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CDKs and the yeast-hyphal decision

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Fungal cells exist in a diverse range of morphologies. Some species, such as *Candida albicans*, are dimorphic capable of growing either in a yeast-like form or as a hypha. Cyclin-dependent kinases (CDKs) have long been thought to play a central role in the yeast-hyphal decision. However, until recently direct links of CDKs with proteins that execute polarized growth were elusive. In this review I will focus on new findings that have established concrete links between CDKs and several key components of the polarity machinery in *C. albicans* and the budding yeast *Saccharomyces cerevisiae.* Inhibitory phosphorylation of the GTPase-activating proteins (GAPs) of Cdc42 has emerged as a common mechanism underlying polarized growth in both organisms. *C. albicans* contains a hyphal-specific cyclin Hgc1. In association with the CDK Cdc28 it ensures hyphal development by phosphorylating the Cdc42 GAP Rga2, two septins and the transcription factor Efg1. This review will discuss both conserved mechanisms and ones specific for hyphal development in *C. albicans*.

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Introduction

Fungal cells attain specific morphologies by directing cell growth to different areas of the cell surface. Spreading growth uniformly (isotropic growth) makes round cells, whereas confining it to a small area (polarized growth) makes tubular cells. But what controls the pattern of growth? Many years of studies on *Saccharomyces cerevisiae* have produced the following prevalent model [1]: the cyclin-dependent kinase Cdk1 (also known as Cdc28) associates sequentially with G1 and G2 cyclins to guide bud growth through polarized and isotropic phases in a cell cycle; and the relative length of each phase dictates the shape of the daughter cell. Although well received and backed by strong evidence, this model faces a challenge in explaining the growth pattern of fungal hyphae that occurs continuously at the growing tips in a cell-cycle-independent manner. In recent years, significant progress has been made in elucidating the mechanisms that govern hyphal development in *Candida albicans*. This fungus can grow as yeast, pseudohyphae or hyphae depending on environmental circumstances [2]. It also exhibits dramatic elongation when the cell cycle is perturbed [3]. This high morphological plasticity has greatly facilitated discoveries of molecular mechanisms that control growth patterns in fungi.

A challenging task in understanding how CDKs control cell morphogenesis is to identify their direct regulatory targets. This review will discuss CDKs' roles in fungal morphogenesis with an emphasis on recent findings that have unveiled molecular links between CDKs and several key proteins of the cell's polarity machinery from the studies of both *C. albicans* and *S. cerevisiae*.

CDKs control cell morphogenesis

The concept that CDKs have a role in cell morphogenesis was first formulated and developed in the study of bud formation in *S. cerevisiae* [4]. Cdk1 directs bud growth via association with different cyclins through the cell cycle [5,6]. $Cdk1^{G1}$ promotes bud emergence and polarized growth at the tip, whereas $Cdk1^{G2}$ later switches the growth to isotropic expansion. Timing of the switch is critical in shaping the bud: a premature switch produces round buds, whereas a delayed one causes bud elongation. This control involves CDKs through the morphogenesis checkpoint, which delays mitotic entry via Swe1-mediated inhibitory phosphorylation of Cdk1 [7,8].

In *C. albicans*, CDKs also play important roles in morphogenesis during both yeast and hyphal growth. *C. albicans* contains two G1 (Ccn1 and Cln3) and two G2 (Clb2 and Clb4) cyclins for cell-cycle control in contrast to three G1 (Cln1–3) and six G2 (Clb1–6) cyclins in *S. cerevisiae*. Perhaps owing to reduced redundancy as well as an intrinsic capacity for filamentation, deleting some of the cyclin genes causes dramatic morphological changes in *C. albicans*. Depletion of Cln3, Clb2 or Clb4 in yeast cells results in strong filamentation in the absence of hyphal-inducing signals [9–11], and deletion of *CCN1* impairs the maintenance of hyphal growth [12,13••]. Whether a Swe1-mediated morphogenesis checkpoint operates in *C. albicans* remains unclear, because deleting *SWE1* has not significantly affected any form of polarized growth [14]. However, other cell-cycle checkpoints may be involved. Activation of the DNA-damage, DNA-replication or spindle-assembly checkpoint in yeast cells all

provoke filamentous growth [15•,16]. Zheng *et al.* [17] discovered that *C. albicans* contains a novel G1 cyclin gene, *HGC1*, specifically used for promoting hyphal growth. Unlike the cell-cycle-regulated expression of other cyclin genes, *HGC1* is activated by hyphal-inducing signals. Evolution of this control has proved to be crucial for ensuring the cell-cycle-independent hyphal extension (see below).

The Cdc42 GTPase module plays a central role in polarized growth

The Rho-type GTPase Cdc42 is a central regulator of cell polarity in fungi [18]. It orchestrates multiple cellular activities, including polarization of the actin cytoskeleton and delivery of secretory vesicles to the sites of growth [19]. In *S. cerevisiae*, cells of *cdc42* null mutants cannot polarize growth, but instead grow isotropically, resulting in large, round cells [20,21]. Conversely, overexpression of a constitutively active form of Cdc42 can induce initial polarization in cells lacking $Cdk1^{G1}$ [22]. Therefore, activation of Cdc42 is both necessary and sufficient to promote cell polarization. Cdc42 cycles between a GTP-bound active and a GDP-bound inactive state. The levels of Cdc42-GTP are regulated positively by guanine-nucleotide exchange factors (GEFs) and negatively by GTPase-activating proteins (GAPs). Cdc24 is the sole Cdc42 GEF in *S. cerevisiae*. Temperature-sensitive mutants of *CDC24* exhibit polarization defects similar to those of *CDC42* at the restrictive temperature [23]. *S. cerevisiae* contains three GAPs specific for Cdc42: Rga1, Rga2, and Bem3, which have overlapping functions in morphogenesis [19]. Significant bud elongation occurs only when two or three of them are deleted simultaneously [24].

The Cdc42 module also plays an essential role in *C. albicans* hyphal development. Lowering the expression of *CDC42* or *CDC24* to suboptimal levels can abolish hyphal growth without a significant effect on yeast growth [25]. This observation not only shows the essentiality of both proteins for hyphal growth, but also indicates that hyphal growth requires more Cdc42 than yeast growth does. *C. albicans* contains two Cdc42 GAPs Bem3 and Rga2. *bem3*Δ/Δ and *rga2*Δ/Δ mutants are normal in hyphal growth, but the latter produces elongated yeast cells [26••,27••]. The *bem3*Δ/Δ *rga2*Δ/Δ double mutant grown under pseudohyphal-promoting conditions displays a hyphal morphology [26••], suggesting that high levels of Cdc42-GTP stimulate hyphal development. A key role of Rga2 in hyphal growth was discovered when the deletion of *RGA2* in *hgc1*Δ/Δ cells was found to restore hyphal growth [27••]. It suggests that Hgc1 promotes hyphal growth by inactivating Rga2.

CDKs target components of the Cdc42 GTPase module

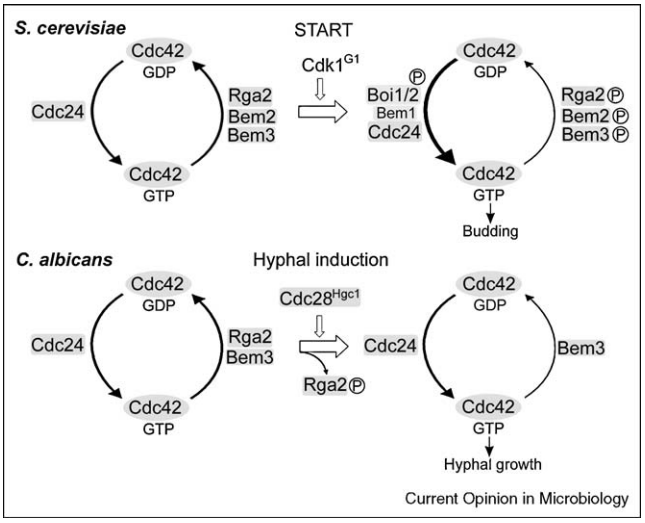
Early studies in *S. cerevisiae* provided the initial evidence that CDK^{G1} might activate cell polarization by targeting the Cdc42 module [28]. However, a daunting task is to identify the substrates of CDK^{G1}. Cdc24 has been an appealing candidate because of its CDK^{G1}-dependent behavior. In haploid cells, Cdc24 is sequestered in the nucleus by Far1 in G1 phase [29]. Phosphorylation of Far1 by CDK^{G1} at START triggers its degradation and export of Cdc24 to a site of polarization where it recruits and activates Cdc42 [30]. However, a cytoplasmic form of Cdc24 is unable to induce polarized growth in cells lacking CDK^{G1} [29]. Thus, CDK^{G1} must target proteins other than Far1 for Cdc24 polarization. Cdc24 itself might be a substrate, because it can be phosphorylated by CDK^{G1} complexes *in vitro*. However, mutating six potential CDK phosphorylation sites on Cdc24 did not affect its function *in vivo* [28]. Therefore, either Cdc24 might contain additional CDK phosphorylation sites or Cdc24-associated factors are CDK^{G1} substrates. McCusker *et al.* [31••] identified physical associations of Cdc24 at bud emergence with Boi1 and Boi2, two homologous proteins that are involved in regulating Cdc42 and Rho3 for polarized exocytosis [32]. Importantly, Boi1 and Boi2 undergo CDK^{G1}-dependent phosphorylation both *in vivo* and *in vitro*; and Boi1 mutants lacking CDK phosphorylation sites fail to polarize, a defect similar to the inactivation of Cdc24.

Recently, several groups reported that CDK^{G1} activates polarized growth by phosphorylating Cdc42 GAPs. In *S. cerevisiae*, Bem3 and Rga2 are hyperphosphorylated at the time of bud emergence in a CDK-dependent manner, and expression of an unphosphorylatable version of either protein in wild-type cells produces unpolarized morphologies [33••,34••]. Rga2 is a substrate of two sets of CDKs containing the Cdc28 or Pho85 CDK in complex with their respective G1 cyclins [34••]. Overexpression of *RGA2* in the absence of functional Pho85 or Cdc28 CDK complexes is toxic, because of an inability to polarize growth. The interpretation of these observations is that CDK^{G1} phosphorylates and inhibits the GAP activity of Bem3 and Rga2, thereby increasing the amount of Cdc42-GTP and leading to actin polarization. Inhibition of a Cdc42 GAP by CDK phosphorylation also underlies the control of hyphal development in *C. albicans*. Hyphal growth requires sustained activation of Cdc42 at the growing tips. This is achieved by a simple mechanism [27••]: the hypha-specific $Cdc28^{Hgc1}$ phosphorylates and prevents Rga2 from localizing to hyphal tips where Cdc42 is concentrated, resulting in a local increase of Cdc42-GTP. Together, these new findings indicate that the inhibition of GAPs by CDKs may be a conserved mechanism that promotes polarized growth in fungi [35] (Figure 1).

CDKs target septins

Septins are a family of GTP-binding, filament-forming proteins [36]. Although best known for their roles in cytokinesis, septins have also been closely linked to

Figure 1



CDKs promote polarized growth through inhibitory phosphorylation of Cdc42 GAPs both during bud formation in *S. cerevisiae* and hyphal growth in *C. albicans*. See text for details.

polarized growth [19]. In yeast cells, they polarize to form a cortical ring that marks the site of bud emergence [19,37]. In many mutants with elongated buds septins nearly always exist at the tips, and mutations that disrupt septin organizations often cause cell elongation [19,37,38]. In *C. albicans*, septins polarize to the site of germ-tube formation and then localize persistently at hyphal tips [13••,39]. Deletion of a non-essential septin gene *CDC10* or *CDC11* impairs hyphal growth [40]. Knocking down septins also blocks the formation of dendritic spines, finger-like surface protrusions, in mammalian neurons [41,42]. These observations suggest an evolutionarily conserved role for septins in polarized growth. This role is clearly defined in the recent discovery of direct phosphorylation of Cdc11 by CDKs in *C. albicans* [13••]. In response to hyphal induction $Cdc28^{Ccn1}$ immediately phosphorylates Cdc11 at Ser395, after which the hypha-specific $Cdc28^{Hgc1}$ is required to sustain this phosphorylation throughout hyphal growth. Without Hgc1, Ser395 phosphorylation is quickly lost, terminating hyphal extension. Here two CDKs regulate hyphal development through temporally controlled phosphorylation of a septin. This finding unveils a molecular event that demonstrates the significance of placing *HGC1* expression under the control of hyphal-inducing signals. There is evidence that septins at the septum and the tip in apical hyphal cells compete in attracting exocytosis [43•]. Ser395 phosphorylation is thought to reduce the affinity of the septin ring for the exocyst landmark Sec3, thereby favoring growth at the tip [43•,44]. Septins as CDK substrates and their role in polarized exocytosis seem to be highly conserved. They associate with exocyst in mammalian neurons [45], and Cdk5 phosphorylates the septin SEPT5 and modulates exocytosis [46].

$Cdc28^{Hgc1}$ also participates in the phosphorylation of the septin Sep7, which is required for the assembly of hyphal-specific septin rings to prevent cell separation after cytokinesis [47••]. In *S. cerevisiae*, there has been no evidence that CDKs directly phosphorylate septins. But they may control septin ring assembly indirectly through Cdc42 and its effector kinase Cla4 [19,48,49].

$Cdc28^{Hgc1}$ phosphorylates the transcription factor Efg1

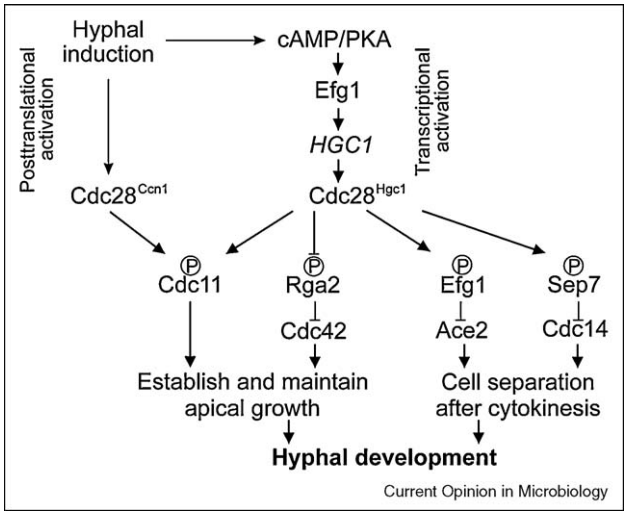
To prevent cell separation in hyphae, genes encoding enzymes responsible for the post-cytokinesis septum degradation during yeast growth are suppressed. Some of these genes are controlled by the transcription factor Ace2 [50,51]. $Cdc28^{Hgc1}$ plays a role in downregulating Ace2-activated genes by phosphorylating another transcription factor Efg1 [52••]. The phosphorylated Efg1 binds strongly to the promoters of these genes, suppressing their expression.

Cell-cycle checkpoints and polarized growth

C. albicans yeast cells switch to filamentous growth when treated with genotoxins such as the DNA-replication inhibitor hydroxyurea (HU) and DNA-damaging agents [15•,53]. This phenotype requires the DNA-replication and damage checkpoints and is abolished in mutants lacking the Rad53 effector kinase [15•]. Several recent findings suggest that Rad53 may have a direct role in regulating polarized growth. First, *rad53*Δ/Δ cells have defects in hyphal growth [15•]. Second, mutating certain amino acids in the FHA1 domain abolishes HU-induced filamentation without significantly compromising other responses [15•]. This observation is important, because it suggests that like cell-cycle arrest and activation of DNA repair, filamentous growth might be another checkpoint-activated cellular response to genotoxic stresses. Third, *S. cerevisiae* also grows elongated buds when treated with HU, and *rad53*Δ/Δ cells exhibit aberrant morphologies even under unperturbed conditions [54,55,56••].

How might Rad53 activate polarized growth? A recent study shows that *S. cerevisiae* Rad53 directly binds septins and localizes to the bud neck in a manner enhanced by genotoxic stress [56••]. Overexpression of the FHA1 domain results in elongated cells lacking normal septin structures [57••]. These data suggest that Rad53 may influence polarized growth via septins. Consistently, Rad53 can phosphorylate Sep7 *in vitro*, and deleting *SEP7* suppresses the morphological defect resulted from FHA1 overexpression. Thus, Sep7 is likely a direct target of Rad53. The septin ring anchors components of the morphogenesis checkpoint [1,8], providing a link to Cdk1. Rad53 also associates with septins in *C. albicans*

Figure 2



In *C. albicans* two CDKs $Cdc28^{Ccn1}$ and $Cdc28^{Hgc1}$ work in a temporally controlled manner to promote and ensure continuous hyphal extension. They act through direct phosphorylation of multiple proteins crucial for polarized growth and inhibition of cell separation after cytokinesis, two defining features of hyphal growth. Whereas $Cdc28^{Hgc1}$ is regulated at the transcriptional level, $Cdc28^{Ccn1}$ is activated immediately in response to hyphal induction to phosphorylate Cdc11, strongly suggesting a post-translational control.

(WJ Li, Y Wang, unpublished results), but the significance remains to be investigated.

Many other conditions that interfere with the cell cycle can cause cell elongation in *C. albicans*. Some are mediated by well-defined checkpoint pathways [15•,57••], while others are not [9–11,58–61]. Great challenges lie ahead in establishing the pathways linking the events that disturb the cell cycle with the polarity machinery.

Conclusion

In the past few years, exciting advances have been achieved in understanding how CDKs control morphogenesis in fungi by establishing concrete links between CDKs and some key components of the polarity machinery. One conserved mechanism has emerged that CDKs promote polarized growth through inhibitory phosphorylation of Cdc42 GAPs, although the specific mechanisms of inhibition vary. In *C. albicans*, evolution of the hyphal-specific $Cdc28^{Hgc1}$ provides a mechanism to ensure the continuous, cell-cycle-independent hyphal growth. The identification of a Cdc42 GAP, two septins and the transcription factor Efg1 as the substrates of $Cdc28^{Hgc1}$ has revealed molecular events crucial for establishing and maintaining hyphal development (Figure 2). The phosphorylation of Cdc11 by $Cdc28^{Ccn1}$ immediately after hyphal induction indicates that certain modifications of the polarity machinery occur before the expression of hyphal-specific genes. Past efforts on the identification of hypha-specific genes have produced few genes important for hyphal growth. Perhaps it is time to shift our focus to post-transcriptional and post-translational levels to unearth mechanisms underlying the yeast-hyphal decision in fungi. To this end, endeavors in identifying substrates of CDKs have proved to be productive in yielding mechanistic insights.

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