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REVIEW

Hgc1-Cdc28–how much does a single protein kinase do in the regulation of hyphal development in *Candida albicans*?

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The fungal human pathogen *Candida albicans* can cause invasive infection with high mortality rates. A key virulence factor is its ability to switch between three morphologies: yeast, pseudohyphae and hyphae. In contrast to the oval-shaped unicellular yeast cells, hyphae are highly elongated, tube-like, and multicellular. A long-standing question is what coordinates all the cellular machines to construct cells with distinct shapes. Hyphal-specific genes (HSGs) are thought to hold the answer. Among the numerous HSGs found, only *UME6* and *HGC1* are required for hyphal development. *UME6* encodes a transcription factor that regulates many HSGs including *HGC1*. *HGC1* encodes a G1 cyclin which partners with the Cdc28 cyclin-dependent kinase. Hgc1-Cdc28 simultaneously phosphorylates and regulates multiple substrates, thus controlling multiple cellular apparatuses for morphogenesis. This review is focused on major progresses made in the past decade on Hgc1's roles and regulation in *C. albicans* hyphal development and other traits important for infection.

Keywords: *Candida albicans*, yeast-to-hyphae growth transition, cyclin-dependent kinase, polarized growth, protein phosphorylation

Introduction

Candida albicans is a predominant opportunistic fungal pathogen in humans (Bassetti *et al*., 2010). It is also a member of the human microbiota. Together with trillions of commensal microbial cells belonging to >1,000 species, *C. albicans* colonizes and thrives in many parts of our body such as the skin and mucosal surfaces in nearly all healthy individuals. Although it is not clear whether *C. albicans* does anything that may benefit the host like many commensal microbes do, it is responsible for a wide range of superficial infections. Approximately 75% of women will suffer at least one episode of vulvovaginal candidiasis in their life time (Brown *et al*., 2012). Although most of the superficial infections can be treated and cured easily, *C. albicans* can cause life-threatening invasive disease in individuals under certain medical conditions. In patients with severely compromised immunity, *C. albicans* can enter the circulation and is able to colonize virtually all organs, leading to death of the patient. In the past 3 decades, due to the AIDS pandemic and the ever-increasing use of powerful anticancer and immunosuppressive therapies and broad-spectrum antibiotics in hospitals, *C. albicans* has become the most common fungal pathogen for nosocomial blood-stream infection with mortality rates often exceeding 50% in spite of the use of antifungal therapies (Brown *et al*., 2012). Currently, options of antifungal drugs are limited, populations of susceptible patients are growing rapidly, and drug-resistant strains are emerging worldwide, posing a great challenge to medical sciences to develop new effective antifungal strategies. To combat this terrible disease, scientists need to uncover, understand and eventually design approaches to target the key mechanisms underlying *C. albicans* pathogenesis.

A defining feature of *C. albicans* is its ability to grow and rapidly switch among three main morphological forms: budding yeast, pseudohyphae, and true hyphae, in response to environmental cues (Sudbery *et al*., 2004). A general understanding is that the different forms of *C. albicans* play different roles during infection. Because of their unicellular nature, small sizes and oval shape, yeast cells can easily travel through the circulation system, thus disseminating to initiate colonization in many parts of the body, whereas hyphae possess strong penetrative power which facilitates both the invasion of host tissues and evasion of host immune cells (Sudbery, 2011). Consistently, mutants locked in either the yeast or the hyphal form of growth have diminished virulence. Now it is well established that the yeast-to-hyphae transition is a key virulence determinant of *C. albicans*.

For decades, great efforts have been invested in elucidating the factors and mechanisms in both the host and *C. albicans* that trigger or control the yeast-to-hyphae transition. Many nutritional and environmental cues have been found that activate this growth transition such as serum (Taschdjian *et al*., 1960), peptidoglycan (Wang and Xu, 2008; Xu *et al*., 2008), N-acetylglucosamine (Simonetti *et al*., 1974), neutral pH (Buffo *et al*., 1984), host physiological temperature, nu-

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trient starvation (Lu *et al*., 2011), hypoxia, and CO_2 (Klengel *et al*., 2005). These signals activate a number of signal transduction pathways (Whiteway, 2000), which converge to activate multiple transcription regulators, turning on the expression of hundreds of hyphae-specific genes (HSGs). Among these pathways, the Ras-cAMP-protein kinase A (PKA) signalling cascade plays an essential role (Sudbery, 2011). A central component of this pathway is the adenylate cyclase Cyr1 that has the capacity to sense and integrate several major hyphal inducing signals (Wang, 2013).

Currently, how the cellular machines are switched rapidly from a mode that generates the oval-shaped unicellular yeasts to one that constructs the highly elongated multicellular hyphae remains largely unclear. Mechanistic understanding of the yeast-to-hyphae transition has much broader importance than just finding ways to treat *C. albicans* infection; it will help elucidate the molecular mechanisms that control some fundamental biological processes such as cell morphogenesis, polarity establishment and maintenance, and membrane trafficking. Since late 1990's, great enthusiasm has persisted in the *C. albicans* field to search for HSGs using various technologies, because HSGs are thought to hold the key to unlocking the mysteries shrouding the yeast-to-hyphae transition. To date, mainly by means of microarray and RNA-Seq, hundreds of HSGs have been found in diverse experimental settings (Murad *et al*., 2001; Nantel *et al*., 2002; Kodash and Johnson, 2005; Bruno *et al*., 2010). Surprisingly, although a significant number of HSGs have been found to be responsible for properties associated with hyphal cells such as adhesion to host tissues (Staab *et al*., 1999), secretion of lytic enzymes (Naglik *et al*., 2003), and acquisition of iron (Ramanan and Wang, 2000), only a few genes are required for hyphal growth such as *HGC1* and *UME6* (Zheng *et al*., 2004; Carlisle and Kodash, 2010). *HGC1* encodes a G1 cyclin that partners with the cyclin-dependent kinase (CDK) Cdc28. *UME6* encodes a transcription factor and plays its role in hyphal growth mainly, if not entirely, through maintaining the expression of *HGC1* throughout hyphal development (Banerjee *et al*., 2008). This implies that *HGC1* plays a central role in the control of the hyphal development. How does a single protein control hyphal morphogenesis which requires complex regulation and coordination of multiple cellular machines? Cdc28 is a highly conserved master regulator of cell cycle progression and capable of regulating multiple, highly complex cellular processes including DNA replication, chromosome segregation, and cytokinesis (Nurse, 2002). Thus, it can be expected that the Hgc1-Cdc28 kinase, once turned on by inducing signals, is capable of activating and organizing the cellular machines for hyphal construction. *HGC1* was identified 10 years ago, and the time is ripe for a review of the many progresses that have been made to elucidate its roles in *C. albicans* hyphal development.

Discovery of *HGC1* as a key regulator of hyphal development

Possibly owing to its low expression level in hyphal cells, *HGC1* was not found among the HSGs in early microarray-based studies. Its discovery came from a hypothesis-driven approach guided by knowledge of how bud formation is controlled in *Saccharomyces cerevisiae*. In early 1990's, Lew and Reed (1993) proposed that Cdc28 in association with different cyclins plays a central role in determining how a bud takes a shape (Fig. 1, yeast growth). In the beginning of a cell cycle, the G1 cyclin-Cdc28 kinases, Cln1-Cdc28 and Cln2-Cdc28, promote bud emergence and confine cell growth to the bud apex, leading to bud elongation, whereas in a later stage the G2 kinases, Clb1-Cdc28 and Clb2-Cdc28, take over to terminate the apical growth and stimulate a switch to isotropic bud expansion (Lew and Reed, 1995). Timing of the apical-to-isotropic switch is critical in shaping the bud: an early switch produces round buds, whereas a delayed one causes bud elongation. If the switch is blocked, a hypha-like long bud can be formed. Based on this model, Zheng *et al*. (2004) hypothesized that a G1 cyclin might be able to drive apical extension of hyphae in *C. albicans* if its activity is activated and kept high by hyphal induction signals. To test this hypothesis, they first examined whether any of the G1 cyclin genes was up-regulated or expressed specifically in hyphal cells. In an early version of the *Candida* genome database (Inglis *et al*., 2012) three homologs of G1 cyclin genes annotated as *CCN1*, *CLN3*, and *CLN21* were found. By Northern blot analysis, comparable levels of *CCN1* and *CLN3* mRNA were detected in yeast and hyphal cells, but *CLN21* mRNA could only be detected in hyphal cells. Using synchronized cells, it was further found that while *CCN1* expression was cell-cycle phase dependent in both yeast and hyphal cells, *CLN21* was constitutively expressed throughout the cell cycle. The specific and constitutive expression of *CLN21* in hyphae satisfied the basic requirements, hypothesized above, for a G1 cyclin to drive hyphal development (Fig. 1, hyphal growth). *CLN21* was then renamed *HGC1* for Hyphal-specific-G1 Cyclin 1 (Zheng *et al*., 2004). Deletion

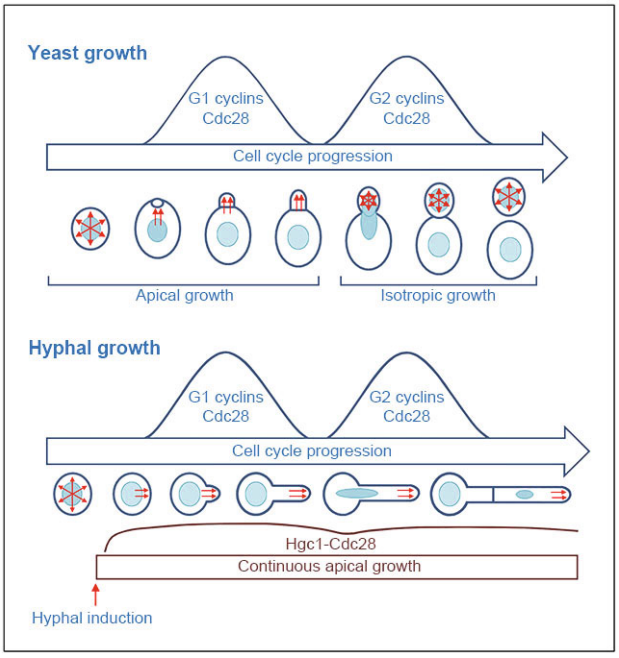

Fig. 1. Cyclin-CDK complexes control morphogenesis in yeasts. *C. albicans* uses a specialized, hyphal-specific G1 cyclin Hgc1 to promote persistent tip growth throughout hyphal development. See the text for detailed explanation.

of *HGC1* caused severe defects in hyphal development under all experimental conditions tested and blocked the constitutive filamentous growth in mutants deleted for the hyphal repressor gene *TUP1* (Braun and Johnson, 1997). These results established *HGC1* as a positive regulator of hyphal growth. Like most other HSGs, *HGC1* expression is activated through the cAMP-PKA pathway and the transcription factor Efg1 and kept off by the transcription repressor complex Tup1/Nrg1 (Braun *et al*., 2001). This finding is important, because it indicates that evolution has placed multiple traits important for virulence and infection under the same control.

Co-immunoprecipitation (co-IP) experiments showed that Hgc1 physically associates with Cdc28, the master cell-cycle regulator (Zheng *et al*., 2004). However, *hgc1Δ/Δ* mutants exhibited no obvious defects in cell cycle progression. One reasonable explanation is that during evolution Hgc1 has lost its function in cell cycle control and become specialized in promoting polarized growth during hyphal development. The constitutive expression of *HGC1* throughout hyphal development is also consistent with the continuous, cell-cycle independent hyphal tip extension. While *HGC1* is required for hyphal growth, is it sufficient? Constitutive expression of *HGC1* under the control of *ACT1* promoter failed to cause hyphal growth (Zheng *et al*., 2004). However, our unpublished data showed that when driven by the strong TET-Off promoter, *C. albicans* showed significant filamentous growth.

C. albicans cells homozygous at the Mating Type Locus (MTL) can undergo a switch between white and opaque morphological forms (Johnson, 2003). White yeast cells are round, and opaque yeast cells are ellipsoidal. Both types of yeast cells can switch to hyphal growth, but they respond to different environmental triggers and involve two distinct sets of genes with only a few overlaps (Si *et al*., 2013). Interestingly, among the few genes involved in both programs are found *HGC1* and *UME6*, and deleting either one completely blocks the hyphal growth in opaque cells. In another study, Guan *et al*. (2013) demonstrated that opaque cells deleted for the transcription factor gene *BCR1* exhibit constitutive filamentous growth in a manner strictly dependent on *HGC1*. Furthermore, *HGC1* overexpression using a TET-On promoter was sufficient to drive strong filamentous growth in opaque cells under non-inducing conditions. Together, the data strongly support a role for Hgc1 as a master regulator of cellular processes that drive filamentous growth.

HGC1 provides definitive evidence that hyphal growth is an important for virulence

hgc1Δ/Δ mutants exhibited markedly reduced virulence in the mouse model of systemic infection (Zheng *et al*., 2004). The data not only demonstrate *HGC1*'s importance for virulence, but also help establish a definitive role for the yeast-to-hyphae transition as a key virulence factor (Zheng *et al*., 2004). Although many previous studies had reported that mutants defective in the yeast-to-hyphae transition had diminished virulence, most of the mutations affected the upstream signalling pathways that control HSG expression. Thus, these mutations impair not only hyphal morphogenesis but also other virulence traits, rendering the role of the yeast-to-hyphae transition in virulence inconclusive. As *HGC1* is a HSG itself and plays a specific role in hyphal growth, deleting *HGC1* is expected to abolish hyphal development without affecting the expression of other HSGs, thus providing a conclusive test on the role of the yeast-to-hyphae transition in virulence. Diminished virulence of *hgc1Δ/Δ* mutants were also observed in a zebra fish model of *C. albicans* systemic infection (Chao *et al*., 2010).

Hgc1-Cdc28 phosphorylates and regulates multiple cellular machines for polarized growth

One successful approach to untangle the complex mechanisms by which Hgc1-Cdc28 controls hyphal morphogenesis is to first identify its substrates. Extensive previous studies of key regulators of cell morphogenesis in *S. cerevisiae* helped tremendously to shorten the list of the candidates of Hgc1-Cdc28 substrates. A highly successful approach to identifying Hgc1-cdc28 substrates has been to use western-blotting to detect electrophoretic mobility shift of a candidate specifically in wild-type strains but not in *hgc1Δ/Δ* mutants under hyphal-induction conditions.

Rga2

The Rho GTPase Cdc42 is a highly conserved regulator of cell polarity in eukaryotic cells (Etienne-Manneville, 2004) and is essential for budding in *S. cerevisiae* and for both budding and hyphal growth in *C. albicans* (Bassilana *et al*., 2005; Court and Sudbery, 2007). A main effector of Cdc42 is the formin Bni1 that assembles actin cables for secretory vesicle transport to the growth site at the bud and hyphal tip (Evangelista *et al*., 1997; Li *et al*., 2005). Cdc42 works as a molecular switch cycling between an active ATP-bound form and an inactive ADP-bound form. ATP hydrolysis by Cdc42 is activated by GTPase-activating proteins (GAPs), leading to the formation of ADP-Cdc42, whereas the conversion of ADP-Cdc42 to ATP-Cdc42 is catalysed by guanine-nucleotide exchange factors (GEFs) (Park and Bi, 2007). Thus, activation of Cdc42 can be achieved by either activating a GEF or inactivating a GAP. *C. albicans* has two Cdc42 GAPs, Rga2 and Bem3. Zheng *et al*. (2007) discovered that hyphal induction causes Hgc1-Cdc28 hyperphosphorylation of Rga2, resulting in its physical separation from Cdc42 at the hyphal tip. During the yeast growth, Rga2 colocalizes with Cdc42 at the bud tip and the bud neck. In contrast, unlike Cdc42 which persistently localizes to the hyphal tip (Crampin *et al*., 2005; Pulver *et al*., 2013), Rga2 is found in the cytoplasm. Mutation of the phosphorylation sites in Rga2 revealed that Hgc1-Cdc28 phosphorylation prevents Rga2 from localizing to the hyphal tip. Thus, a model was proposed that keeping Rga2, a negative regulator of Cdc42, away from the hyphal tip allows persistent local activation of Cdc42 (Zheng *et al*., 2007). Deleting *RGA2* or inactivating its GAP activity can fully restore hyphal growth in *hgc1Δ/Δ* mutants, indicating that Rga2 is a hyphal repressor which is inactivated by Hgc1-Cdc28 upon hyphal induction. Rga2 also undergoes transient Cdc28-dependent hyperphosphorylation at bud emergence, suggesting that regulating a GAP(s) of Cdc42 by CDKs may play an important role in governing different forms of polarized morphogenesis in yeasts. *rga2Δ/Δ* yeast cells are of an elongated morphology, consistent with enhanced Cdc42 activity at the bud tip. This study estab-

lished a direct molecular link of Hgc1-Cdc28 to the Cdc42 module, a central regulator of cell polarity.

CDK phosphorylation of GAPs seems to be a conserved mechanism that controls polarized growth in yeasts. In *S. cerevisiae*, two GAPs Bem2 and Bem3 are hyperphosphorylated at the time of bud emergence in a CDK-dependent manner, and expression of an unphosphorylatable version of either GAP in wild-type cells leads to unpolarised morphologies (Knaus *et al.*, 2007). Because non-phosphorylatable Bem3 mutants are hyperactive and interfere with Cdc42 activation, the authors propose that Cln-Cdc28 kinase contributes to site-specific activation of Cdc42 at bud emergence through inhibitory phosphorylation of its GAPs. *RGA2* overexpression in the absence of functional Cdc28 is toxic as a result of failure to control polarized growth (Sopko *et al.*, 2007). By mapping and then mutating phosphorylated residues, the authors provide evidence that CDK-dependent phosphorylation restrains Rga2 activity to ensure appropriate activation of Cdc42 during cell polarity establishment.

Cdc11 and Sep7

Septins are a family of GTP-binding and filament-forming proteins, best studied for their roles in cytokinesis (Fung *et al.*, 2014). In fungi, they also take part in polarized growth. In yeasts, they are among the first proteins that polarize to the presumptive budding sites, forming a ring that encircles the area for initial bud growth (Sudbery, 2001). Interestingly, in filamentous fungi including the hyphae of *C. albicans*, septins often localize to hyphal tips, strongly suggesting a role in polarized growth (Warenda and Konopka, 2002). *C. albicans* has the same five mitotic septins as *S. cerevisiae* including Cdc3, Cdc10, Cdc11, Cdc12, and Sep7. Using 2D Western blotting, Sinha *et al.* (2007) discovered that Cdc11 undergoes phosphorylation immediately following hyphal induction and this phosphorylation is maintained throughout hyphal growth. In *hgc1Δ/Δ* mutants, this rapid Cdc11 phosphorylation can still occur in response to hyphal induction, but it is quickly lost, suggesting that Hgc1-Cdc28 is required to keep Cdc11 in the phosphorylated state for hyphal development. This is consistent with the observation that *hgc1Δ/Δ* yeast cells can respond to hyphal induction and form short and swollen protrusion but fail to form hyphae (Zheng *et al.*, 2004). A series of elegant genetic and biochemical studies revealed that the G1 cyclin Ccn1-Cdc28 kinase phosphorylates Cdc11 at serine 395 upon hyphal induction and the maintenance of this phosphorylation is required for normal hyphal development. As Ccn1 is synthesized in G1 and degraded in later phases, it is unable to fulfil the persistent Cdc11 phosphorylation throughout hyphal growth. This job was found to be taken over by Hgc1-Cdc28 which is hyphal-specific and cell-cycle independent. Currently, how septins at the hyphal tip influence hyphal growth remains unclear. One mechanism could be that septins help to recruit the exocyst to the hyphal tip and thus assist exocytosis (Li *et al.*, 2007). In mammals, some isoforms of septins are localized to the growth cones of growing neurites and have been found to physically associate with components of the exocyst in rat brain tissues (Hsu *et al.*, 1998; Xue *et al.*, 2004).

González-Novo *et al.* (2008) reported that upon hyphal induction, septin rings are converted to a hyphal-specific state, in which Sep7, Cdc3, and Cdc12 form a frozen core, whereas Cdc10 undergoes highly dynamic exchange between the ring and the cytoplasm. This hyphal-specific state of septin ring is important for preventing cell separation after cytokinesis so that long hyphae can be formed. This modification of septin ring dynamics during hyphal growth is dependent on Sep7, and Hgc1-Cdc28 takes part by controlling Sep7 phosphorylation. Whether Hgc1-Cdc28 phosphorylation of Cdc11 also has a role in assembling the hyphal-specific septin ring remains a possibility to be tested.

Sec2 and Exo84

C. albicans hyphal growth requires membrane trafficking from the endoplasmic reticulum (ER) to Golgi and finally to the site of cell growth at the bud or hyphal tip. Rab GTPases play a central role in multiple steps of this ER to plasma mem-

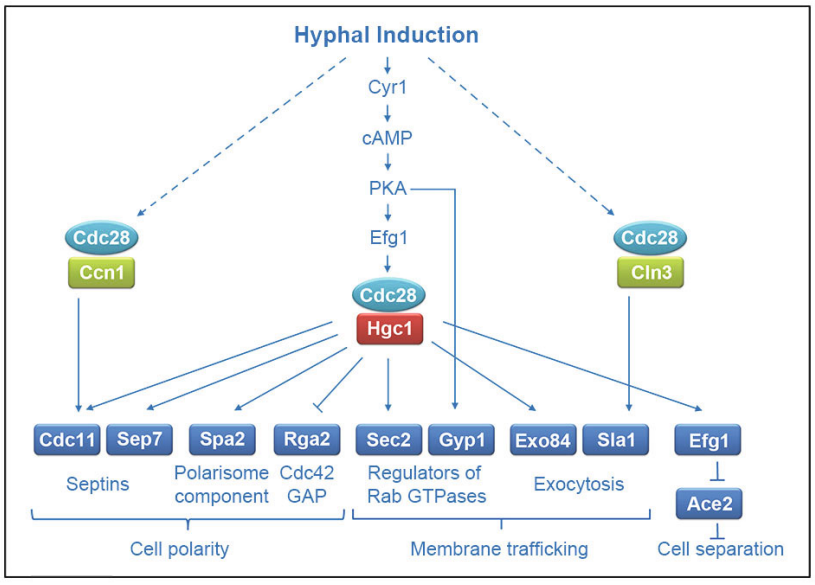

Fig. 2. Hgc1-Cdc28 directly regulates multiple cellular processes important for hyphal morphogenesis.

brane transport (Hutagalung and Novick, 2011). The Rab GTPase Sec4 is a resident protein on the surface of secretory vesicles and specifically mediates the post-Golgi stage of transport. Sec2 is a GEF that activates Sec4 (Ortiz *et al*., 2002). Bishop *et al*. (2010) used a combination of deletion mapping, *in vitro* mutagenesis, an analogue-sensitive allele of Cdc28 and *in vitro* kinase assays and gained strong evidence that Hgc1-Cdc28 phosphorylates Sec2 and regulates its localization. Blocking this phosphorylation causes abnormal hyphal development. This study uncovered a novel molecular link of Hgc1-Cdc28 to mechanisms that control polarized secretion.

When delivered to the growth site, secretory vesicles are tethered to a multiprotein complex called exocyst before fusion with the plasma membrane. One component of the exocyst is Exo84. In *S. cerevisiae*, phosphorylation of Exo84 by Clb2-Cdc28 causes the exocyst to disassemble. Caballero-Lima and Sudbery (2014) discovered that during *C. albicans* hyphal growth Hgc1-Cdc28 phosphorylates Exo84 and alters its affinity for phosphatidylserine, allowing it to recycle at the plasma membrane. This phosphorylation is required for efficient hyphal extension.

Efg1

Fungal filaments are chains of elongated cells whose formation requires suppression of cell separation after cytokinesis. In both *S. cerevisiae* and *C. albicans*, the transcription factor Ace2 turns on the genes encoding enzymes such as chitinases and glucanases for septum degradation, leading to separation of mother and daughter cells at the end of a cell cycle (Weiss, 2012; Calderón-Noreña *et al*., 2015). Wang *et al*. (2009) discovered a fascinating mechanism that prevents Ace2 from activating its target genes. Efg1 is a transcription factor well studied for its role in activating HSG expression (Stoldt *et al*., 1997). Interestingly, Hgc1-Cdc28 was found to phosphorylate Efg1 at a single site, and this phosphorylation confers Efg1 specificity for occupying the promoters normally controlled by Ace2, thus blocking the expression of Ace2 controlled genes and preventing septum degradation.

The above studies demonstrate that Hgc1-Cdc28 has the capacity to directly phosphorylate and regulate in a coordinated manner multiple cellular processes responsible for different aspects of hyphal development (Fig. 2).

Role of Hgc1 in biofilm formation and cell adhesion to substrate

C. albicans can grow on both biotic and abiotic surfaces, forming three-dimensional multicellular communities known as biofilms (Bonhomme and d'Enfert, 2013). Microbial biofilms are a major contributory factor to therapeutic failure due to higher resistance of the biofilm mode of growth to drugs and the host immunity (Mathé and Van Dijck, 2013). Understanding mechanisms governing the biofilm formation is of high importance for designing new therapies which can effectively target and kill *C. albicans* cells embedded in the biofilm. There have been several recent publications reporting an important role for Hgc1 in biofilm formation. Transcription factors Cph1 and Tec1 are key regulators of biofilm formation in *C. albicans* (Lin *et al*., 2013). To find genes controlled by both transcription factors, Lin *et al*. (2013) identified 196 candidates; and interestingly, later validation identified *HGC1* being the only gene required for the formation of both conventional and pheromone-stimulated biofilms. In another study of the role of *UME6* in biofilm formation, Banerjee *et al*. (2013) reported that overexpression of *UME6* strongly enhances biofilm formation of *C. albicans* in a manner dependent on *HGC1*.

How does Hgc1 regulate biofilm formation? *C. albicans* biofilm is multicellular architectures with both hyphal and yeast cells as building blocks, and mutants defective in hyphal growth are known to be defective in biofilm formation. Thus, a simple and straightforward explanation of Hgc1's role in biofilm formation is its direct role in the control of hyphal development. However, there are data suggesting that Hgc1 may do more. Ability of adhesion of *C. albicans* yeast cells to host tissues is a first step of biofilm formation. Wilson and Hube (2010) used an *in vitro* circulatory *C. albicans*-endothelium interaction model to study the fungal and host factors which contribute to *C. albicans* adhesion to endothelium. They obtained evidence that Hgc1 regulates a specific temporal event in the yeast-to-hyphal transition critical for *C. albicans*-endothelium adhesion during circulation. In a different experimental setting, Nagy *et al*. (2014) observed that *hgc1Δ/Δ* cells exhibit greatly reduced ability to adhere to the surface of culture flasks. These experiments demonstrated significant defects of *hgc1Δ/Δ* mutants in adhesion to both biotic and abiotic surfaces, supporting the idea that Hgc1 has a role in the control of substrate adhesion. However, the underlying molecular mechanisms remain to be elucidated.

Regulation of *HGC1* expression

HGC1 expression is hyphae specific (Zheng *et al*., 2014). Under hyphal-induction conditions, *HGC1* mRNA cannot be detected in *cyr1Δ/Δ* and *efg1Δ/Δ* mutants, but is expressed normally in *cph1Δ/Δ* cells. Furthermore, *HGC1* is constitutively expressed in *tup1Δ/Δ* and *nrg1Δ/Δ* mutants. Together, the data indicate that hyphal-induction signals activate *HGC1* expression via the cAMP-PKA pathway and its downstream transcription factor *Efg1*. In yeast cells, *HGC1* is repressed by the Tup1-Nrg1 repressor complex. However, the mitogen-activated protein kinase (MAPK) pathway and its effector transcription factor Cph1 are not involved. Recent studies reveal that hyphal development in *C. albicans* requires two temporally linked regulations for initiation and maintenance of the hyphal transcriptional program. Hyphal initiation requires rapid but temporary disappearance of Nrg1, while hyphal maintenance requires exclusion of Nrg1 binding to promoters of HSGs or reduced *NRG1* expression (Lu *et al*., 2011). Both transcriptional downregulation and protein degradation contribute to the initial Nrg1 disappearance, and the exclusion of Nrg1 from promoters of HSGs involves complex modification and remodelling of chromatins, in which histone deacetylases play an important role (Lu *et al*., 2011, 2014). To date, the exact mechanisms that control *HGC1* expression at its promoter remain undetermined. The transcription factor Ume6 may play a direct role. Carlisle and Kodash (2010) demonstrated that although *HGC1* expression is activated immediately after hyphal induction, its

expression level drops quickly in the absence of Ume6, indicating that Ume6 is not involved in the initial activation of *HGC1* expression but is required to maintain its expression during hyphal development. Intriguingly, a study using epitope-tagged Tpk1 or Tpk2, catalytic subunits of PKA, in genome-wide chromatin immunoprecipitation on chip (ChIP chip) detected genomic binding sites mostly within the promoters of genes encoding regulators of morphogenesis including *HGC1* (Schaekel *et al*., 2013). However, what is phosphorylated by PKA at these sites and how it affects transcription remain to be elucidated.

HGC1 expression is not only hyphae specific but also apical cell specific. By using indirect immunofluorescence staining, Wang *et al*. (2007) detected Hgc1 in the apical cells but not subapical compartments of hyphae. Interestingly, transcription from *HGC1* native promoter is essential for this asymmetric localization, as it is not observed when *HGC1* expression is driven by the *MAL2* promoter. Consistently, fluorescence in situ hybridization could only detect *HGC1* mRNA in the apical cells, indicating differential expression of *HGC1* is, at least, one mechanism underlying the asymmetric Hgc1 localization in hyphae. This is an interesting mechanism that makes sense, because cell growth only occurs at the tip of apical cells in true hyphae, while the subapical cells are arrested.

Conclusions

The past decade since the discovery of *HGC1* has witnessed many progresses in elucidating the role of Hgc1 as a major regulator of the hyphal development in *C. albicans*. To adapt to a polymorphic life style that demands swift changes in growth form in response to environmental stimuli, Hgc1 has evolved from a multifunctional cell cycle regulator to a protein specialized in promoting hyphal morphogenesis. The transcription control circuit has also been rewired to place *HGC1* under the control of hyphal induction signals to ensure constitutive expression of *HGC1* under conditions favourable for hyphal growth. Importantly, the requirement of *HGC1* for virulence and its co-regulation with other virulence genes underscore its role in the pathogenicity of *C. albicans*. Switching to and maintaining hyphal growth need coordination of multiple cellular processes. In complex with the CDK Cdc28, Hgc1 possesses the ability to directly regulate via phosphorylation multiple cellular machines. To date, direct molecular links have been discovered of Hgc1-Cdc28 to several cellular machines responsible for different aspects of hyphal development, including polarity regulators, organizers of actin cables, septin filaments, secretory vesicles, the exocyst, and regulators of cell separation (Fig. 2). Elucidation of the physiological consequence of Hgc1-Cdc28 phosphorylation of target proteins has yielded insightful mechanistic understanding about how each of the above processes is regulated for hyphal construction. In future studies, application of new technologies such as stable isotope labelling by amino acids in cell culture (SILAC) for mass spectrometry-based phosphoproteomics will certainly help scientists to complete the repertoire of Hgc1-Cdc28 substrates. In spite of Hgc1's major role in hyphal morphogenesis, several studies have shown that molecular modifications of some cellular apparatuses occur prior to the activation of Hgc1-Cdc28. For example, Cdc11 phosphorylation by Ccn1-Cdc28 (Sinha *et al*., 2007), Gyp1 phosphoryation by PKA (Huang *et al*., 2014), and Sla1 dephosphorylation (Zeng *et al*., 2012) were all detected within minutes of hyphal induction. Blocking any of these events impairs hyphal development. Thus, to gain a full understanding of the hyphal development, it is necessary to elucidate how Hgc1-Cdc28 cooperates with other regulators. As the hyphal growth is an important virulence factor, Hgc1 regulated processes may be targeted for developing novel antifungal therapies.

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