

Growth of *Candida albicans* hyphae

Peter E. Sudbery

Abstract | The fungus *Candida albicans* is often a benign member of the mucosal flora; however, it commonly causes mucosal disease with substantial morbidity and in vulnerable patients it causes life-threatening bloodstream infections. A striking feature of its biology is its ability to grow in yeast, pseudohyphal and hyphal forms. The hyphal form has an important role in causing disease by invading epithelial cells and causing tissue damage. This Review describes our current understanding of the network of signal transduction pathways that monitors environmental cues to activate a programme of hypha-specific gene transcription, and the molecular processes that drive the highly polarized growth of hyphae.

Genetic toolbox

Describing methods that can be used to investigate *Candida albicans*. As *C. albicans* is an obligate diploid, to generate null strains it is necessary to delete both copies of a gene, designated Δ in this Review. Strains with multiple auxotrophic markers have facilitated the generation of null strains. Other advances include using gene fusion to generate fluorescent and epitope-tagged proteins, and regulatable promoters.

Candida albicans is a commensal fungus that is frequently a benign member of the skin and mucosal flora. However, *C. albicans* can cause disease of mucosal membranes^{1,2}. Vulvovaginal candidiasis is commonplace and may affect up to 75% of women at least once in their lifetime³. A small subset of women (5–10%) experience chronic recurrent episodes that substantially affect their quality of life. Patients with AIDS are prone to oral and oesophageal candidiasis and such infections are also commonly associated with oral cancers, the use of dentures and terminally ill patients who fail to produce sufficient saliva². Patients suffering from burns and newborn (especially premature) babies are also subject to *C. albicans* skin infections. In vulnerable groups of patients and frail patients in intensive care units, *C. albicans* can cause a bloodstream infection known as candidaemia, which can develop into disseminated candidiasis when the infection spreads to internal organs⁴. Candidaemia and disseminated candidiasis are extremely serious medical conditions with mortality rates documented in different surveys of between 30–50%; some surveys have found them to be the second most common cause of death from nosocomial infections^{5–8}.

A striking feature of *C. albicans* is its ability to grow either as a unicellular budding yeast or in filamentous pseudohyphal and hyphal forms^{9,10} (FIG. 1). Pseudohyphae are morphologically distinguishable from hyphae because pseudohyphae have constrictions at the sites of septation and are wider than hyphae. By contrast, hyphae form long tube-like filaments with completely parallel sides and no constrictions at the site of septation (FIG. 1). As discussed later, there are also fundamental differences between hyphae and pseudohyphae in their cell cycle organization and mechanisms of polarized growth. The morphological plasticity of *C. albicans* is a virulence determinant, as the hyphal form has key roles in the infection process (BOX 1). During mucosal infections, the hyphal forms

invade epithelial and endothelial cells and cause damage, probably through the release of hydrolytic enzymes^{11–14}. Access to the bloodstream to establish candidaemia requires penetration of mucosal barriers, whereas infection of internal organs requires penetration of endothelia. *In vitro* studies with both reconstituted epithelia and endothelia show that it is specifically the hyphal form that is invasive¹¹. In addition, biopsy samples from patients with mucosal infection show that only hyphal forms are found in epithelial cells¹⁵. Furthermore, when yeast cells are engulfed by macrophages they escape by switching to the hyphal form¹⁶. Despite these documented roles of hyphae during infection, whether the hyphal form is necessary for virulence is still controversial (BOX 2).

In the past 10 years, important technical advances have facilitated the investigation of the cell and molecular biology of hyphal induction and growth, and of hyphal interactions with the human host. These advances include the availability of genomic and transcriptomic sequence data, improvements in the genetic toolbox (reviewed in REF. 17) and advances in live cell imaging. Our understanding has been enhanced by studies in model fungal organisms such as the budding yeast *Saccharomyces cerevisiae* and filamentous species such as *Neurospora crassa* and *Aspergillus nidulans*. This Review describes how these studies are providing increasing information about the signal transduction pathways that induce hyphal growth, the molecular and cell biology of hyphal growth itself, the role of hyphal growth during the infection process and the way that the host responds to such infections.

Controlling hyphal gene transcription

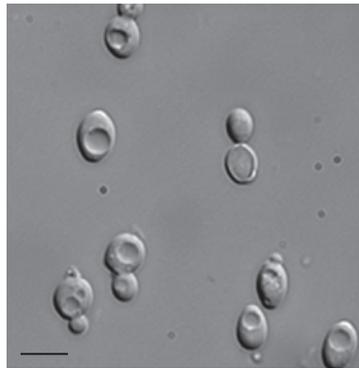
Environmental cues inducing hyphal growth. *C. albicans* is exquisitely adapted to growth in its human host and forms hyphae under a range of environmental conditions

Department of Molecular Biology and Biotechnology, Sheffield University, Western Bank, Sheffield S10 2TN, UK.
e-mail: P.Sudbery@shef.ac.uk
doi:10.1038/nrmicro2636
Published online
16 August 2011;
corrected 20 August 2011

Pseudohyphae



Yeast



Hyphae

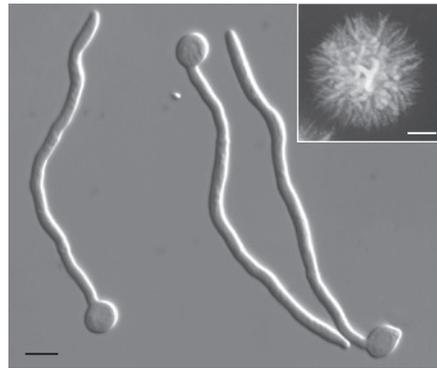


Figure 1 | Morphology of yeast, hyphal and pseudohyphal forms. The inset in the hyphae panel shows the appearance of a hyphal colony that has been growing for 5 days on Spider medium. Scale bars in the main panels represent 5 μm , and in the inset on the hyphae panel represents 1 mm. Figure is reproduced, with permission, from REF. 144 © (2008) Wiley-Blackwell.

that reflect the diversity of the microenvironments that it encounters in the host. For instance, hyphae form in response to the presence of serum¹⁸, neutral pH¹⁹, 5% CO₂ (the partial pressure of CO₂ in the bloodstream)²⁰, *N*-acetyl-D-glucosamine (GlcNAc)²¹ and growth in an embedded matrix or in microaerophilic conditions under a coverslip in strains lacking the transcriptional regulator enhanced filamentous growth protein 1 (Efg1)^{22,23}. In addition, hyphal growth is often induced in synthetic growth media such as Lee's medium (which contains a mixture of amino acids)²⁴, Spider medium (a semi-synthetic medium based on mannitol as a carbon source)²⁵ and mammalian tissue culture media such as M199. Generally, hyphal growth requires a temperature of 37 °C; an exception is filamentation in an embedded matrix and hypoxic growth, which occurs at 25 °C. The serum and 37 °C combination generates a powerful and robust signal for germ tube formation from yeast cells and forms the basis for a classic diagnostic test for the presence *C. albicans* in medical microbiology.

Two different stages of hyphal growth can be experimentally distinguished. The first stage is the ability to establish and maintain hyphal growth in the short term. This involves the production of germ tubes and hyphae in liquid medium when yeast cells are challenged with hypha-inducing conditions such as serum at 37 °C (FIG. 1). In this case, the experimental observations are restricted to a few hours after hyphal induction. The second stage is the ability to maintain hyphal growth in the long term by assessing colony morphology after 5 days growth on a solid medium such as Spider medium or in serum at 37 °C. Hyphal formation on solid medium is manifested by feathery or spidery outgrowths from

Germ tube

In this Review, a narrow, tube-like projection from a mother yeast cell that forms up to the end of the first cell cycle when an unbudded yeast cell is placed in hypha-inducing conditions.

the main colony, which has an extensively crenulated or crinkled appearance (FIG. 1). Mature hyphal colonies will invade the agar, a phenomenon that can be readily tested by examining the persistence of cells in the agar after the surface colony has been washed off. The ability to form hyphae is often assessed either by the ability of the fungus to form hyphae in liquid culture or by the production of hyphal colonies on solid medium. The liquid culture assay tests the immediate ability of the organism to respond to environmental cues and initiate the highly polarized growth characteristic of hyphae. The solid medium assay tests the ability of the organism to maintain hyphal growth in the complex and changing microenvironments in and around a colony as it develops. It also tests the ability to invade a solid substratum. It takes into account factors such as the changing availability of nutrients, oxygen tension, CO₂ generation and the production of, and response to, quorum sensing molecules. A mutation that does not directly affect the hyphal growth process could nevertheless affect hyphal growth indirectly as a result of a change in any of these complex microenvironments.

The morphological switch between yeast and hyphal forms is also regulated by interaction with the microbiological flora that *C. albicans* encounters in its environment. Sophisticated mechanisms result in communication not only with other *C. albicans* cells but also with bacterial cells (reviewed in REF. 26). *C. albicans* cells sense the density of the surrounding *C. albicans* population by a quorum sensing mechanism that is based on the sesquiterpene farnesol, which is secreted into the environment and inhibits hyphal formation²⁷. By contrast, the aromatic alcohol tyrosol shortens the lag phase of quiescent yeast cells and promotes germ tube formation and hyphal formation in biofilms²⁸. *C. albicans* also frequently forms mixed infections with bacterial species. For example, *C. albicans* is often found in combination with *Pseudomonas aeruginosa* in biofilms forming in catheters, and both species are often recovered from lung infections of patients with cystic fibrosis or infections of patients with burns²⁹. *P. aeruginosa* cells attach to *C. albicans* germ tubes and form a biofilm that results in the death of the germ tube. This process is specific to germ tubes; the yeast form is not affected. In response, *C. albicans* senses *P. aeruginosa* by the presence of 3-oxo-homoserine lactone, which is a component of the *P. aeruginosa* quorum sensing mechanism; this leads to the repression of hyphal formation and the promotion of yeast-form growth^{29–32}.

Regulation of hyphal morphogenesis. The pathways that operate to transduce hypha-inducing signals and the transcription factors that are targeted have been reviewed recently in detail³³, and a summary is provided in FIG. 2. Hypha-specific gene expression is negatively regulated by a complex consisting of the general transcriptional corepressor Tup1 in association with Nrg1 or Rox1p-like regulator of filamentous growth (Rfg1) (REFS 34–38) (FIG. 2). Cells lacking any of these of repressors grow constitutively as long pseudohyphae, and expression of hypha-specific genes is derepressed. Positive regulation of hypha-specific gene expression is

Box 1 | The role of hyphae during infection

Candida albicans yeast forms are commonly found on the mucosal and skin surfaces, where they grow benignly and are tolerated by the host immune system. Mucosal disease involves the adherence and invasion of epithelial cells, and results in tissue damage (reviewed in REF. 14). Invasive hyphal forms are not tolerated by the immune system and induce a specific immune response that is mediated by macrophages¹³⁶. Systemic candidaemia is thought to originate from the gastrointestinal tract¹³⁷ and therefore involves invasion of endothelial cells that line the gastrointestinal tract. Yeast cells adhere to both oral and intestinal epithelia and then rapidly switch to hyphal growth¹¹. Adherence is mediated by a range of cell wall proteins that are expressed on the surface of hyphal cells. These include members of the agglutinin-like (Als) family of adhesins such as Als3 and Als5 (REF. 138), hyphal wall protein 1 (Hwp1), which forms covalent bonds with the host cell through the action of host cell transglutaminases¹³⁹, and eIF4E-associated protein 1 (Eap1), which is a hypha-specific protein that confers adhesive properties to *Saccharomyces cerevisiae* cells when heterologously expressed¹⁴⁰. Hyphal but not yeast cells are found in epithelial cells at sites of infection¹⁵. Two separate routes of epithelial cell invasion have been shown to operate¹¹. First, hyphal cells induce endocytosis by the host cell¹⁴¹. This is a process that requires the adhesin Als3 (REF. 13); however, endocytosis of hyphal cells is not an active fungal process since heat-killed cells are still taken up by this route. It is an active process by the host cells and requires the presence of an active endocytosis pathway and accumulation of microfilaments around the site of endocytosis leading to the extrusion of pseudopodia that envelop the germ tube. (The fate of the endocytosed *C. albicans* cells has not been investigated.) The second route of hyphal invasion of epithelial cells is active penetration of the plasma membrane. This process requires live fungal cells¹¹. Invasion of oral epithelial cells occurs through both routes, with induced endocytosis occurring predominantly soon after contact. By contrast, active penetration is the only route by which *C. albicans* invades intestinal epithelial cells¹¹.

carried out by a panel of transcription factors, including Efg1 (REF. 39), Cph1 (REFS 25,40), Cph2 (REF. 41), Tec1 (REF. 42), Flo8 (REF. 21), Czf1 (REF. 43), Rim101 (REFS 44–46) and Ndt80 (REF. 47) (FIG. 2). Efg1 is required for hyphal formation in response to serum, CO₂, neutral pH and GlcNAc in liquid media, and on solid media such as Spider medium^{39,48}. Cph1 and its upstream activating pathway are only required for hyphal formation on solid Spider medium but not in liquid media^{25,40}. Consequently, Efg1 is thought to be the major regulator of hyphal formation under most conditions⁴⁹. Efg1 and Cph1 are activated by distinct upstream signalling pathways. In the case of Efg1 the pathway is based on cyclic AMP, whereas in the case of Cph1 the pathway depends on a mitogen-activated protein kinase (MAPK) signalling pathway (FIG. 2). Ras1 stimulates both the cAMP and MAPK pathways^{50,51}.

C. albicans expresses a single adenylyl cyclase, which is encoded by *CYR1* (also known as *CDC35*). Cyr1 integrates environmental signals from a range of sources and is essential for hyphal formation but not yeast-form growth⁵² (FIG. 2). Serum-mediated induction of hyphal formation apparently stimulates Cyr1 in two separate ways. The first way is through Ras1, which binds to a conserved Ras-association domain in Cyr1 (REF. 53). Second, bacterial peptidoglycan, which has been shown to be present in serum, stimulates Cyr1 directly in a Ras1-independent fashion, by binding to a leucine-rich repeat region similar to that found in the innate immunity proteins of higher organisms, such as toll-like receptor 1 (TLR1) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2)⁵⁴. CO₂ and HCO₃⁻ also stimulate Cyr1 activity directly by interacting with

its catalytic domain⁵⁵. Cyr1 forms a tripartite complex along with adenylyl cyclase-associated protein 1 (Cap1) and actin monomers (G-actin)⁵⁶. This observation raises the possibility that once hyphal formation has been initiated there may be feedback from the polarized actin cytoskeleton at the hyphal tips to the signalling pathway that induces hyphal growth.

The response to the quorum sensing compounds farnesol and dodecanol may also be mediated by the cAMP pathway, as the repressive effects of these compounds on hyphal formation are reversed by the addition of exogenous cAMP⁵⁷. Farnesol and dodecanol seem to act downstream of Ras1 as they still inhibit hyphal formation in a strain that carries a dominant active *RAS1*^{13V} allele, which causes hyperactivation of the cAMP pathway and constitutive hyphal formation⁵⁰. Consistent with this observation, both farnesol and 3-oxo-homoserine lactone inhibit the catalytic activity of recombinant adenylyl cyclase *in vitro*⁵⁸. By contrast, dodecanol seems to act through the transcription factor Sfl1, which has been shown to be a negative regulator of hyphal formation⁵⁸. Farnesol may also act through the negative regulators of hyphal formation because neither *tup1*^{-/-} nor *nrg1*^{-/-} mutants respond to the presence of farnesol and both mutants continue to show the constitutive pseudohyphal phenotype that is characteristic of these mutants. Furthermore, *TUP1* transcription is induced by farnesol in wild-type cells⁵⁹.

Elevated temperature is a requirement of all hypha-inducing conditions except growth in an embedded matrix. Temperature seems to be sensed by heat shock protein 90 (Hsp90), as pharmacological inhibition of Hsp90 by geldanamycin leads to hyphal growth⁶⁰. Furthermore, strains that are engineered to have a moderate reduction in Hsp90 levels form hyphae in response to serum at 30 °C rather than 37 °C. Hsp90 signalling requires an intact cAMP pathway as a mutation in any of the components upstream of Efg1 blocked the hypha-inducing effects of Hsp90 inhibition. Interestingly, an *efg1*^{-/-} mutant still formed hyphae when Hsp90 was inhibited, suggesting that an alternative downstream target is activated by Hsp90 signalling.

Key outputs of the signal transduction pathways. The signal transduction pathways described above result in a programme of hypha-specific gene transcription. [Supplementary information S1](#) (table) lists the combined data from two studies that used microarray analysis to identify genes that were upregulated when yeast cells were induced to form hyphae by incubation at 37 °C in the presence of serum^{35,61}. The most highly expressed genes encode the glycosylphosphatidylinositol-anchored cell wall protein hyphal wall protein 1 (Hwp1), the secreted aspartyl protease (SAP) family proteins Sap4, Sap5 and Sap6, adhesin agglutinin-like protein 3 (Als3) and cell elongation protein 1 (Ece1), which is a protein of unknown function. Although it likely that these proteins will collectively give rise to the special properties of hyphae that are important for virulence, none of these genes alone are required for hyphal formation and maintenance. However, three genes that are induced

Box 2 | Are hyphae necessary for virulence?

Despite the roles of hyphae during infection, described in BOX 1, formal proof that the hyphal form is required for virulence has proved difficult to obtain, and there is a countervailing view. It is important to note that virulence can be defined experimentally by different infection models. The most commonly used is the mouse tail vein injection model of haematogenously disseminated candidiasis. In this model, *Candida albicans* cells are injected directly into the bloodstream and so its ability to invade epithelia is not tested. Moreover, the model is uninformative about their capability to cause mucosal disease. Other models such as the rat vaginal infection model or the *ex vivo* model of oral mucosal infection may be informative about different aspects of virulence. Formal proof is sometimes inferred from the observation that a double mutant for the enhanced filamentous growth protein 1 (*efg1*) and *cph1* genes, which is unable to form hyphae in most environments, is avirulent in the mouse tail vein infection model⁴⁸. The problem with this observation is that this mutant will also be defective for the whole of the transcription programme that accompanies hyphal growth, such as the expression of cell wall proteins, adhesins and secreted aspartyl proteases. Moreover, such mutants can still be filamentous when growing embedded in a matrix, which may allow the formation of hyphae *in vivo* at crucial stages of the infection. A similar argument applies to the use of a construct in which expression of the *NRG1* repressor was forced using the regulatable TET promoter, thus locking the cells into the yeast state⁴². However, an interesting observation from this study was that the yeast form was able to transit from the bloodstream into internal organs, suggesting that, contrary to expectation, the yeast form is capable of invasion. (It is possible that hyphae formed but were not detected in this experiment.) Perhaps the best formal proof that hyphae are an important virulence factor is the avirulence of the hyphal G1 cyclin protein 1 (*HGC1*)-deficient strain, which is unable to form normal hyphae but in which expression of a panel of other hypha-specific genes was not affected⁶⁷.

There remain two arguments that challenge the view that hyphae are essential for virulence. First, *Candida glabrata*, which is the second most common cause of serious life-threatening yeast infections, does not form hyphae so hyphal growth is not essential in principle for pathogenicity. Second, a recent systematic study generated deletions affecting 674 genes (11%) of the *C. albicans* genome¹⁴³. The relative virulence of cells with these deletions was examined by a competition experiment in which pools of bar-coded deletion strains were injected into mice. Mutants with reduced virulence were identified through their selective depletion in the host. The deletion collection was also screened for the ability of mutant strains to produce filamentous colonies on Spider medium. The pool of filamentation-defective mutants did show an enriched presence in the pool of virulence-defective mutants, but the correlation was imperfect. Of the 24 mutants showing the most profound defect in filamentation, two-thirds showed normal virulence, casting doubt on the hypothesis that filamentation is necessary for virulence. However, there are two issues with this experiment. First, filamentation was only assessed by the appearance of colonies on Spider medium, which tests the long-term ability to maintain hyphal growth in the complex microenvironments in a colony, but not the ability to form hyphae in the short term. Second, the virulence testing was restricted to the mouse infection model and other infection models, which test the ability to invade epithelia, may have generated different results.

have a key role in hyphal formation: *UME6*, hyphal G1 cyclin protein 1 (*HGC1*) and *EED1*. The null mutants *eed1*^{-/-} and *ume6*^{-/-} each initiate germ tube formation in liquid culture on hyphal induction, but hyphal growth cannot be maintained^{62,63}. On solid Spider medium these mutants form smooth colonies with no evidence of filamentation. *UME6* and *EED1* are negatively regulated by Tup1 and Nrg1 (REFS 63–65). *UME6* expression is reduced on hyphal induction in the absence of any one of the transcription factors that mediate the expression of hypha-specific genes, such as *Efg1*, *Cph1*, *Cph2*, *Czf1* and *Flo8* (REF. 66). Importantly, ectopic overexpression of *UME6* leads to hyphal formation in conditions that normally favour yeast-form growth⁶⁴. The molecular function of *Eed1* remains unknown but it is required

for expression of *UME6* (REF. 62). Overexpression of *EED1* partially rescues the filamentation defect of an *efg1*^{-/-} mutant, and overexpression of *UME6* rescues the phenotype of an *eed1*^{-/-} mutant. Thus, *Eed1* seems to act in a pathway between *Efg1* and *Ume6*. *Hgc1* is the cyclin partner of the kinase cell division control 28 (*Cdc28*) (also known as *Cdk1*)⁶⁷. On hyphal induction *hgc1*^{-/-} mutants only form very short germ tubes before reverting to non-polarized growth⁶⁷. *Hgc1* has multiple roles in the polarized growth and repression of cell separation that are characteristic of hyphae (reviewed later). Expression of *HGC1* is detectable within 5 minutes of hyphal induction, whereas expression of *UME6* is not detectable until 15 minutes after induction⁶⁵. In an *ume6*^{-/-} mutant, *HGC1* expression is initially induced but is not sustained as it is in a *UME6* strain. Thus maintenance of *HGC1* expression is dependent on *Ume6*, but the initial induction must depend on an alternative *Ume6*-independent pathway. The hypha-specific gene expression of *Hgc1* provides a partial explanation of how the signal from the transduction pathways results in activation of the cell pathways that lead to hyphal growth, which are described in the following section. However, it is likely that other genes that show hypha-specific gene expression also have key roles in hyphal growth in ways that have yet to be recognized.

Cell biology of hyphal growth

When unbudded yeast cells are induced to form hyphae, a single germ tube evaginates from the mother cell (FIG. 3). The incipient point of evagination is marked by a patch of septins, which then form a band at the base of the germ tube and a cap at its elongating tip^{68,69} (FIG. 3a,e). The germ tube elongates exclusively from its tip and thus exhibits highly polarized growth⁷⁰. Evagination occurs before the start of the cell cycle; therefore, the first cell cycle is initiated when the germ tube has already formed and may be as long as 15–20 μm ^{71,72}. Apparently coincident with the start of the cell cycle, the cap of septins at the germ tube tip forms a ring around the germ tube that remains fixed in position as the tip continues to elongate^{68,71} (FIG. 3b,e). The nucleus in the mother cell then migrates into the germ tube, where the first mitosis occurs (FIG. 3b,e); after mitosis, one daughter nucleus migrates back into the mother cell, while the other nucleus migrates to a position that is on the apical side of the septin ring^{68,71} (FIG. 3e). The septin ring divides into two rings between which an actomyosin ring is formed; this ring is composed of actin and myosin 1 (*Myo1*; which is a type II myosin) with its regulatory side chain, myosin light chain 1 (*Mlc1*),^{73,74}. The actomyosin ring contracts (Supplementary information S2 (movie)), guiding the formation of the primary septum between the two septin rings, which are composed of chitin made by chitin synthase 1 (*Chs1*) or *Chs3*. (REF. 75) (FIG. 3c). Following the formation of the primary septum, invaginating cell wall growth forms secondary septa. After the formation of the secondary septa, the primary septum is not hydrolysed, as it is in yeast cells, so the two daughter compartments remain firmly attached to each other. Cytokinesis does not result in any constriction, so the

Septins

A family of related proteins that form structures consisting of heteromeric filaments; first identified in *Saccharomyces cerevisiae*, in which they form a ring at the bud neck. Just before cytokinesis, the ring splits in two and this organizes the formation of the septum. The septin ring also acts as a diffusion barrier to the movement of proteins along the inner side of the plasma membrane.

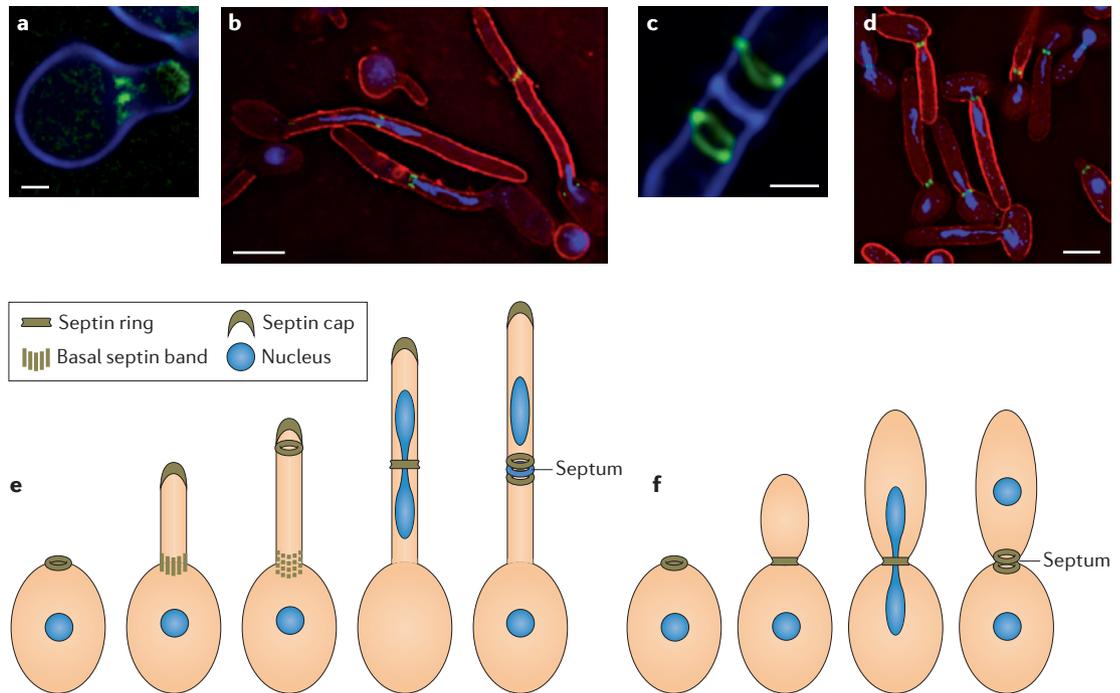


Figure 3 | Cell biology of hyphal development. **a** | As the mother cell germinates, septin bars are visible at the germ tube base, as well as a septin cap at the tip. Septin is visualized by cell division control 10 (Cdc10)–yellow fluorescent protein (YFP; green) and the hyphae are outlined by counterstaining with Concanavalin-Alexafluor 388 (blue). **b** | As the germ tube extends, the septin bars (visualized with Cdc10–YFP) disappear and a septin ring appears in the hypha, and the nucleus (stained with 4',6-diamidino-2-phenylindole (DAPI); blue) migrates out of the mother cell to undergo mitosis in the germ tube. Hyphae are outlined by counterstaining with Concanavalin-Texas Red. **c** | The septin ring divides in two and the septum forms between the two rings. Components stained as in part **a**. **d** | In pseudohyphae, the septin ring and septum form across the mother bud neck, forming the plane across which nuclear division takes place. Components stained as in part **a**. **e** | Schematic of the first cell cycle of hyphal formation. **f** | Schematic of a pseudohyphal cell cycle. Scale bars in parts **a** and **c** represent 1 μm and in parts **b** and **d** represent 5 μm . Images in parts **a–d** are reproduced, with permission, from REF. 144 © 2008, Wiley-Blackwell.

(FIG. 4d). The exocyst components may act to tether vesicles that have exited the Spitzenkörper and encountered the surface at the tip (FIG. 4a). A prediction of this model is that vesicle components will be highly dynamic, as there is a constant flux of vesicles arriving and exiting the Spitzenkörper, whereas polarisome and exocyst components will be more static. This prediction is supported by experiments using fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) experiments⁸³. Exocyst components were less dynamic than Spitzenkörper components but more dynamic than polarisome components, which suggests that exocyst components are transported to the cell surface on vesicles, but that not all vesicles carry exocyst components. Treatment with the actin-disrupting drugs cytochalasin A^{73,86} or latrunculin A⁸⁷ causes tip swelling as polarized growth reverts to isotropic growth. In the case of latrunculin A treatment, GFP–Cdc42 became dispersed from the tip⁸⁷. Long microtubules run the length of the hypha⁸⁸; however, there are conflicting reports as to whether microtubule inhibitors such as benomyl and nocadazole affect hyphal extension^{73,86,89,90}. Thus, the role of microtubules in hyphal tip growth is presently unclear.

Actin patches which form the sites of endocytosis are also polarized towards the tip (FIG. 4e). Endocytosis is

important for hyphal formation because deletion of genes encoding the actin cytoskeleton-regulatory complex protein Sla1 (REF. 91), Sla2 (REF. 92) the actin cytoskeleton-regulatory complex protein Pan1 (REF. 93), Wal1 (REF. 94), verprolin (Vrp1) (REF. 95) or Myo5 (REF. 96) caused defects in both hyphal growth and endocytosis (the *S. cerevisiae* orthologues of these proteins localize to the actin patches that form the sites of endocytosis⁹⁷). Interestingly, deletion of the gene encoding either actin-related protein 2 (Arp2) or Arp3 also leads to an inability to form hyphae, but endocytosis as judged by FM4-64 uptake still occurred, albeit after a delay⁹⁸. The requirement for endocytosis may reflect a necessity to reabsorb excess membrane that is inserted into the tip by the fusion of secretory vesicles or the need to recycle membrane proteins, such as v-SNAREs carried by secretory vesicles and will be required for further cycles of vesicle fusion. These possibilities are not mutually exclusive.

In *S. cerevisiae*, Cdc42 and related Rho family GTPases orchestrate polarized growth and morphogenesis at multiple levels, including polarization of the actin cables and patches to sites of polarized growth, formation of the exocyst complex, docking of secretory vesicles, the formation of the septin ring before bud emergence, and activation of the MAPK module through Ste20 during

v-SNAREs

(Vesicle-membrane soluble N-ethyl-maleimide-sensitive attachment protein receptors). Highly α -helical proteins that mediate the specific fusion of vesicles with target membranes. SNAREs have been classified into two complementary classes that are referred to as vesicle-membrane SNAREs (v-SNAREs) and target-membrane SNAREs (t-SNAREs).

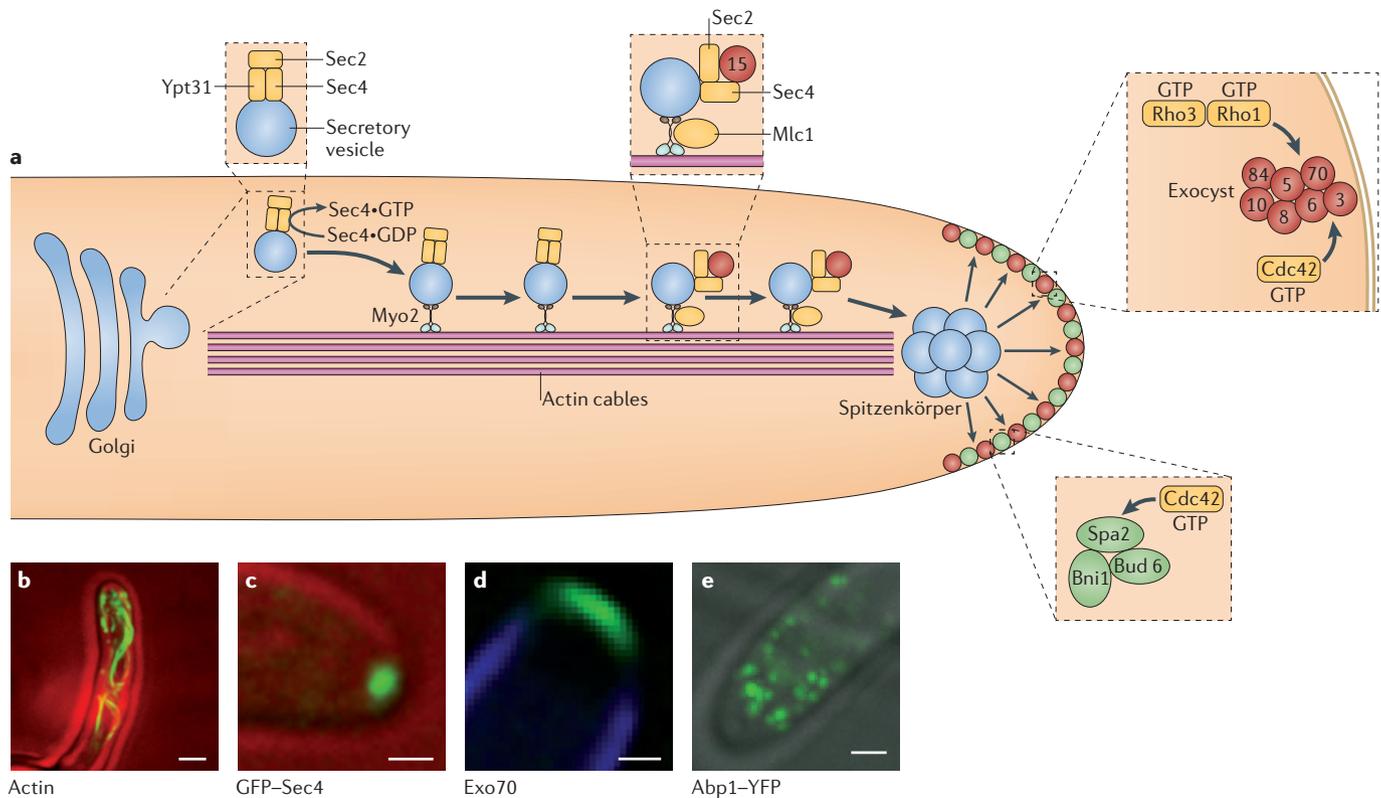
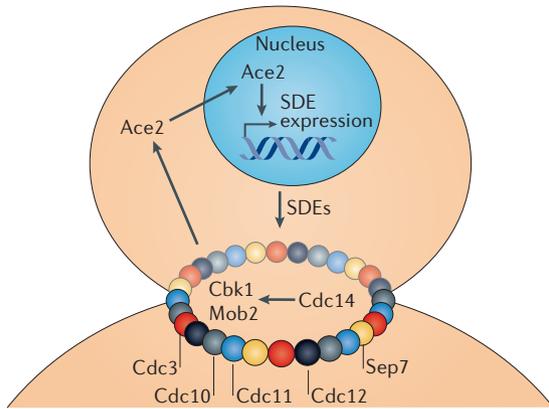


Figure 4 | The polarized growth machinery. **a** | Model of secretory vesicle delivery to the hyphal tip incorporating knowledge of polarized growth from *Saccharomyces cerevisiae* (reviewed in REF. 99). At the *trans*-Golgi network (TGN), the GTPase Ypt31 recruits Sec2 to the surface of the nascent vesicle. Sec2 activates the Rab GTPase Sec4, and the vesicle is licensed to leave the TGN. The exocyst component Ypt31 is displaced by Sec15, which physically interacts with both Sec2 and Sec4. The vesicles are transported towards the tip along actin cables. These cables are nucleated by the formin Bni1, assisted by the polarisome, which is located at the cell surface and consists of Spa2 and bud site selection protein 6 (Bud6)^{145–148}. Note that there is no *Candida albicans* homologue of the Pea2 protein, which is found in the *S. cerevisiae* polarisome. Myosin 2 (Myo2; a type V myosin), which forms a complex with the regulatory myosin light chain 1 (Mlc1), provides the motive force for vesicle transport. The vesicles accumulate in a subapical cluster called the Spitzenkörper before docking with the exocyst, which is composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 (for clarity, the exocyst components are shown by red spheres with a number without the Sec or Exo prefix). Initial contact upon docking is thought to be between Sec15 and Sec10. Fusion with the plasma membrane is mediated by a v-SNARE (Sec9) on the vesicle and target-membrane SNAREs (Sec9 and Sso2) on the plasma membrane. The Rho GTPase cell division control 42 (Cdc42) orchestrates multiple functions that are required for polarized growth, as described in the text. **b** | The actin cytoskeleton as revealed by GFP–Lifeact fusion protein¹⁴⁹, which was expressed from the constitutive *TEF1* promoter. Note cables arising at a region near the tip and extending to subapical regions but not the full length of the hypha. Actin patches are also visible that are absent from the region at the very apex of the hypha (seen at high magnification in panel **e**). **c** | Localization of the vesicle-associated protein GFP–Sec4 to an apical spot. **d** | Localization of the exocyst protein Exo70 to a surface crescent. **e** | Actin patches visualized using Abp1–YFP by differential interference contrast microscopy. Scale bar in all images is 1 μm . Image in part **b** is courtesy of S. Lensing, D. Caballero-Lima and J. Greig, University of Sheffield, UK, and P.E.S. Images in parts **c** and **d** are reproduced, with permission, from REF. 83 © (2010) American Society for Microbiology. Image in part **e** is courtesy of S. Lensing and D. Caballero-Lima.

pseudohyphal growth (reviewed in REFS 99, 100). In *C. albicans*, Cdc42 and its guanine exchange factor (GEF) Cdc24 localize to the tips of hyphae^{73,87,101} and are required for viability^{102,103}. However, decreasing the expression of Cdc42 or Cdc24 using a regulatable *MET3* promoter revealed that a reduced level of expression permits vegetative yeast-form growth but not hyphal growth¹⁰². Cdc42 GTPase-activating proteins (GAPs) in *C. albicans* are encoded by *RGA2* and bud emergence protein 3 (*BEM3*). A mutant lacking both of these GAPs formed hyphae under conditions that would normally

only allow pseudohyphal formation¹⁰⁴. Rga2 is phosphorylated by the Hgc1–Cdc28 kinase, which prevents its localization to the hyphal tip¹⁰⁵, providing one mechanism by which a hypha-specific gene (that is, *HGC1*) could lead to the sustained polarized-growth characteristic of hyphae. In *S. cerevisiae*, localized activation of Cdc42 depends on Cdc24; this activation is also dependent on another GTPase, the Ras-related protein Rsr1, its GEF bud site selection protein 5 (Bud5) and its GAP Bud2 (REF. 99). *C. albicans* mutants that lack Rsr1 or Bud2 form hyphae that are wider than wild-type hyphae and

a Yeast cells



b Hyphae

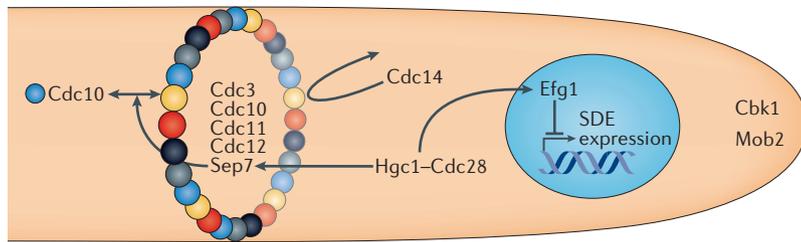


Figure 5 | Mechanism of cell separation suppression in hyphae. a | Cell separation in yeast cells. The five septin subunits that form the ring, cell division control 3 (Cdc3), Cdc10, Cdc11, Cdc12 and Sep7, are denoted by five different coloured spheres. Cdc14 at the septin ring dephosphorylates Mob2–Cbk1, which then licenses Ace2 to enter the nucleus, where it transcribes genes encoding septum-degrading enzymes (SDEs). These enzymes hydrolyse the primary septum, allowing mother and daughter to separate. **b** | Hyphal G1 cyclin protein 1 (Hgc1)–Cdc28 acts in two ways to suppress degradation of the primary septum. First, it phosphorylates enhanced filamentous growth protein (Efg1), which then binds to Ace2-target genes, inhibiting their transcription. Second, it phosphorylates Sep7, which then modifies the properties of the septin ring so that Cdc10 becomes dynamic. In this state, Cdc14 is unable to localize to the septin ring and Mob2–Cbk1 at the septum cannot license Ace2 to enter the nucleus. Mob2–Cbk1 remains at the hyphal tip, where it promotes polarized growth.

are unable to maintain directed growth¹⁰⁶. This observation suggests that Cdc24 localization is partly, but not fully, dependent on Rsr1 and Bud2. These mutants are also defective in hyphal guidance, showing a complete lack of thigmotropism and galvanotropism¹⁰⁷. Two other Rho-family GTPases play a part in hyphal growth. Rho3 is required for actin polarization and polarized growth¹⁰⁸, and Rac1 and its GEF, Dck1, are required for hyphal growth when embedded in a matrix^{109,110}.

Control of septation in hyphae. Hyphal growth is characterized by the inhibition of cell separation after cytokinesis, resulting in a mature hypha that consists of a linear chain of elongated cells. Research in *S. cerevisiae* has shown that cell separation occurs in yeast cells when the primary septum, which is composed of chitin, is hydrolysed by septum-degrading enzymes (SDEs) (such as a chitinase encoded by endochitinase 1 (*CTS1*) and a glucanase encoded by *DSE4*), leading to separation of the mother and daughter cells. Expression of SDEs is daughter cell specific and is programmed by the transcription factor Ace2, which is targeted to the daughter nucleus by the action of a kinase, Cbk1, and its regulatory subunit Mob2.

In turn, expression of Cbk1 is dependent on triggering of the mitotic exit network by the release of the phosphatase Cdc14 from the nucleolus¹¹¹.

In *C. albicans*, the inhibition of cell separation in hyphae is marked by a change in the properties of the septin ring and the action of the hypha-specific cyclin, Hgc1. Cdc14 translocates to the septin ring in the bud neck after mitosis in yeast cells but not to the septin ring in hyphae¹¹² (FIG. 5). The exclusion of Cdc14 from the hyphal septin ring depends on Hgc1. In yeast cells, FRAP experiments show that Cdc3, Cdc10, Cdc12 and Sep7 subunits of the septin ring are static (that is, no exchange takes place with free subunits)¹¹³. However, in hyphae the Cdc10 subunits are dynamic. Sep7 seems to play a key part in modifying the properties of the hyphal septin ring, as in *sep7*^{-/-} mutants Cdc10 became less dynamic and the expression of SDEs and consequent cell separation increases. Moreover, phosphorylation of Sep7 is at least in part dependent on Hgc1. Hgc1 has another role in controlling cell separation after cytokinesis by phosphorylating the transcription factor Efg1, resulting in the downregulation of SDE expression. On phosphorylation by Hgc1–Cdc28, Efg1 associates with Ace2-target promoters, presumably acting to suppress transcription of the SDE-associated genes¹¹⁴. A model for cell separation is shown in FIG. 5, in which the recruitment of Cdc14 to the septum in yeast is posited to activate Mob2–Cbk1 by dephosphorylation, allowing Ace2 to be targeted to the nucleus and the consequent expression of SDEs. In hyphae, Cbk1 is localized to the tip, where it promotes polarized growth. It is also still localized to the septum. However, the Sep7- and Hgc1-dependent exclusion of Cdc14 from the septum and the action of Hgc1–Cdc28 on Efg1 prevent activation of the SDEs.

Hyphal growth and the cell cycle. Polarized growth in hyphae is continuous throughout the ensuing cell cycle, in contrast to yeast and pseudohyphal buds⁷⁰. Accordingly, in hyphae the actin cytoskeleton remains continuously polarized^{72,115} and the polarisome and Spitzenkörper are continuously present⁷³ (Supplementary information S2 (movie)). An extended G2 phase is thought to be responsible for the elongated growth of pseudohyphal buds in *S. cerevisiae*¹¹⁶. However, this does not explain the highly polarized growth of *C. albicans* hyphae because after the cell cycle is initiated there is little difference in the timing of progression of the S and G2 phases when yeast cells and hyphal cells are compared. Moreover, there is no change in the degree of inhibitory Tyr18 phosphorylation of Cdc28 mediated by the mitosis inhibitor protein kinase Swe1 (REF. 72). Consistent with this observation, deletion of Swe1 does not abolish hyphal growth¹¹⁷.

Although alteration of cell cycle kinetics does not seem to have a role in normal hyphal growth, a striking feature of *C. albicans* biology is that a range of different mutations or drug treatments that interrupt nuclear cycle progression result in unscheduled polarized or highly polarized growth. In some cases, these phenotypic alterations are accompanied by the expression of hypha-specific genes (reviewed in REF. 118). To some extent, the

Thigmotropism

The ability of hyphae to sense and grow along topographical cues in the environment such as cracks and ridges. This ability may help growth towards entry points in epithelia and endothelia.

Galvanotropism

The ability to sense and orientate along an electric field; *Candida albicans* hyphae grow towards the cathode.

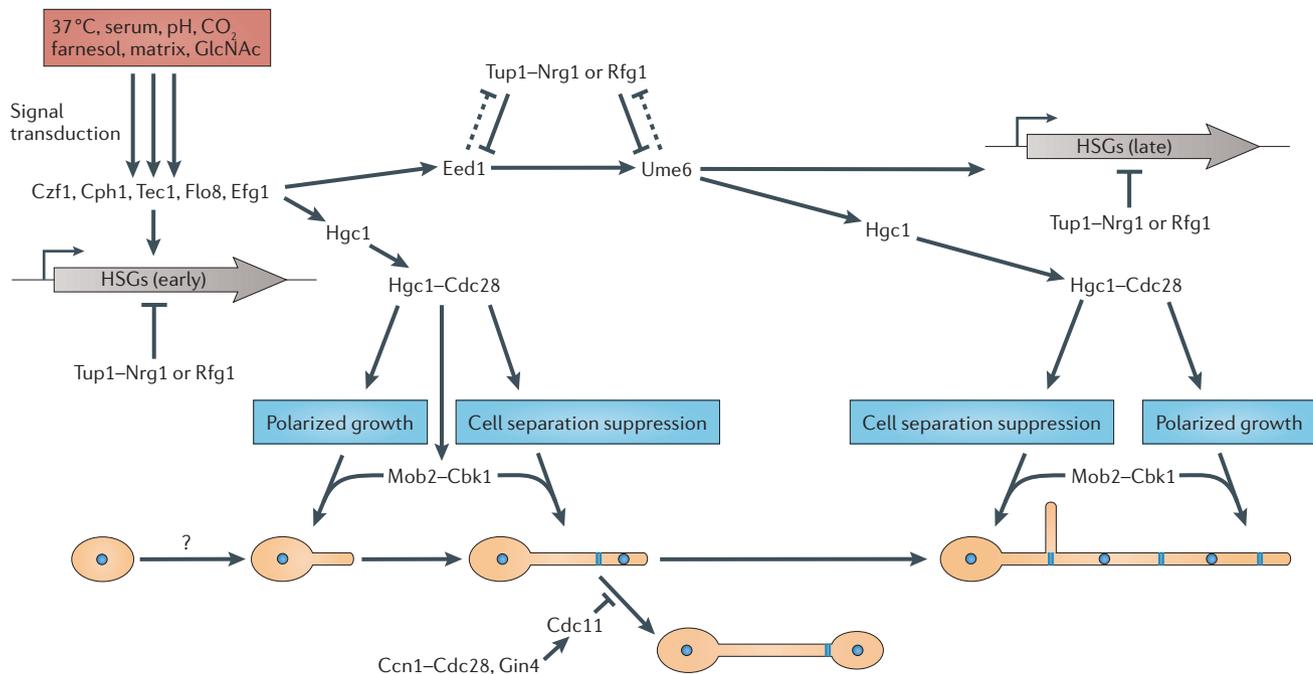


Figure 6 | The hyphal induction programme. Hyphal growth requires mechanisms for initiation and long-term maintenance. The transduction of environmental cues leading to early expression of hypha-specific genes (HSGs) is set out in detail in FIG. 2. Long-term maintenance of HSG expression requires the activation of *UME6* expression through *Eed1*. Evagination of a polarized germ tube occurs in the absence of hyphal G1 cyclin protein 1 (*Hgc1*) and when cell division control 28 (*Cdc28*) activity is inhibited. Thus, the immediate events leading to germ tube evagination remained to be elucidated. *Efg1*, enhanced filamentous growth protein 1; *Gin4*, growth-inhibitory protein 4; *GlcNAc*, *N*-acetyl-D-glucosamine; *Rfg1*, *Rox1p*-like regulator of filamentous growth.

phenotype reflects the position in the cycle at which cells are arrested. For example, cells arrested in G1 (such as G1-S-specific cyclin *Cln3* (*Cln3*)-depleted cells^{119,120} or *cdc34*^{-/-} cells¹²¹) arrest in an apparent hyphal morphology. Cells arrested in mitosis, such as *cdc5*^{-/-} mutants¹²², G2-mitotic-specific cyclin 2 (*Clb2*)-depleted cells¹²³ and *cdc20*^{-/-} mutants¹²⁴, also have a highly elongated morphology, although the elongated daughter cells are wider than true hyphae and have constrictions at their base. Conditions that cause an S-G2 arrest display a pseudo-hyphal phenotype. These conditions include DNA arrest with hydroxyurea¹¹⁹, forkhead protein homologue 2 mutants (*fkh2*^{-/-}) (*Fkh2* is necessary for cell cycle progression)¹²⁵ and *clb4*^{-/-} mutants¹²³. A possibly related phenomenon is the pseudo-hyphal phenotype of growth-inhibitory protein 4 (*gin4*^{-/-} or *hsl1*^{-/-} mutants^{117,126}). The cause of these elongated-morphology phenotypes and their connection to normal hyphal growth is still unknown.

The role of kinases in hyphal formation

It is becoming increasingly clear that kinases play a key part in mediating the extreme polarized growth of hyphae, and a number of kinase-target relationships have been recently defined (FIG. 6). The cyclin-dependent kinase *Cdc28* has a central role (reviewed in REF. 127). Hyphal formation is disrupted in cells in which the expression of *Cdc28* has been repressed or inhibited. Cells that lack the *Cdc28* cyclin *Hgc1* initiate germ tubes but quickly become depolarized. Deletion

of G1-S-specific cyclin *Ccn1* (also known as *Cln1*), another *Cdc28* cyclin, results in cells with apparently normal germ tubes that extend for a substantially longer period than those of *hgc1*^{-/-} mutants, but growth eventually becomes depolarized leading to a failure to form filamentous colonies on Lee’s medium¹²⁸. The role of *Hgc1*-*Cdc28* in phosphorylating and inhibiting the *Cdc42* GAP, *Rga2*, and in targeting *Efg1* to repress the expression of SDEs was described above. *Hgc1*-*Cdc28* also targets *Sec2*, which is the *Sec4* GEF, to promote the flux of secretory vesicles to the hyphal tip⁸². It has also recently been shown to phosphorylate *Mob2*¹²⁹.

Ccn1-*Cdc28* has also been shown to play a key part in hyphal development, in this case in conjunction with the kinase *Gin4*. *Gin4* phosphorylates the septin *Cdc11* on residue Ser395 (REF. 130). This phosphorylation is a necessary prerequisite for *Ccn1*-*Cdc28* to phosphorylate *Cdc11* on the adjacent Ser394 residue. These phosphorylation events occur within a few minutes of hyphal induction. If these events are prevented, by deletion of *Ccn1* or by substituting non-phosphorylatable residues at Ser394 and/or Ser395, then germ tubes evaginate normally but growth subsequently becomes depolarized. The role of *Cdc11* in polarized growth is further elaborated by the effect of deleting the exocyst component *Sec3*, which in *S. cerevisiae* acts as landmark for exocyst localization. In *C. albicans* *sec3*^{-/-} mutants the germ tube tip swells after the first septin ring forms¹³¹. This phenotype is rescued by deletion of either of the *CDC11* or *CDC12* septin genes. Taken together, these

two studies reveal that Cdc11 has a negative role in polarized growth that must be inhibited through phosphorylation by Ccn1–Cdc28 and by the presence of Sec3 at the hyphal tip.

Although Cdc28 clearly has multiple roles in hyphal growth, a germ tube still evaginates when Cdc28 activity is inhibited and in *hgc1*^{-/-} or *ccn1*^{-/-} strains. Loss of Cbk1 kinase activity has an even more profound effect on hyphal growth^{132,133}. In *S. cerevisiae*, Cbk1 is a member of the regulation of Ace2-dependent transcription and morphogenesis (RAM) complex of proteins (which includes Kic1, Sog1, Hym1, Mob2 and Sog1)¹³⁴. Kic1 is a kinase that is required for phosphorylation of a conserved C-terminal motif in Cbk1. This phosphorylation is not required for kinase activity or localization but is required for Cbk1 to perform its normal function¹³⁵. In addition to this phosphorylation, Cbk1 is activated by association with its regulatory subunit Mob2 and autophosphorylation of its T-loop, which separates the catalytic domain of Cbk1 into two subdomains. In *C. albicans*, loss of any of the components of the RAM complex resulted in a range of pleiotropic phenotypes¹³³, including a complete loss of hyphal growth, which is associated with a failure to express genes regulated by Nrg1 and Tup1. Although the role of Cbk1 remains mysterious, the phosphorylation of its regulatory subunit Mob2 partner by Hgc1–Cdc28 provides a connection between these two key kinases.

Conclusions and challenges

This Review describes the signal transduction pathways that induce hyphal growth, the roles of hyphae in the infection process and the cellular mechanisms that operate to promote the extreme polarized growth of

hyphae. The connections between the signal transduction pathways and the endpoint of modification of polarized growth mechanisms were once opaque but now are starting to become clear. It is now possible to sketch a model that encompasses the perception of inducing signals such as temperature, serum, CO₂ and starvation, the transduction of these signals through pathways leading to transcription factors such as Efg1, Eed1 and Ume6, through to the outputs of Hgc1 expression, which are required for polarized growth and inhibition of cell separation. A summary of the hyphal induction programme is shown in FIG. 6.

Of course much remains to be understood. We remain largely ignorant of the ways in which the output of the signal transduction pathway results in the formation of the Spitzenkörper and the way that polarized growth becomes continuous throughout the cell cycle. Although roles of Hgc1 in promoting hyphal growth such as phosphorylation of Rga2, Mob2, Efg1 and Sec2 have been uncovered, the processes so far documented cannot be the whole story. There are likely to be other targets of Cdc28 yet to be identified, and the role of Mob2–Cbk1 in polarized growth remains mysterious. As loss of Cbk1 causes a profound defect in hyphal growth, its role is one of the most urgent issues that needs to be addressed.

A second area of ignorance is the identity and role of other genes for which regulated expression is required for hyphal growth. Although a long list of genes has been identified by microarray experiments, few if any of the functions of these genes suggest an obvious involvement with polarized growth. An exciting possibility is that a gene of unknown function has a role, opening up new pathways to polarized growth that are distinct from those described in the *S. cerevisiae* model.

- Odds, F. C. *Candida and Candidosis* (Balliere Tindall, London, 1988).
- Runke, M. in *Candida and Candidiasis* (ed. Calderone, R.) 307–325 (ASM Press, Washington, 2002).
- Sobel, J. D. Vaginitis. *N. Engl. J. Med.* **337**, 1896–1903 (1997).
- Kullberg, B. J. & Filler, S. G. in *Candida and Candidiasis* (ed. Calderone, R. A.) 327–340 (ASM Press, Washington DC, 2002).
- Beck-Sague, C. M. & Jarvis, W. R. National nosocomial infections surveillance system. Secular trends in the epidemiology of nosocomial fungal infections in the United States 1980–1990. *J. Inf. Dis.* **167**, 1247–1251 (1993).
- Kibbler, C. C. *et al.* Management and outcome of bloodstream infections due to *Candida* species in England and Wales. *J. Hosp. Infect.* **54**, 18–24 (2003).
- Pfaller, M. A., Jones, R. N., Messer, S. A., Edmond, M. B. & Wenzel, R. P. National surveillance of nosocomial blood stream infection due to species of *Candida* other than *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE program. *Diagn. Microbiol. Infect. Dis.* **30**, 121–129 (1998).
- Wisplinghoff, H. *et al.* Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin. Infect. Dis.* **39**, 309–317 (2004).
- Odds, F. C. *Candida and Candidosis*. 42–59 (Balliere Tindall, London, 1988).
A masterly review from one of the founding fathers of modern *C. albicans* research sets out many of the observations concerning morphogenesis that are now taken for granted.
- Sudbery, P. E., Gow, N. A. R. & Berman, J. The distinct morphogenic states of *Candida albicans*. *Trends Microbiol.* **12**, 317–324 (2004).
- Dalle, F. *et al.* Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cell. Microbiol.* **12**, 248–271 (2010).
- Filler, S. G. & Sheppard, D. C. Fungal invasion of normally non-phagocytic host cells. *PLoS Pathog.* **2**, e129 (2006).
- Phan, Q. T. *et al.* Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. *PLoS Biol.* **5**, e64 (2007).
- Zhu, W. D. & Filler, S. G. Interactions of *Candida albicans* with epithelial cells. *Cell. Microbiol.* **12**, 273–282 (2010).
- Scherwitz, C. Ultrastructure of human cutaneous candidosis. *J. Invest. Dermatol.* **78**, 200–205 (1982).
- Lorenz, M. C., Bender, J. A. & Fink, G. R. Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot. Cell* **3**, 1076–1087 (2004).
- Noble, S. M. & Johnson, A. D. Genetics of *Candida albicans*, a diploid human fungal pathogen. *Annu. Rev. Genet.* **41**, 193–211 (2007).
- Taschdjian, C. L., Burchill, J. J. & Kozinn, P. J. Rapid identification of *Candida albicans* by filamentation on serum and serum substitutes. *AMA J. Dis. Child.* **99**, 212 (1960).
- Buffo, J., Herman, N. & Soll, D. R. A characterization of pH regulated dimorphism in *Candida albicans*. *Mycopathologia* **85**, 21–30 (1985).
- Mardon, D., Balish, E. & Phillips, A. W. Control of dimorphism in a biochemical variant of *Candida albicans*. *J. Bacteriol.* **100**, 701–707 (1969).
- Simonneti, N., Stripolli, V. & Cassone, E. A. Yeast-mycelial conversion induced by *N*-acetyl- β -glucosamine in *Candida albicans*. *Nature* **250**, 344–346 (1974).
- Brown, D. H. Jr, Giusani, A. D., Chen, X. & Kumamoto, C. A. Filamentous growth of *Candida albicans* in response to physical environmental cues and its regulation by the unique *CZF1* gene. *Mol. Microbiol.* **34**, 651–662 (1999).
- Sonneborn, A., Bockmuhl, D. P. & Ernst, J. F. Chlamydospore formation in *Candida albicans* requires the Efg1 p morphogenetic regulator. *Infect. Immun.* **67**, 5514–5517 (1999).
- Lee, K. L., Buckley, H. R. & Cambell, C. C. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. *Sabouraudia* **13**, 148–153 (1975).
- Liu, H. P., Kohler, J. R. & Fink, G. R. Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. *Science* **266**, 1723–1726 (1994).
- Shareck, J. & Belhumeur, P. Modulation of morphogenesis in *Candida albicans* by various small molecules. *Euk Cell* 3 Jun 2011 (doi:10.1128/EC.05030-11).
- Hornby, J. M. *et al.* Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl. Environ. Microbiol.* **67**, 2982–2992 (2001).
The first identification of a quorum sensing compound, farnesol. This opened up a new field studying intercellular communication between *C. albicans* cells and with bacterial cells.
- Chen, H., Fujita, M., Feng, Q. H., Clardy, J. & Fink, G. R. Tyrosol is a quorum-sensing molecule in *Candida albicans*. *Proc. Natl Acad. Sci. USA* **101**, 5048–5052 (2004).
- De Sordi, L. & Muhschlegel, F. A. Quorum sensing and fungal-bacterial interactions in *Candida albicans*: a communicative network regulating microbial coexistence and virulence. *FEMS Yeast Res.* **9**, 990–999 (2009).
- Hogan, D. A., Vik, Å. & Kolter, R. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. *Mol. Microbiol.* **54**, 1212–1223 (2004).

31. Hogan, D. A. & Kolter, R. *Pseudomonas candida* interactions: an ecological role for virulence factors. *Science* **296**, 2229–2232 (2002).
32. Hogan, D. A. Talking to themselves: autoregulation and quorum sensing in fungi. *Eukaryot. Cell* **5**, 613–619 (2006).
33. Shapiro, R. S., Robbins, N. & Cowen, L. E. Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiol. Mol. Biol. Rev.* **75**, 213–267 (2011).
This review provides a recent, exhaustive and detailed description of the complex network of pathways that regulate the transcription of hypha-specific genes.
34. Braun, B. R. & Johnson, A. D. Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. *Science* **277**, 105–109 (1997).
The first identification of a protein that negatively controls hyphal growth and hypha-specific gene expression.
35. Kadosh, D. & Johnson, A. D. Induction of the *Candida albicans* filamentous growth program by relief of transcriptional repression: a genome-wide analysis. *Mol. Biol. Cell* **16**, 2903–2912 (2005).
36. Braun, B. R., Kadosh, D. & Johnson, A. D. NRG1, a repressor of filamentous growth in *C. albicans*, is down-regulated during filament induction. *EMBO J.* **20**, 4753–4761 (2001).
The identification of Nrg1 as a co-repressor of hypha-specific gene transcription.
37. Murad, A. M. A. *et al.* Nrg1 represses yeast–hypha morphogenesis and hypha-specific gene expression in *Candida albicans*. *EMBO J.* **20**, 4742–4752 (2001).
38. Kadosh, D. & Johnson, A. D. Rfg1, a protein related to the *Saccharomyces cerevisiae* hypoxic regulator Rox1, controls filamentous growth and virulence in *Candida albicans*. *Mol. Cell Biol.* **21**, 2496–2505 (2001).
39. Stoldt, V. R., Sonneborn, A., Leuker, C. E. & Ernst, J. F. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. *EMBO J.* **16**, 1982–1991 (1997).
The first identification of the Efg1 transcription factor, which is required for hypha-specific gene expression.
40. Leberer, E. *et al.* Signal transduction through homologs of the Ste20p and Ste7p protein kinases can trigger hyphal formation in the pathogenic fungus *Candida albicans*. *Proc. Natl Acad. Sci. USA* **93**, 13217–13222 (1996).
41. Lane, S., Zhou, S., Pan, T., Dai, Q. & Liu, H. The basic helix–loop–helix transcription factor Cph2 regulates hyphal development in *Candida albicans* partly via Tec1. *Mol. Cell Biol.* **21**, 6418–6428 (2001).
42. Lane, S., Birse, C., Zhou, S., Matson, R. & Liu, H. P. DNA array studies demonstrate convergent regulation of virulence factors by Cph1, Cph2, and Efg1 in *Candida albicans*. *J. Biol. Chem.* **276**, 48988–48996 (2001).
43. Cao, F. *et al.* The Flo8 transcription factor is essential for hyphal development and virulence in *Candida albicans*. *Mol. Biol. Cell* **17**, 295–307 (2006).
44. Davis, D., Wilson, R. B. & Mitchell, A. P. RIM101 dependent and independent pathways govern pH responses in *Candida albicans*. *Mol. Cell Biol.* **20**, 971–978 (2000).
45. Davis, D. Adaptation to environmental pH in *Candida albicans* and its relation to pathogenesis. *Curr. Genet.* **44**, 1–7 (2003).
46. El Barkani, A. *et al.* Dominant active alleles of RIM101 (PRR2) bypass the pH restriction on filamentation of *Candida albicans*. *Mol. Cell Biol.* **20**, 4635–4647 (2000).
47. Sellam, A. *et al.* Role of transcription factor Cndt80p in cell separation, hyphal growth, and virulence in *Candida albicans*. *Eukaryot. Cell* **9**, 634–644 (2010).
48. Lo, H. J. *et al.* Nonfilamentous *C. albicans* mutants are avirulent. *Cell* **90**, 939–949 (1997).
49. Braun, B. R. & Johnson, A. D. TUP1, CPH1 and EFG1 make independent contributions to filamentation in *Candida albicans*. *Genetics* **155**, 57–67 (2000).
50. Feng, Q. H., Summers, E., Guo, B. & Fink, G. Ras signaling is required for serum-induced hyphal differentiation in *Candida albicans*. *J. Bacteriol.* **181**, 6339–6346 (1999).
51. Leberer, E. *et al.* Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus *Candida albicans*. *Mol. Microbiol.* **42**, 673–687 (2001).
52. Rocha, C. R. C. *et al.* Signaling through adenyllyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus *Candida albicans*. *Mol. Biol. Cell* **12**, 3631–3643 (2001).
53. Fang, H. M. & Wang, Y. RA domain-mediated interaction of Cdc35 with Ras1 is essential for increasing cellular cAMP level for *Candida albicans* hyphal development. *Mol. Microbiol.* **61**, 484–496 (2006).
54. Xu, X. L. *et al.* Bacterial peptidoglycan triggers *Candida albicans* hyphal growth by directly activating the adenyllyl cyclase Cyr1p. *Cell Host Microbe* **4**, 28–39 (2008).
55. Klengel, T. *et al.* Fungal adenyllyl cyclase integrates CO₂ sensing with cAMP signaling and virulence. *Curr. Biol.* **15**, 2021–2026 (2005).
56. Zou, H., Fang, H. M., Zhu, Y. & Wang, Y. *Candida albicans* Cyr1, Cap1 and G-actin form a sensor/effector apparatus for activating cAMP synthesis in hyphal growth. *Mol. Microbiol.* **75**, 579–591 (2010).
57. Davis-Hanna, A., Piispanen, A. E., Stateva, L. I. & Hogan, D. A. Farnesol and dodecanol effects on the *Candida albicans* Ras1-cAMP signalling pathway and the regulation of morphogenesis. *Mol. Microbiol.* **67**, 47–62 (2008).
58. Hall, R. A. *et al.* The quorum sensing molecules farnesol/homoserine lactone and dodecanol operate via distinct modes of action in *Candida albicans*. *Eukaryot. Cell* 10 Jun 2011 (doi: 10.1128/EC.05060–11).
59. Keabaara, B. W. *et al.* *Candida albicans* Tup1 is involved in farnesol-mediated inhibition of filamentous growth induction. *Eukaryot. Cell* **7**, 980–987 (2008).
60. Shapiro, R. S. *et al.* Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1–PKA signaling. *Curr. Biol.* **19**, 621–629 (2009).
61. Nantel, A. *et al.* Transcription profiling of *Candida albicans* cells undergoing the yeast-to-hyphal transition. *Mol. Biol. Cell* **13**, 3452–3465 (2002).
62. Martin, R. *et al.* The *Candida albicans* specific gene *EED1* encodes a key regulator of hyphal extension. *PLoS ONE* **6**, e18394 (2011).
The identification of a hypha-specific gene (EED1) that is required for hyphal growth. Epistasis analysis places it downstream of EFG1 but upstream of UME6.
63. Banerjee, M. *et al.* *UME6*, a novel filament-specific regulator of *Candida albicans* hyphal extension and virulence. *Mol. Biol. Cell* **19**, 1354–1365 (2008).
64. Carlisle, P. L. *et al.* Expression levels of a filament-specific transcriptional regulator are sufficient to determine *Candida albicans* morphology and virulence. *Proc. Natl Acad. Sci. USA* **106**, 599–604 (2009).
A description of UME6, a hypha-specific gene that is required for hyphal growth. Importantly, this reference shows that overexpression of UME6 is sufficient for hyphal growth, suggesting that UME6 expression is a critical limiting step for hyphal growth.
65. Carlisle, P. L. & Kadosh, D. *Candida albicans* Ume6, a filament-specific transcriptional regulator, directs hyphal growth via a pathway involving Hgc1 cyclin-related protein. *Eukaryot. Cell* **9**, 1320–1328 (2010).
66. Zeidler, U. *et al.* *UME6* is a crucial downstream target of other transcriptional regulators of true hyphal development in *Candida albicans*. *FEMS Yeast Res.* **9**, 126–142 (2009).
67. Zheng, X., Wang, Y. & Wang, Y. Hgc1, a novel hypha-specific G1 cyclin-related protein regulates *Candida albicans* hyphal morphogenesis. *EMBO J.* **23**, 1845–1856 (2004).
A key paper that, for the first time, identifies a gene that is only expressed in hyphae and is also required for hyphal development.
68. Sudbery, P. E. The germ tubes of *Candida albicans* hyphae and pseudohyphae show different patterns of septin ring localisation. *Mol. Microbiol.* **41**, 19–31 (2001).
This study shows that although pseudohyphae may superficially resemble hyphae, there are fundamental differences in the underlying cellular organization. The article describes a qualitative test to distinguish between hyphae and pseudohyphae.
69. Warena, A. J. & Konopka, J. B. Septin function in *Candida albicans* morphogenesis. *Mol. Biol. Cell* **13**, 2732–2746 (2002).
70. Soll, D. R., Herman, M. A. & Staebell, M. A. The involvement of cell wall expansion in the two modes of mycelium formation of *Candida albicans*. *J. Gen. Microbiol.* **131**, 2367–2375 (1985).
- A classic paper that demonstrates that hyphal growth is polarized to the tip.**
71. Finley, K. R. & Berman, J. Microtubules in *Candida albicans* hyphae drive nuclear dynamics and connect cell cycle progression to morphogenesis. *Eukaryot. Cell* **4**, 1697–1711 (2005).
72. Hazan, I., Sepulveda-Becerra, M. & Liu, H. P. Hyphal elongation is regulated independently of cell cycle in *Candida albicans*. *Mol. Biol. Cell* **13**, 134–145 (2002).
A meticulous analysis that shows that cell cycle events occur with the same kinetics in yeast and hyphae, so that changes in cell cycle organization are not responsible for hyphal morphology. Furthermore, this analysis shows that germ tube evagination can occur before the start of the cell cycle, and thus a germ tube is not a modified bud.
73. Crampin, H. *et al.* *Candida albicans* hyphae have a Spitzenkörper that is distinct from the polarisome found in yeast and pseudohyphae. *J. Cell Sci.* **118**, 2935–2947 (2005).
This investigation shows that hyphal growth is driven by a Spitzenkörper; therefore, the mechanism of polarized growth is different in hyphae and in yeast and pseudohyphae. The article illustrates the power of high-resolution imaging of fluorescently tagged proteins.
74. Crampin, H. The identification of the Spitzenkörper in *Candida albicans* and the partial characterization of the contractile ring components. Thesis, Univ. Sheffield (2006).
75. Lenardon, M. D. *et al.* Phosphorylation regulates polarisation of chitin synthesis in *Candida albicans*. *J. Cell Sci.* **123**, 2199–2206 (2010).
76. Merson-Davies, L. A. & Odds, F. C. A morphology index for cell shape in *Candida albicans*. *J. Gen. Microbiol.* **135**, 3143–3152 (1989).
77. Odds, F. C. Morphogenesis in *Candida albicans*. *Crit. Rev. Microbiol.* **12**, 45–93 (1985).
78. Gow, N. A. R. & Gooday, G. W. A model for the germ tube formation and mycelial growth form of *Candida albicans*. *Sabouraudia* **22**, 137–143 (1984).
79. Gow, N. A. R. & Gooday, G. W. Vacuolation, branch production and linear growth of germ tubes of *Candida albicans*. *J. Gen. Microbiol.* **128**, 2195–2198 (1982).
80. Soll, D. R., Stasi, M. & Bedell, G. The regulation of nuclear migration and division during pseudo-mycelium outgrowth in the dimorphic yeast *Candida albicans*. *Exp. Cell Res.* **116**, 207–215 (1978).
81. Martin, R., Walther, A. & Wendland, J. Ras1-induced hyphal development in *Candida albicans* requires the formin Bni1. *Eukaryot. Cell* **4**, 1712–1724 (2005).
82. Bishop, A. *et al.* Hyphal growth in *Candida albicans* requires the phosphorylation of Sec2 by the Cdc28–Ccn1/Hgc1 kinase. *EMBO J.* **29**, 2930–2942 (2010).
83. Jones, L. A. & Sudbery, P. E. Spitzenkörper, exocyst and polarisome components in *Candida albicans* hyphae show different patterns of localization and have distinct dynamic properties. *Eukaryot. Cell* **9**, 1455–1465 (2010).
84. Virag, A. & Harris, S. D. The Spitzenkörper: a molecular perspective. *Mycol. Res.* **110**, 4–13 (2006).
85. Bartnicki-Garcia, S., Hergert, F. & Gierz, G. Computer-simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. *Protoplasma* **153**, 46–57 (1989).
86. Akashi, T., Kanbe, T. & Tanaka, K. The role of the cytoskeleton in the polarized growth of the germ tube in *Candida albicans*. *Microbiology* **140**, 271–280 (1994).
87. Hazan, I. & Liu, H. P. Hyphal tip-associated localization of Cdc42 is F-actin dependent in *Candida albicans*. *Eukaryot. Cell* **1**, 856–864 (2002).
88. Barton, R. & Gull, K. Variation in cytoplasmic microtubular organisation and spindle length between the two forms of the dimorphic fungus *Candida albicans*. *J. Cell Sci.* **91**, 211–220 (1988).
89. Rida, P. C. G., Nishikawa, A., Won, G. Y. & Dean, N. Yeast-to-hyphal transition triggers formin-dependent golgi localization to the growing tip in *Candida albicans*. *Mol. Biol. Cell* **17**, 4364–4378 (2006).
90. Yokoyama, K., Kaji, H., Nishimura, K. & Miyaji, M. The role of microfilaments and microtubules in apical growth and dimorphism of *Candida albicans*. *J. Gen. Microbiol.* **136**, 1067–1075 (1990).
91. Reijntj, P., Walther, A. & Wendland, J. Functional analysis of *Candida albicans* genes encoding SH3-domain-containing proteins. *FEMS Yeast Res.* **10**, 452–461 (2010).

92. Asleson, C. M. *et al.* *Candida albicans* INT1-induced filamentation in *Saccharomyces cerevisiae* depends on Sla2p. *Mol. Cell Biol.* **21**, 1272–1284 (2001).
93. Martin, R. *et al.* Functional analysis of *Candida albicans* genes whose *Saccharomyces cerevisiae* homologues are involved in endocytosis. *Yeast* **24**, 511–522 (2007).
94. Walther, A. & Wendland, J. Polarized hyphal growth in *Candida albicans* requires the Wiskott–Aldrich syndrome protein homolog Wal1p. *Eukaryot. Cell* **3**, 471–482 (2004).
95. Borth, N. *et al.* *Candida albicans* Vrp1 is required for polarized morphogenesis and interacts with Wal1 and Myo5. *Microbiology* **156**, 2962–2969 (2010).
96. Oberholzer, U., Marcil, A., Leberer, E., Thomas, D. Y. & Whiteway, M. Myosin I is required for hypha formation in *Candida albicans*. *Eukaryot. Cell* **1**, 213–228 (2002).
97. Robertson, A. S., Smythe, E. & Ayscough, K. R. Functions of actin in endocytosis. *Cell. Mol. Life Sci.* **66**, 2049–2065 (2009).
98. Epp, E. *et al.* Forward genetics in *Candida albicans* that reveals the Arp2/3 complex is required for hyphal formation, but not endocytosis. *Mol. Microbiol.* **75**, 1182–1198 (2010).
99. Park, H. O. & Bi, E. F. Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Microbiol. Mol. Biol. Rev.* **71**, 48–96 (2007).
100. Leberer, E., Thomas, D. Y. & Whiteway, M. Pheromone signalling and polarized morphogenesis in yeast. *Curr. Opin. Genet. Dev.* **7**, 59–66 (1997).
101. Bassilana, M., Hopkins, J. & Arkowitz, R. A. Regulation of the Cdc42/Cdc24 GTPase module during *Candida albicans* hyphal growth. *Eukaryot. Cell* **4**, 588–603 (2005).
102. Bassilana, M., Blyth, J. & Arkowitz, R. A. Cdc24, the GDP–GTP exchange factor for Cdc42, is required for invasive hyphal growth of *Candida albicans*. *Eukaryot. Cell* **2**, 9–18 (2003).
103. Ushinsky, S. C. *et al.* CDC42 is required for polarized growth in the human pathogen *Candida albicans*. *Eukaryot. Cell* **1**, 95–104 (2002).
104. Court, H. & Sudbery, P. Regulation of Cdc42 GTPase activity in the formation of hyphae in *Candida albicans*. *Mol. Biol. Cell* **18**, 265–281 (2007).
105. Zheng, X. D., Lee, R. T. H., Wang, Y. M., Lin, Q. S. & Wang, Y. Phosphorylation of Rga2, a Cdc42 GAP, by CDK/Hgc1 is crucial for *Candida albicans* hyphal growth. *EMBO J.* **26**, 3760–3769 (2007). **This paper provides the first example of a molecular mechanism in which a protein that is only expressed in hyphae promotes hyphal growth.**
106. Hausauer, D. L., Gerami-Nejad, M., Kistler-Anderson, C. & Gale, C. A. Hyphal guidance and invasive growth in *Candida albicans* require the Ras-like GTPase Rsr1p and its GTPase-activating protein Bud2p. *Eukaryot. Cell* **4**, 1273–1286 (2005).
107. Brand, A. *et al.* An internal polarity landmark is important for externally induced hyphal behaviors in *Candida albicans*. *Eukaryot. Cell* **7**, 712–720 (2008).
108. Dunkler, A. & Wendland, J. *Candida albicans* Rho-type GTPase-encoding genes required for polarized cell growth and cell separation. *Eukaryot. Cell* **6**, 844–854 (2007).
109. Bassilana, M. & Arkowitz, R. A. Rac1 and Cdc42 have different roles in *Candida albicans* development. *Eukaryot. Cell* **5**, 321–329 (2006).
110. Hope, H., Bogliolo, S., Arkowitz, R. A. & Bassilana, M. Activation of Rac1 by the guanine nucleotide exchange factor Dck1 is required for invasive filamentous growth in the pathogen *Candida albicans*. *Mol. Biol. Cell* **19**, 3638–3651 (2008).
111. Brace, J., Hsu, J. & Weiss, E. L. Mitotic exit Control of the *Saccharomyces cerevisiae* Ndr/LATS kinase Cbk1 regulates daughter cell separation after cytokinesis. *Mol. Cell Biol.* **31**, 721–735 (2011).
112. Clemente-Blanco, A. *et al.* The Cdc14p phosphatase affects late cell-cycle events and morphogenesis in *Candida albicans*. *J. Cell Sci.* **119**, 1130–1143 (2006).
113. Gonzalez-Novo, A. *et al.* Sep7 is essential to modify septin ring dynamics and inhibit cell separation during *Candida albicans* hyphal growth. *Mol. Biol. Cell* **19**, 1509–1518 (2008).
114. Wang, A., Raniga, P. P., Lane, S., Lu, Y. & Liu, H. P. Hyphal chain formation in *Candida albicans*: Cdc28–Hgc1 phosphorylation of Efg1 represses cell separation genes. *Mol. Cell Biol.* **29**, 4406–4416 (2009). **This paper, together with reference 113, describes molecular mechanisms for the suppression of cell separation in hyphae**
115. Anderson, J. & Soll, D. R. Differences in actin localization during bud and hypha formation in the yeast *Candida albicans*. *J. Gen. Microbiol.* **132**, 2035–2047 (1986).
116. Kron, S. J., Styles, C. A. & Fink, G. R. Symmetrical cell-division in pseudohyphae of the yeast *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **5**, 1003–1022 (1994).
117. Wightman, R., Bates, S., Amnorrattapan, P. & Sudbery, P. E. In *Candida albicans*, the Nim1 kinases Gin4 and Hsl1 negatively regulate pseudohypha formation and Gin4 also controls septin organization. *J. Cell Biol.* **164**, 581–591 (2004).
118. Berman, J. Morphogenesis and cell cycle progression in *Candida albicans*. *Curr. Opin. Microbiol.* **9**, 595–601 (2006).
119. Bachewich, C., Nantel, A. & Whiteway, M. Cell cycle arrest during S or M phase generates polarized growth via distinct signals in *Candida albicans*. *Mol. Microbiol.* **57**, 942–959 (2005).
120. Chapa y Lazo, B., Bates, S. & Sudbery, P. E. CLN3 regulates hyphal morphogenesis in *Candida albicans*. *Eukaryot. Cell* **4**, 90–94 (2005).
121. Atir-Lande, A., Gildor, T. & Kornitzer, D. Role for the SCF/CDC4 ubiquitin ligase in *Candida albicans* morphogenesis. *Mol. Biol. Cell* **16**, 2772–2785 (2005).
122. Bachewich, C. & Whiteway, M. Cyclin Cln3p links G1 progression to hyphal and pseudohyphal development in *Candida albicans*. *Eukaryot. Cell* **4**, 95–102 (2005).
123. Bensen, E. S., Clemente-Blanco, A., Finley, K. R., Correa-Bordes, J. & Berman, J. The mitotic cyclins Clb2p and Clb4p affect morphogenesis in *Candida albicans*. *Mol. Biol. Cell* **16**, 3387–3400 (2005).
124. Chou, H., Glory, A. & Bachewich, C. Orthologues of the APC/C coactivators Cdc20p and Cdh1p are important for mitotic progression and morphogenesis in *Candida albicans*. *Eukaryot. Cell* **10**, 696–709 (2011).
125. Bensen, E. S., Filler, S. G. & Berman, J. A forkhead transcription factor is important for true hyphal as well as yeast morphogenesis in *Candida albicans*. *Eukaryot. Cell* **1**, 787–798 (2002).
126. Umeyama, T. *et al.* *Candida albicans* protein kinase CaHsl1p regulates cell elongation and virulence. *Mol. Microbiol.* **55**, 381–395 (2005).
127. Wang, Y. CDKs and the yeast-hyphal decision. *Curr. Opin. Microbiol.* **12**, 644–649 (2009).
128. Loeb, J. J., Sepulveda-Becerra, M., Hazan, I. & Liu, H. P. A G1 cyclin is necessary for maintenance of filamentous growth in *Candida albicans*. *Mol. Cell Biol.* **19**, 4019–4027 (1999).
129. Gutiérrez-Escribano, P. *et al.* Cdk-dependent phosphorylation of Mob2 is essential for hyphal development in *Candida albicans*. *Mol. Biol. Cell* **22**, 2458–2469 (2011).
130. Sinha, I. *et al.* Cyclin-dependent kinases control septin phosphorylation in *Candida albicans* hyphal development. *Dev. Cell* **13**, 421–432 (2007).
131. Li, C. R., Lee, R. T.-H., Wang, Y. M., Zheng, X. D. & Wang, Y. *Candida albicans* hyphal morphogenesis occurs in Sec5p-independent and Sec5p-dependent phases separated by septin ring formation. *J. Cell Sci.* **120**, 1898–1907 (2007).
132. McNemar, M. D. & Fonzi, W. A. Conserved serine/threonine kinase encoded by *CBK1* regulates expression of several hypha-associated transcripts and genes encoding cell wall proteins in *Candida albicans*. *J. Bacteriol.* **184**, 2058–2061 (2002). **This paper is the first to demonstrate that Cbk1 is absolutely required for hyphal growth. The role of this kinase remains mysterious, but its action clearly critical for hyphal formation.**
133. Song, Y. *et al.* Role of the RAM network in cell polarity and hyphal morphogenesis in *Candida albicans*. *Mol. Biol. Cell* **19**, 5456–5477 (2008).
134. Nelson, B. *et al.* RAM: A conserved signaling network that regulates Ace2p transcriptional activity and polarized morphogenesis. *Mol. Biol. Cell* **14**, 3782–3803 (2003).
135. Jansen, J. M., Barry, M. F., Yoo, C. K. & Weiss, E. L. Phosphoregulation of Cbk1 is critical for RAM network control of transcription and morphogenesis. *J. Cell Biol.* **175**, 755–766 (2006).
136. Moyes, D. L. *et al.* A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of *Candida albicans* in epithelial cells. *Cell Host Microbe* **8**, 225–235 (2010).
137. Nucci, M. & Anaissie, E. Revisiting the source of candidemia: skin or gut? *Clin. Infect. Dis.* **33**, 1959–1967 (2001).
138. Hoyer, L. L. The ALS gene family of *Candida albicans*. *Trends Microbiol.* **9**, 176–180 (2001).
139. Staab, J. F., Bradway, S. D., Fidel, P. L. & Sundstrom, P. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* **283**, 1535–1538 (1999).
140. Li, F. & Palecek, S. P. Distinct domains of the *Candida albicans* adhesin Eap1p mediate cell–cell and cell–substrate interactions. *Microbiology* **154**, 1193–1203 (2008).
141. Drago, L. *et al.* *Candida albicans* cellular internalization: a new pathogenic factor? *Int. J. Antimicrob. Agents* **16**, 545–547 (2000).
142. Saville, S. P., Lazzell, A. L., Monteagudo, C. & Lopez-Ribot, J. L. Engineered control of cell morphology *in vivo* reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot. Cell* **2**, 1053–1060 (2003).
143. Noble, S. M., French, S., Kohn, L. A., Chen, V. & Johnson, A. D. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nature Genet.* **42**, 590–598 (2010).
144. Gladfelter, A. S. & Sudbery, P. E. In *The Septins* (eds. Hall, P. A., Russell, S. E. & Pringle, J. R.) 125–146 (Wiley-Blackwell, Chichester, 2008).
145. Evangelista, M., Pruyne, D., Amberg, D. C., Boone, C. & Bretscher, A. Formins direct Arp2/3-independent actin filament assembly to polarize cell growth in yeast. *Nature Cell Biol.* **4**, 32–41 (2002).
146. Pruyne, D. Role of formins in actin assembly: nucleation and barbed-end association. *Science* **297**, 612–615 (2002).
147. Sagot, I., Klee, S. K. & Pellmar, D. Yeast formins regulate cell polarity by controlling the assembly of actin cables. *Nature Cell Biol.* **4**, 42–50 (2002).
148. Sheu, Y. J., Santos, B., Fortin, N., Costigan, C. & Snyder, M. Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. *Mol. Cell Biol.* **18**, 4053–4069 (1998).
149. Riedl, J. *et al.* Lifeact: a versatile marker to visualize F-actin. *Nature Methods* **5**, 605–607 (2008).

Acknowledgements

Work in the author's laboratory is supported by the UK Biotechnology and Biological Sciences Research Council grant BB-F007892.

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

Peter E. Sudbery's homepage:
<http://www.sheffield.ac.uk/mbf/staff/sudbery>

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (movie) | S3 (movie) | S4 (movie)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF