of this kingdom in the tree of life (Institute of Medicine (US) Forum on Microbial Threats 2011).

Morphotype switching is a prominent strategy adopted by phytopathogenic fungi for host invasion and dissemination. In *Ophiostoma ulmi*, the causal agent of Dutch elm disease, the mycelial form is required to penetrate adjacent xylem vessels. In contrast, the conidia/yeast form (conidia bud in a yeast-like fashion) is used to translocate within the individual xylem vessels via the host transpiration stream (Kulkarni and Nickerson 1981). Production of conidia or yeast-like blastoconidia in planta by *Verticillium* species is also implicated in the rapid dissemination of the pathogen within the host vascular tissue in diverse plant species (Schnathorst 1981; Pegg and Brady 2002). *Mycosphaerella graminicola*, an important wheat pathogen, switches from a yeast-like form to the infectious hyphal form to invade leaf tissue through stomata (Mehrabi and Kema 2006). In *Taphrina deformans*, the causal agent of leaf curl in peach and almond, yeast cells land on leaves and undergo a mitotic nuclear division to establish a binucleate condition. The resulting dikaryotic cells subsequently switch to parasitic filamentous growth in planta (Rodrigues and Fonseca 2003). Similarly, in the biotrophic maize pathogen *Ustilago maydis*, haploid yeast cells of compatible mating types fuse to form a dikaryon, which subsequently generates the infectious dikaryotic hyphae to continue growth and development in vivo (Banuett 1991). Although the hyphal form is used by many plant pathogens to invade the host, filamentous growth itself may not be adequate, or even required, for pathogenesis. For example, *Holleya sinecauda*, the dimorphic pathogen of mustard seeds, is isolated almost exclusively in the yeast form in plant lesions (Holley et al. 1984). Mutations that enable *U. maydis* to grow solely in a filamentous form do not support *Ustilago*

pathogenicity (Barrett et al. 1993). Thus, either or both of the morphotypes could be associated with infection, depending on the infection strategy adopted by a particular species in a particular host microenvironment.

Likewise, the ability to undergo morphotype transitions is often required for human fungal pathogens to adapt to the host environment, elude host immune defense systems, and inhabit different niches in the host. The classic thermally dimorphic fungi, such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, and *Penicillium marneffei*, grow as saprophytic molds in the environment. After host infection, conidia or hyphal fragments convert to yeasts to replicate intracellularly and disseminate through the host (Gauthier and Klein 2008). (See Sil and Andrianopoulos [2015] for details about the association between morphotype changes and virulence in these dimorphic human fungal pathogens.) However, the role of morphotype switching in pathogenesis goes beyond these classic dimorphic pathogens. For instance, *Malassezia* yeasts typically superficially colonize the skin and can also be taken up by keratinocytes to exist as facultative intracellular parasites. Excessive sebum from the host could induce hyphal formation in *Malassezia globosa* to reroot the infection in nutrient-rich, deep cornified layers (Brand 2012).

Similarly, the yeast form of *C. albicans* is associated with the commensal state, and the appearance of filaments is associated with invasive disease. The escape of *C. albicans* from phagocytic cells requires the switch from the yeast form to the hyphal form (Lorenz et al. 2004). Failure to sustain polarized hyphal growth leads to the entrapment of *C. albicans* intracellularly (Zakikhany et al. 2007). Although *C. albicans* hyphae predominate at the infiltration site, yeast cells are seen on the epithelial cell surface, as well as emerging from penetrating hyphae (Scherwitz 1982; Ray and Payne 1988). Yeast cells also produce proteins (e.g., Ywp1) that weaken adherence and thus promote dissemination (Granger et al. 2005). This bidirectional morphotype conversion likely boosts the ability of *C. albicans* to invade and disseminate.

Dermatophytes (e.g., *Trichophyton rubrum*) are known to grow as hyphae when infecting the stratum corneum or hair follicle. However, during invasive or deep dermal infections, dermatophytes also manifest morphological diversity as broad, pleomorphic hyphae with budding yeast–like arthrospores are observed (King et al. 1975; Bibel et al. 1977; Lillis et al. 2010; Marconi et al. 2010; Brand 2012). Such morphological switching in the dermatophyte *Trichophyton mentagrophytes* can also be observed under certain in vitro conditions (Bibel et al. 1977). In contrast, *Cryptococcus* species grow as encapsulated yeasts in animals/humans. Its filamentous form is only occasionally seen in host tissues and considered less virulent (Lin 2009; Magditch et al. 2012; Wang et al. 2012). For this intracellular pathogen, the ability to hijack host cells bypasses the requirement of the hyphal form for host invasion. Thus, *Cryptococcus* can translocate, invade, escape, and disseminate in the host, all in the yeast form.

In term of pathogenesis, fungal morphotype transitions can alter the host–pathogen interaction by the differential presentation of pathogen-associated molecular patterns in different morphotypes. For example, in *B. dermatitidis*, *H. capsulatum*, and *P. brasiliensis*, the transition from the hyphal to the pathogenic yeast form is accompanied by increased α-1,3-glucan deposition in the cell wall (Kanetsuna and Carbonell 1971; Seider et al. 2010), which masks the immunostimulatory β-glucan (Rappleye et al. 2007). Additionally, these different fungal morphotypes can display altered tolerance of certain host physiological conditions, as well as varying abilities to disseminate in different host tissues (Klein and Tebbets 2007). The pathogenic *Histoplasma* yeast cells, and not the saprophytic hyphal cells, secrete Cbp1 to acquire calcium to better survive within phagolysosomes (Batanghari et al. 1998). Cbp1 supports the growth of intracellular yeast cells and is required for *Histoplasma* virulence in animal models (Sebghati et al. 2000). *Histoplasma* yeast cells also produce Yps3 to promote extrapulmonary dissemination (Holbrook and Rappleye 2008). Similarly, the pathogenic *Blastomyces* yeast cells produce adhesin WI-1/Bad1 to suppress

phagocyte proinflammatory responses, and Bad1 is indispensable for virulence of the fungus (Klein 2000; Rooney et al. 2001; Finkel-Jimenez et al. 2002).

The yeast and hyphal forms of *C. albicans* also differentially interact with the host and its immune cells. For example, the hyphal form of this fungus does not expose the immunostimulatory β-glucan (Gantner et al. 2005). Instead, it produces a suite of hypha-specific factors such as Als3, Hyr1, and Hwp1 to assist in host invasion (Staab et al. 1999; Luo et al. 2010). Als3 is an adhesin and invasin that induces host endocytosis (Hoyer et al. 1998; Argimon et al. 2007; Phan et al. 2007; Liu and Filler 2011). Als3 also helps *Candida* acquire iron by binding to host ferritin (Almeida et al. 2008).

In *Aspergillus fumigatus*, both hydrophobins and melanin coat the conidial surface to help the cell type evade host detection and remain protected within host phagolysosomes (Aimanianda et al. 2009; Volling et al. 2011; Carrion et al. 2013). On germination and hyphal growth, cell wall protein CspA is unmasked, helping to mitigate hyphal damage induced by neutrophils (Levdansky et al. 2010). Thus, factors produced during morphogenesis, in addition to the morphotype transition itself, help fungi adapt to host conditions.

ENVIRONMENTAL REGULATION OF HYPHAL MORPHOGENESIS

Sensing Nutritional and Environmental Signals

Pathogens must be proficient at sensing and adapting to their surroundings to survive changing host microenvironments. *C. albicans* cells undergo the yeast to hypha transition in response to many nutritional and environmental signals, including an increase in temperature to 37°C, neutral pH, serum, nutrients, *N*-acetylglucosamine (GlcNAc), hypoxia, and CO_2. Sensors for many of these signals have been identified in *C. albicans* (Fig. 3) (Cottier and Muhlschlegel 2009). Many of the strong hypha-inducing signals are sensed and integrated by the adenylate cyclase Cyr1, which is indis-
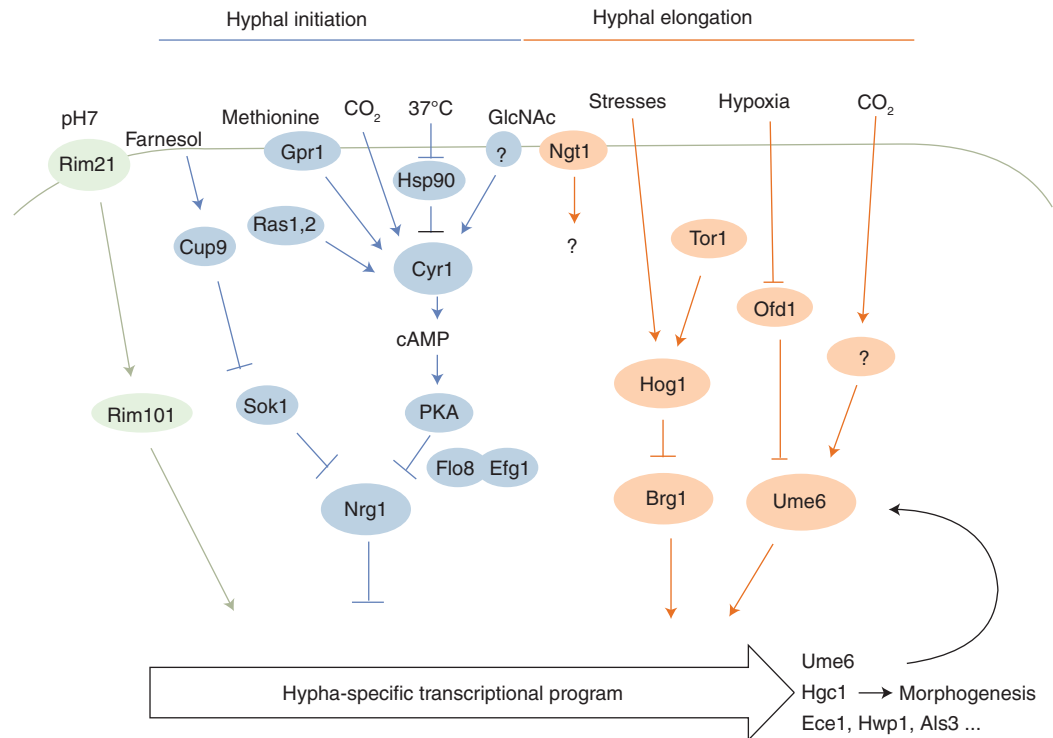
Figure 3. Signal transduction pathways integrating various signals for morphogenesis in *C. albicans*. Selective signal transduction pathways and regulators of morphogenesis are shown. Arrows indicate activation. Bars indicate inhibition. Hyphal-specific regulator Ume6 sustains the hypha-specific transcriptional program and Hgc1 promotes hyphal morphogenesis.

pensable for hyphal growth under all conditions (Bahn and Sundstrom 2001; Rocha et al. 2001; Hogan and Sundstrom 2009; Zou et al. 2009). CO_2/HCO_3^- directly stimulates Cyr1p activity by binding to the catalytic domain of Cyr1 (Klengel et al. 2005). The cyclase activity is also regulated by the small GTPases (Ras1, Ras2), G-protein coupled receptor Gpr1, and Gα protein Gpa2 in response to nutrients (Feng et al. 1999; Sanchez-Martinez and Perez-Martin 2002; Miwa et al. 2004; Maidan et al. 2005a; Zhu et al. 2009). Gpr1 in *C. albicans* is responsive to methionine, but not to glucose (Maidan et al. 2005b), suggesting that this signal is likely encountered by this fungus in vivo. The rapid increase in temperature to 37°C is also essential for hyphal induction, likely by relieving Hsp90-mediated repression of the Ras1-Cyr1 pathway (Shapiro et al. 2009, 2012). The cAMP-dependent protein kinase A (PKA) is a

major target of Cyr1, consisting of one regulatory subunit (Bcy1) and two catalytic subunits (Tpk1 and Tpk2). Each PKA subunit has distinct functions in *C. albicans* hyphal development, indicating even further adaptability of this otherwise highly conserved signaling system (Sonneborn et al. 2000; Bockmuhl et al. 2001; Cassola et al. 2004). A major function of the *C. albicans* cAMP-PKA pathway is to down-regulate the expression level of *NRG1* (Lu et al. 2011), the major repressor of hyphal morphogenesis (Braun et al. 2001; Murad et al. 2001). This down-regulation of *NRG1* expression requires the transcription factors Efg1 and Flo8 (Lu et al. 2011).

The yeast-to-hypha transition in *C. albicans* must be initiated and then maintained (Lu et al. 2011). Hyphal initiation requires temporary clearing of Nrg1, which needs a transient activation of the cAMP-PKA pathway. In contrast,

hyphal maintenance requires continuous and active sensing of the surrounding environment. During hyphal initiation, when the Nrg1 protein disappears, the expression of a GATA family transcription factor Brg1 is activated in response to serum, starvation, or treatment with rapamycin via reduced Tor1 signaling (Lu et al. 2011, 2012; Su et al. 2013). The accumulated Brg1 recruits the Hda1 histone deacetylase to promoters of hypha-specific genes, leading to nucleosome repositioning, obstruction of Nrg1 binding sites, and sustained hyphal development (Lu et al. 2011, 2012). The conserved Tor1-signaling pathway functions as a global regulator of cellular growth in response to nutrient availability, and it controls different cellular processes in fungi (Rohde and Cardenas 2004; Rohde et al. 2008). Therefore, hyphal development is controlled by two major nutrient-responsive and growth-regulating pathways.

These same signaling pathways have been co-opted by other pathogens to regulate different phenotypic outputs that are required for pathogenesis. In contrast to *C. albicans*, in which the yeast-hyphal morphological transition is essential for pathogenesis, *Cryptococcus* uses the cAMP-signaling axis to control expression of the polysaccharide capsule, a predominantly virulence-associated phenotype (Alspaugh et al. 1997). The hyphal transition during cryptococcal mating is also dependent on cAMP-Nrg1 signaling (Cramer et al. 2006), although the detailed signaling mechanisms of this observation have yet to be defined.

In addition to PKA signaling, other kinase-directed pathways also control fungal morphological transitions and host response. The high osmolarity glycerol (HOG) mitogen-associated protein kinase pathway plays a central role in stress responses in *C. albicans* (Alonso-Monge et al. 1999; Smith et al. 2004; Arana et al. 2005; Enjalbert et al. 2006). Hog1 is activated by osmotic, oxidative, and heavy metal stress. Hog1 is required for the survival of *C. albicans* cells when they encounter these host-relevant stresses. In contrast to stress-induced rapid Hog1 activation, rapamycin treatment leads to a down-regulation of Hog1 basal activity for a prolonged period of time through the functions of the two Hog1 tyrosine phosphatases, Ptp2 and Ptp3 (Su et al. 2013). Hog1 and its upstream kinases, Pbs2 and Ssk2, play a repressive role in hyphal elongation (Alonso-Monge et al. 1999; Eisman et al. 2006). Hog1 phosphorylation/activation in response to stresses, such as osmotic or oxidative stress, blocks hyphal maintenance (Su et al. 2013). This effect is dominant over rapamycin-induced Hog1 dephosphorylation and hyphal elongation. Active Hog1 represses the expression of *BRG1* via the transcriptional repressor Sko1 (Su et al. 2013), which in turn represses the yeast-to-hypha transition (Alonso-Monge et al. 2010; Su et al. 2013). Therefore, reduced Tor1 signaling lowers Hog1 basal activity via Hog1 phosphatases to activate *BRG1* expression for hyphal elongation. The HOG pathway is required for similar stress resistance phenotypes in other fungi such as *C. neoformans*. Also, mutations in HOG signaling result in repression of hyphal differentiation in *C. neoformans* mating reactions (Bahn et al. 2005), similar to its hyphal suppressive effects in *C. albicans*.

Other environmental factors also control morphological decisions in human fungal pathogens. The combination of hypoxia and high CO_2, but neither condition alone, maintains *C. albicans* hyphal elongation, even in mutants lacking the nutrient responsive chromatin-remodeling pathway (Lu et al. 2013). One key downstream target of Hda1-mediated chromatin regulation is Ume6, a hypha-specific transcription factor that controls the level and duration of hypha-specific transcription (Banerjee et al. 2008; Carlisle et al. 2009; Zeidler et al. 2009; Lu et al. 2012). Ume6 is stabilized via regulation by Ofd1, a prolyl hydroxylase family member that is inhibited by hypoxia and an uncharacterized pathway that senses high CO_2. The Ume6 stabilization and chromatin-remodeling pathways act in parallel to govern hyphal maintenance and elongation. Virulence and hyphal elongation in vivo are attenuated only when both pathways are blocked. *C. albicans* Ofd1 acts as an oxygen sensor that regulates hyphal development in a mechanism similar to that of *S. pombe* Ofd1. Both Ofd1 proteins have two functional domains: an amino-termi-

nal dioxygenase domain with a conserved Fe^{2+} binding motif essential for O_2 sensing and a carboxy-terminal domain that promotes protein degradation. The amino-terminal domain inhibits the degradative activity of the carboxy-terminal domain in hypoxia (Hughes and Espenshade 2008; Henri et al. 2010). The closest structural homolog to the amino-terminal dioxygenase domain is human $PHD2_{cat}$, a prolyl 4-hydroxylase that acts as an oxygen-sensing component and hydroxylates the hypoxia-inducible transcription factor $HIF1\alpha$ in the presence of oxygen, thus leading to its degradation by the proteasome (Schofield and Ratcliffe 2005; Ozer and Bruick 2007; Henri et al. 2010). Ofd1 orthologs with sequence conservation in both amino- and carboxy-terminal domains are found only in fungi, including *C. neoformans* and *A. fumigatus.* The mechanism of CO_2 sensing during hyphal elongation is not clear. CO_2 is hydrolyzed into HCO_3^- inside the cell naturally and through the activity of carbonic anhydrase (Klengel et al. 2005). HCO_3^- then regulates hyphal morphogenesis through its activation of the adenylyl cyclase Cyr1 and, subsequently, the cAMP-PKA pathway (Klengel et al. 2005). HCO_3^- also signals independently of Cyr1 to regulate levels of carbonic anhydrase (Cottier et al. 2012) and promote hyphal development and cell-fate transition (Du et al. 2012a). Stabilization of Ume6 by CO_2 is likely mediated through a Cyr1-independent pathway.

GlcNAc, unlike other sugars, stimulates the yeast-to-hypha transition in *C. albicans* by activating the Cyr1-PKA pathway (Castilla et al. 1998; Gunasekera et al. 2010) through unknown sensors/receptors. GlcNAc also activates a cAMP-independent pathway to induce the expression of genes needed to catabolize itself (Fig. 3) (Yamada-Okabe et al. 2001; Kumar et al. 2009; Gunasekera et al. 2010). One such gene, *NGT1*, encodes a GlcNAc transporter that mediates hyphal induction (Alvarez and Konopka 2007), suggesting that GlcNAc has to be internalized to induce signaling. GlcNAc is also a potent and specific inducer of the yeast-to-filament transition in the thermally dimorphic pathogens, *H. capsulatum* and *B. dermatitidis* (Gilmore et al. 2013). GlcNAc transporters,

NGT1 and *NGT2*, are necessary for *H. capsulatum* cells to robustly filament in response to GlcNAc and in standard glucose medium, suggesting that Ngt1 and Ngt2 monitor endogenous levels of GlcNAc to control filamentous growth in response to temperature (Gilmore et al. 2013). Interestingly, GlcNAc metabolism is not required for GlcNAc signaling in these fungi (Naseem et al. 2011; Gilmore et al. 2013). Current data suggest that either internal GlcNAc acts as a signal or GlcNAc transporters can also function as a signal transducer. Future experiments are needed to differentiate these possibilities.

Extracellular pH is another important environmental factor that regulates fungal growth (Fig. 3). A fungal-specific signaling pathway, characterized by the activation of the Rim101/pacC transcription factor, controls the cellular response to changes in pH. First identified in *S. cerevisiae* and *A. nidulans*, this pathway is also conserved in fungal pathogens and controls important microbial interactions with the host environment. *C. albicans* grows in the yeast form in acidic conditions and forms hyphae in neutral/alkaline conditions. At neutral/alkaline pH, such as that encountered in the host, the Rim101 transcription factor is activated by proteolysis of its carboxy-terminal tail (Li and Mitchell 1997; Davis et al. 2000). Rim8, Rim13, Rim20, and Rim21 are upstream components required for Rim101 activation (Davis et al. 2000; Gomez-Raja and Davis 2012). The 7-transmembrane domain protein, Rim21, in the plasma membrane is the predicted sensor of pH. Mutation of any of these pH-responsive signaling elements in *C. albicans* results in failed morphogenesis and reduced virulence. In other ascomycetes, such as *A. fumigatus*, the Rim/Pal-signaling cascade is conserved and required for pathogenesis (Bignell 2012). Interestingly, the most upstream elements of this pathway are not clearly present in basidiomycetes, such as *U. maydis* and *C. neoformans* (Arechiga-Carvajal and Ruiz-Herrera 2005; O'Meara et al. 2013). Moreover, unlike in *Candida* species, in *C. neoformans*, the Rim/Pal-signaling cascade is not a significant regulator of the yeast–hyphal transition, although it does control major cell wall

events, thereby influencing the host–pathogen interface.

Quorum Sensing

Quorum sensing is the regulation of gene expression and group behavior in response to changes in cell-population density. Quorum sensing in *C. albicans* was established based on the observation that dense cultures display a reduced propensity for the yeast-to-hypha switch (Hornby et al. 2001). The inhibitory activity is caused by the accumulation of a sesquiterpine alcohol, farnesol (Hornby et al. 2001), formed from an intermediate of the sterol biosynthesis pathway (Hornby et al. 2003). Therefore, farnesol is a quorum-sensing molecule secreted to the medium by *C. albicans* cells as a cell density signal (Hornby et al. 2001). At concentrations of $10-250$ μM, farnesol inhibits hyphal initiation, but it does not suppress hyphal elongation (Mosel et al. 2005). Farnesol is reported to exert its inhibitory effects on germ-tube formation through Ras1-Cyr1 (Davis-Hanna et al. 2008). The release from farnesol inhibition when cells are inoculated from dense cultures into fresh media is essential for Nrg1 degradation, which requires the kinase Sok1 (Lu et al. 2014). The *SOK1* transcriptional repressor, Cup9, is rapidly degraded on release from farnesol inhibition through the E3 ubiquitin ligase, Ubr1, leading to the derepression of *SOK1* transcription and Nrg1 degradation (Lu et al. 2014). Therefore, the temporary clearing of Nrg1 protein during hyphal initiation is achieved by two independent regulations: the cAMP-PKA-dependent transcriptional downregulation of *NRG1* and degradation of Nrg1 protein triggered by the release from farnesol inhibition. Both pathways are required for the rapid clearing of Nrg1 protein and, thus, neither one is sufficient for hyphal initiation.

Contact Sensing

In *C. albicans*, the growth direction of hyphae can be dictated through contact with a surface (thigmotropism) (Gow et al. 1994). Thigmotropism is seen when growing hyphae come

into contact with a ridge. Instead of continuing through the ridge, hyphae modify their growth direction (Brand et al. 2007). This response involves two plasma membrane proteins, Mid1 and Cch1, which are components of the high-affinity calcium uptake system, and Fig. 1, a member of the low-affinity calcium system (Brand et al. 2007). The Mid1/Cch1 complex has been suggested to act as a mechanosensitive channel that takes up calcium in response to contact. Future work needs to elucidate how a localized calcium signal can modulate the activity of the Cdc42-GTPase module at the cell apex for tip reorientation.

From Transcription to Hyphal Morphogenesis

Hyphal growth requires sustained activation of Cdc42 at the growing tips. This is achieved by hypha-specific expression of a G_1-type cyclin protein Hgc1. The hypha-specific $Cdk1^{Hgc1}$ phosphorylates and prevents Rgt2 GTPase-activating protein (GAP) from localizing to hyphal tips in which Cdc42 is concentrated, resulting in a local increase of Cdc42-GTP at hyphal tips (Zheng and Wang 2004; Zheng et al. 2007). Therefore, hypha-specific expression of Hgc1 is responsible for maintaining Cdc42-GTP at the hyphal tips and for sustained polarized growth in hyphae. In *S. cerevisiae*, Bem3 and Rga2 GAPs are hypophosphorylated at the time of bud emergence by $Cdk1^{G1}$, and the inhibition of GAP activity by $Cdk1^{G1}$ phosphorylation leads to increased amounts of Cdc42-GTP and actin polarization for bud emergence (Knaus et al. 2007; Sopko et al. 2007). Therefore, inhibition of GAPs via phosphorylation by G_1/CDKs may be a conserved mechanism that promotes polarized growth in fungi (Wang 2009).

MORPHOGENESIS IN THE FUNGAL LIFE CYCLE

The morphotype switch in fungi is often a bidirectional reversible process. The plasticity in fungal morphological differentiation is very different from that in higher eukaryotes, in which cellular differentiation is often unidirectional and irreversible. The "totipotency" allows fungi

to vary the mode of differentiation in response to external signals and timing of morphological transition in their life cycle. For instance, *Aspergillus* competent hyphae can maintain vegetative growth indefinitely, or switch to conidiophores for sporogenesis when environmental conditions become permissible (light and air, etc.). Conversely, *Aspergillus* conidiophores transferred to submerged culture will revert to vigorous hyphal growth. In the black ink mushroom *Coprinopsis cinerea* and *S. cerevisiae*, the addition of ammonium can revert a reproductive state to a vegetative one (Chiu and Moore 1988a). Because morphotype changes in fungi are often an environmentally controlled flexible process, continued development requires genetic reinforcement appropriate for the cellular microenvironment. For instance, sustained polarized hyphal growth requires hyphal initiation factors followed by hyphal maintenance factors, and some factors function at both stages of hyphal development (Momany et al. 1999; Harris and Momany 2004; Lu et al. 2011; Sudbery 2011b). In *Cryptococcus*, *MAT***a** and *MAT*α isolates are both capable of initiating yeast to hypha transition (Tscharke et al. 2003; Lin et al. 2006), but the *MAT*α allele is much more efficient in sustaining hyphal growth (Wickes et al. 1996; Lin et al. 2006), resulting in more efficient and robust fruiting in α isolates. In *C. albicans*, Ras1 and Efg1 are necessary to establish the hyphal growth; Bin1, Hda1, Cln1, Pes1, Eed1, and Ume6 are required to maintain hyphal growth; and Yap1 and Spa2 are involved in both the establishment and the maintenance of hyphal growth (Martin et al. 2005; Goyard et al. 2008; Lu et al. 2011; Jacobsen et al. 2012). Similarly, initiation and maintenance factors are required for sustained hyphal growth in *Aspergillus* (Momany 2002, 2005; Fortwendel et al. 2011).

It is important to note that not all fungal development is reversible. Irreversible commitment does happen, in some cases, during fungal differentiation. Fungal cells become competent when they have internalized necessary molecules in preparation for the next developmental stage. Morphogenesis is initiated when these competent cells are exposed to inducing conditions. Continued differentiation depends on the interaction of environmental factors, like light and nutrients, with genetic factors controlling differentiation. Commitment occurs when cells undergo certain differentiation steps irrespective of culture conditions. An irreversible state can be established when morphogenesis is coupled with the completion of the fungal life cycle (Madhani and Fink 1998). For example, *U. maydis* forms a dikaryon after two mating-compatible yeast cells fuse. The dikaryon switches to hyphal growth only after signals from the host plant release the fungus from cell-cycle arrest (Garcia-Muse et al. 2004; Heimel et al. 2010). *Coprinopsis* basidia are committed to meiosis and sporulation, and they will continue that development pathway even when excised from their parental fruit body (Chiu and Moore 1988b). (Please see Heitman et al. [2014] for more discussion on the link between morphogenesis and sexual reproduction.)

MORPHOGENESIS IN COMMUNITY DEVELOPMENT

Microbial communities are comprised of a heterogeneous population with variations in morphological or physiological states (Fig. 4). A population with cells poised for different contingencies permits the exploration of new niches or an increase in population fitness during perturbations (i.e., a bet-hedging strategy). For instance, a mature *Aspergillus* colony growing on a solid substrate is comprised of several hyphal types (invasive, interface, and aerial hyphae). The formation of septa allows contrasting patterns of differentiation to exist in neighboring cells in an interconnected mycelium that was derived from a single cell. Furthermore, mycelia give rise to aerial conidiophores with multiple cell types (stalks, vesicles, metulae, phialides, and conidia). Highly polarized growth occurs exclusively at hyphal tips, whereas budding/isotropic growth occurs at conidiophores. Similarly, *C. albicans* biofilm colonies often include coexisting cells of varying morphologies such as yeast, pseudohyphae, and hyphae. The proportion of each morphotype and architecture of the biofilm greatly depend on the environmental conditions and genetic makeup
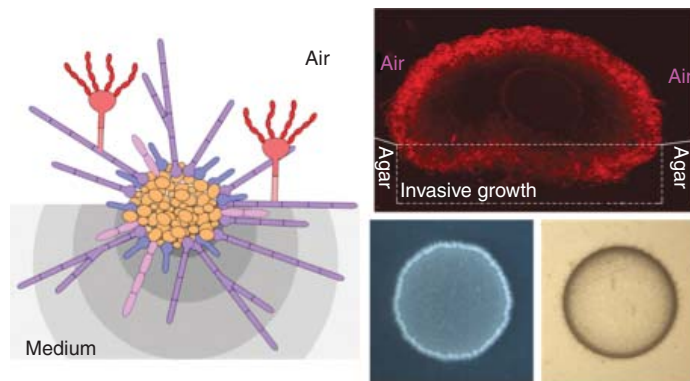
Figure 4. A fungal colony is a heterogeneous population. The *left* panel shows a diagram of a fungal colony composed of multiple morphotypes based on what is known about *Cryptococcus*. Yeast cells (orange) populate the colony center, mixed with some pseudohyphae (pink), germ tubes (blue), and hyphae (purple), which predominate at the periphery of the colony. The filamentous cells also invade the medium (invasive hyphae) or extend into the air (aerial hyphae). Some aerial hyphae further develop into fruiting bodies and produce sexual spores (red). Different microenvironments in the medium are indicated with different shades of gray. Different cell types have different cell surface composition and structure. Even for the same morphotype, cells are phenotypically and physiologically different, based on their microenvironment and spatial position. The *right upper* panel shows a confocal image of a *Cryptococcus* colony derived from a single yeast cell. The bifunctional adhesion protein Cfl1 fused with m-Cherry fluorescent protein is highly expressed in the hyphal subpopulation located at the colony periphery. Yeast cells appear dark because of low levels of Cfl1 expression. The *right lower* panel shows the colony that developed after a yeast-cell suspension was dropped on the filamentation agar. The white fluffy appearance at the colony edge is caused by the presence of aerial hyphae. Invasive growth, detected as cells that remain when the surface is washed, is shown on the *bottom right*. The invasive cells, mostly in the germ tube or hyphal form at the periphery of the colony, penetrate into agar medium forming a ring-like footprint. (Images provided by L. Wang and X. Tian, Texas A&M University.)

of the population (Daniels et al. 2013; Lin et al. 2013). A *Cryptococcus* mating colony also contains yeasts, hyphae, and basidiospores, with yeasts predominantly localized in the colony center and hyphae predominating at the periphery (Fig. 4).

Heterogeneity in gene expression serves as a foundation for the phenotypic heterogeneity in a community derived from otherwise genetically identical cells (Levy et al. 2012; Stovicek et al. 2012). The difference in gene expression among cells within a community could be caused by stochastic factors, determinant factors (e.g., age, cell-cycle stage), or a response to the microenvironment created by gradients of signaling molecules and the extracellular matrix (Kerr et al. 2002; Kim et al. 2008; Shank et al. 2011). For instance, stochastic expression of Wor1 determines white-opaque switch in *Candida* (Zordan et al. 2006; Tuch et al. 2010; Porman et al.

2013), and adhesin Cfl1 in the extracellular matrix stimulates morphogenesis from yeast to hypha in *Cryptococcus* (Wang et al. 2013). The ability to have mixed yeast and hyphal morphotypes was recently shown to enhance *Cryptococcus* competitiveness in vitro (Phadke et al. 2013).

There is an intimate connection between fungal morphogenesis and fungal community development. For instance, genes and compounds that control morphogenesis often also control biofilm development (Giacometti et al. 2011; Du et al. 2012b; Tsang et al. 2012; Connolly et al. 2013; Fazly et al. 2013). Not surprisingly, 68 out of the 122 genes that are involved in biofilm formation are also involved in filamentous growth in *C. albicans* (Inglis et al. 2012). Some of these factors, such as Ras1, Cyr1/Cdc35, and Efg1, regulate *Candida* biofilm formation, filamentous growth, phenotypic switching, and

pathogenesis (Inglis et al. 2012). In *Cryptococcus*, Znf2, the decision maker of the yeast to hypha transition and the mediator of the cryptococcal ability to cause disease, regulates more than half of the potential adhesins encoded in the genome (Wang et al. 2012, 2013; L Wang and X Lin, unpubl.). The Znf2 downstream adhesin Cfl1 also regulates the yeast–hypha transition (Fig. 4) (Wang et al. 2013). Although filamentous growth is not essential for the formation of biofilms (Hawser and Douglas 1994; Reynolds and Fink 2001; Martinez and Casadevall 2007; Cushion et al. 2009), hyphae strengthen the biofilm structure and provide strong and expanded scaffolds for the deposition of extracellular matrix materials and other cells (Ramage et al. 2002; Richard et al. 2005). Hyphae of *C. albicans* are known to penetrate host tissues and even denture materials, and thus it is not surprising that there are many hyphal-specific adhesins that help anchor hyphae firmly to the substrate. Similarly, in *T. mentagrophytes*, hyphae produce adhesion molecules to help the fungus attach to the skin surface (Kaufman et al. 2007). Even in biofilms composed of *Aspergillus* hyphae, the hyphal organization in hyphae formed during aspergilloma infection differs from that formed during invasive aspergillosis. A spheroid mass of highly agglutinated hyphae with increased α-1,3-glucans is present in the former, whereas individual separated hyphae predominate in the latter (Loussert et al. 2010). An in-depth discussion of the genetic regulation of biofilms can be found in Desai et al. (2014).

CONCLUSION

Morphological transitions are commonly observed among diverse fungal species. These cellular responses are accompanied by equally profound changes in cell physiology and structure. As noted above, the highly regulated process of fungal morphogenesis represents an adaptive response to specific stresses encountered in various microenvironments, including that of the infected host. Therefore, morphological and physiological plasticity allows fungi to rapidly adapt to changing extracellular conditions. Species-specific signaling and morpho-

logical features appear to be a direct result of fungal attempts to survive as new microenvironments, and their particular cell stresses, were encountered (Hogan and Klein 1994; Newman et al. 1995; Batanghari et al. 1998; Sebghati et al. 2000; Gow et al. 2002; Brandhorst et al. 2004; Rappleye et al. 2004, 2007; Gantner et al. 2005; Nemecek et al. 2006; Gauthier and Klein 2008; Nather and Munro 2008; Mora-Montes et al. 2011; Wang and Lin 2012; Wang et al. 2012). The concerted action of morphotype and physiological changes in the context of a particular environment are therefore critical for successful fungal adaptation (Butler et al. 2009; O'Connor et al. 2010). Defining the cellular machinery controlling fungal morphogenesis offers unique insight into our basic understanding of fungal life cycles and pathogenesis.

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