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# Morphogenesis and cell cycle progression in *Candida albicans*

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*Candida albicans*, an opportunistic human pathogen, displays three modes of growth: yeast, pseudohyphae and true hyphae, all of which differ both in morphology and in aspects of cell cycle progression. In particular, in hyphal cells, polarized growth becomes uncoupled from other cell cycle events. Yeast or pseudohyphae that undergo a cell cycle delay also exhibit polarized growth, independent of cell cycle progression. The Spitzenkörper, an organelle composed of vesicles associated with hyphal tips, directs continuous hyphal elongation in filamentous fungal species and also in *C. albicans* hyphae. A polarisome mediates cell cycle dependent growth in yeast and pseudohyphae. Regulation of morphogenesis and cell cycle progression is dependent upon specific cyclins, all of which affect morphogenesis and some of which function specifically in yeast or hyphal cells. Future work will probably focus on the cell cycle checkpoints involved in connecting morphogenesis to cell cycle progression.

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## Introduction

*Candida albicans*, the most prevalent human fungal pathogen, can cause life-threatening systemic infections, in addition to superficial mucosal conditions such as thrush and vaginitis. A normal constituent of the gastrointestinal flora, it causes opportunistic infections, primarily in patients with compromised immunity.

It is thought that virulence is only possible in *C. albicans* strains that have the ability to grow with the full repertoire of vegetative morphologic forms: yeast, pseudohyphae and true hyphae (Figure 1) [1,2]. Although it is difficult to distinguish the contributions of cell shape from those of gene expression, the observations that elongated hyphae evade or escape phagocytic cells and that yeast cells disseminate in the bloodstream suggest that morphology

contributes to the survival of *C. albicans* in the broad range of host niches that it inhabits.

These different morphologies are often treated as different developmental states. In the laboratory, cultures grown at low temperature and pH contain mostly ellipsoid yeast cells. Long, narrow hyphae develop from yeast cells grown at 37°C and neutral pH, and in response to external stimuli such as serum. Elongated pseudohyphal cells develop at intermediate temperatures and pH. Pseudohyphae rarely form true hyphae [3] and hyphae rarely produce pseudohyphal buds (Figure 1). Furthermore, pseudohyphal cultures always contain some yeast and/or some hyphal cells (P Amornrattanapan, C Ketel, KR Finley, PE Sudbery, and J Berman, unpublished). Finally, *C. albicans* responds to many types of cell cycle arrest by producing a filamentous cell type with properties of both pseudohyphae and true hyphae.

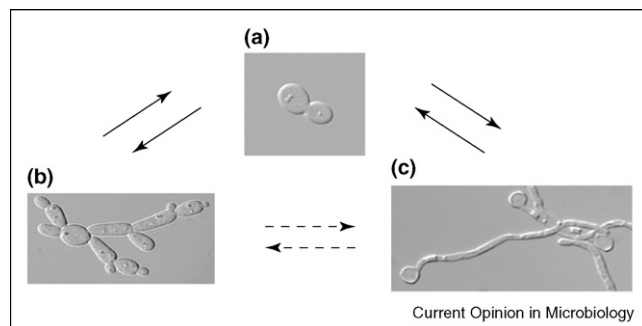
The focus here is on advances in our understanding of how cell cycle progression differs between yeast, pseudohyphae and true hyphae at the cellular and molecular level, highlighting the current view on how cyclins and other proteins regulate cell cycle progression and morphogenesis. Also, there is discussion of the changes to cell morphology that occur in response to cell cycle arrests or delays.

## Cell biology of yeast, pseudohyphae and true hyphae

Yeast and pseudohyphae of *C. albicans* are similar to those of *S. cerevisiae* in shape, size and in the order of cell cycle events. As is the case in *S. cerevisiae* [4], changes in actin-patch distribution reflect a switch from polarized growth at the tip to isotropic growth throughout the bud, and to polarized deposition of cell wall material required for septation. As in *S. cerevisiae*, this switch occurs early in the yeast cell cycle and later in that of pseudohyphae [5,6] (K Finley, PhD thesis, University of Minnesota, 2006).

Yeast cells grow by asymmetric budding, forming smooth, round colonies (Figure 2a). Septin rings appear before bud emergence [7], and nuclei divide across the mother-bud neck [8]. Selection of bud sites in *C. albicans* yeast cells is temperature-dependent. Cultures generally contain a mixture of cells with more cells exhibiting an axial pattern at lower temperatures [9,10]. At START, the transition from G<sub>1</sub> to S phase of the cell cycle, bud emergence is coordinated with the onset of DNA replication and spindle pole body duplication [11]. Yeast cells separate after cytokinesis, when daughter cells have not

Figure 1



Vegetative morphology of *C. albicans* cells. (a) Yeast cells can form both (b) pseudohyphae and (c) true hyphae. Switching between the pseudohyphal and hyphal morphologies is less frequent.

yet reached the size of their mother cells. Daughters enter the next cell cycle slightly later than their mothers, consistent with the idea that a cell size threshold affects the timing of START [6].

*C. albicans* pseudohyphal cells bud in a unipolar pattern (Figure 2b). The cells remain attached after cytokinesis, forming branched chains of elongated buds and colonies that are fibrous or rough. Filaments invade the agar below the colony and extend across the agar from the colony edge. As in yeast cells, septin rings form before bud emergence, and nuclei divide across the neck [8]. As with *S. cerevisiae* pseudohyphae [6], *C. albicans* pseudohyphal cells spend more time growing in a polarized manner and remain in  $G_2$  longer than do yeast cells. These daughters and mothers also reach START when they are a similar size and thus enter the next cell cycle with more synchrony than do yeast cells [6] (KR Finley, PhD Thesis, University of Minnesota, 2006).

Hyphae are narrower than pseudohyphal cells ( $\sim 2 \mu\text{m}$ ) and have parallel walls with no obvious constriction at the site of septation (Figure 2c) [12]. Checkpoints that coordinate bud growth in *S. cerevisiae* do not appear to operate in *C. albicans* hyphae: evagination and elongation of the germ tube is continuous, beginning before other START events, continuing during cytokinesis and not responding to changes in Cdc28 (cyclin-dependent kinase (CDK) protein) Tyr19 phosphorylation [11,13]. When hyphae are induced from yeast cells, a basal septin band, formed by a subset of septins not including Cdc3p and not requiring Gin4p, appears transiently at the mother–germ tube junction [3,10,14]. Septin ring formation, which occurs as the hyphal tip passes the presumptum (presumptive septum), the site where septation will later occur [15], is coordinated with other events of START [11,15]. Nuclei migrate into and divide within the germ tube, usually across the presumptum [15].

## Vacuole inheritance regulates hyphal branching frequency

Hyphae exhibit a linear growth rate because subapical cells remain quiescent in  $G_1$  for several cell cycles before branching [16]. This is as a result of the asymmetric inheritance of vacuoles, such that the apical cell primarily receives cytoplasm and the subapical cell receives the larger vacuoles [16]. The subapical compartments only become competent to branch when the ratio of vacuolar volume to cell volume decreases [16]. Consistent with the idea that a cytoplasmic volume threshold regulates the passage of START, perturbations of vacuolar inheritance alter branching frequencies [17<sup>•</sup>,18<sup>•</sup>]. It will be interesting to determine if factors such as Cln3p, which, in *S. cerevisiae*, regulate the size at which cells commit to START, will also regulate the frequency of hyphal branching.

## The Spitzenkörper: a hyphal-specific organelle

In filamentous fungi, the Spitzenkörper, or ‘tip body’, is a structure just behind the hyphal tip, that mediates growth directionality and hyphal tip morphogenesis by concentrating the delivery of secretory vesicles [19<sup>•</sup>,20<sup>•</sup>]. It is a dynamic structure only associated with actively growing hyphal tips. *C. albicans* hyphae have a Spitzenkörper as well as a cap-shaped polarisome. In yeast and pseudohyphae, a polarisome directs polarized growth in a cell cycle dependent manner (Figure 2) [5<sup>••</sup>]. Continuous polarized tip growth is associated with the presence of the Spitzenkörper, whereas cell cycle dependent polarized growth is associated with the presence of the polarisome. Thus, hyphal growth has properties distinct from those of pseudohyphae, and *C. albicans* hyphae resemble the hyphae of filamentous fungi.

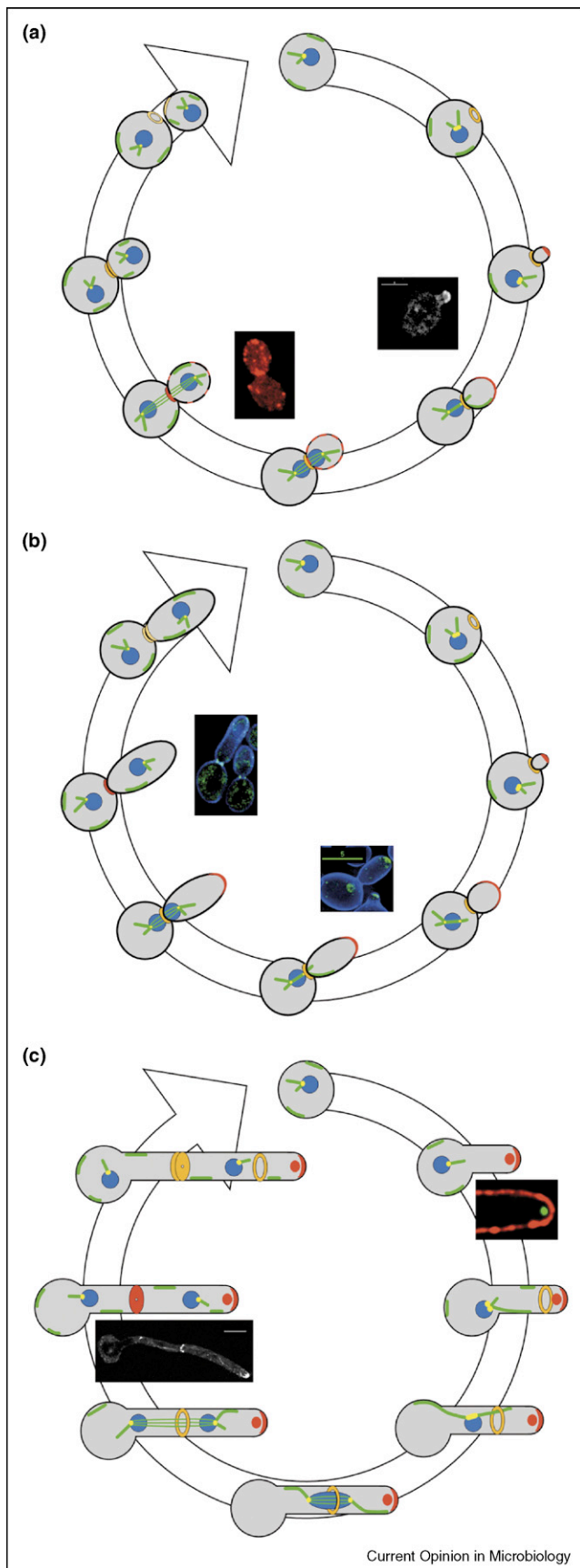
## Spindle dynamics and nuclear migration

Nuclear and spindle movement, including long distance migration of bipolar spindles in hyphae, occurs by repeated sliding of astral microtubules along the cell cortex [15] that is mediated primarily by cytoplasmic dynein [21] (KR Finley, PhD Thesis, University of Minnesota, 2006). By contrast, the mother nucleus returns to the mother cell primarily by spindle elongation forces. Furthermore, in hyphae, the timing of anaphase onset is coordinated with hyphal length and/or volume; hyphal length at anaphase onset remains constant in strains with decreased rates of hyphal elongation [15].

## Induction of and commitment to hyphal growth

In the laboratory, cells diluted into fresh medium from stationary cells that have reached very high cell density ( $\text{OD}_{600} > 13$  [22]) are most responsive to hyphal and pseudohyphal induction signals. This is due, in part, to release of the cells from exposure to farnesol, which is a quorum sensing inhibitor of hyphal growth [23<sup>•</sup>]. Other factors, such as levels of available nitrogen probably affect the efficiency of induction as well [16].

Figure 2



Whether hyphae can be induced from all cell cycle stages is still controversial. In classic experiments, Soll and co-workers [24] found that, when released from starvation at 37°C, small-budded cells formed hyphae, whereas large-budded cells completed a cell cycle before forming hyphae. The crucial transition point occurred when buds reached a size at which they normally switch from polarized growth to isotropic growth [24], suggesting that buds that have switched to isotropic growth can no longer form hyphae. By contrast, when Liu and co-workers [11] treated asynchronous yeast cultures with serum at 37°C, large-budded cells formed a tapered extension, which was interpreted as indicating that hyphal elongation can be induced at any time in the cell cycle. These cells all had constrictions at the neck and might not have exhibited the hallmarks of true hyphae [12]. Importantly, exposure to serum stimulates cell elongation that is independent of hyphal growth: *fh2* (encoding a Fork-head transcription factor) mutants, which are constitutively pseudohyphal, form more polarized buds in the presence of serum than in other hyphal induction conditions [25]. Thus, serum might induce polarized growth, but not true hyphal growth, in large-budded cells. This leaves open the attractive model that a cell cycle restriction point, corresponding to the switch to isotropic growth, limits hyphal formation to the earlier stages of the cell cycle.

### Cell cycle regulators: cyclins, cyclin-dependent kinases and CDC proteins

Although fundamental aspects of cyclin dependent kinase (CDK) activities and substrates are similar across yeast species, the global patterns of transcription for cell

Models for cell cycle progression in yeast, pseudohyphal and hyphal cells. **(a)** Yeast cells traverse START by forming a septin ring (orange), initiating bud emergence directed by a polarisome (red crescent) and duplicating the spindle pole body (yellow). Growth becomes less polarized as sites of growth (red) become distributed around the bud. In G<sub>2</sub> phase the nucleus (blue) moves to the neck assisted by astral microtubule (green) sliding along the cortex and, at anaphase, divides across the neck. At telophase, the spindle disassembles, growth is focused at the neck, the septin ring splits into two and then each ring disappears before appearance of the next ring in G<sub>1</sub>. Polarisome protein Mlc1p-YFP localizes to the tip during early bud growth (right inset). Delocalized actin (red) patches reflect isotropic growth (left inset). **(b)** Pseudohyphal cells have similar features to yeast cells with a few exceptions: the polarisome persists for longer and cells spend more time in G<sub>2</sub> phase, becoming similar in size to mother cells; cells do not separate following cytokinesis. As in yeast cells, sites of growth are cell cycle dependent, leaving the tip and focusing at the bud neck before cytokinesis. Mlc1p-GFP (green) appears at the tips of small and larger buds (right inset). At cytokinesis, Mlc1p-GFP disappears from bud tip and localizes to the neck (left inset). **(c)** Upon induction of hyphal growth from a yeast cell, the Spitzenkörper (red circle) directs germ tube evagination, which persists throughout the cell cycle and initiates before START. A polarisome is also present at hyphal tips. Nuclei migrate to and divide across the presumptum, and the septin ring persists into the next cell cycle. Photomicrograph of Spitzenkörper protein Mlc1p-YFP (green); cell surface is labeled with Texas-red conjugated to Concanavalin A (right inset). During cytokinesis Mlc1p-YFP remains at the growing tip and also appears at the septum (left inset).

cycle genes are very different between *S. cerevisiae* and *C. albicans* [26<sup>••</sup>]. Furthermore, several genes that are essential in *S. cerevisiae* are not required for viability in *C. albicans* (e.g. *CDC4* [27<sup>•</sup>] and *CDC14* [28<sup>•</sup>]). Genes that are essential in *C. albicans*, but not in *S. cerevisiae* (e.g. *CLB4* [29<sup>•</sup>] and *CLN3* [30<sup>•</sup>,31<sup>•</sup>]) can be explained by the genome duplication in *S. cerevisiae* that resulted in many pairs of genes with redundant functions [32].

The G<sub>1</sub> phase cyclins have a very different division of labor in *C. albicans* than in *S. cerevisiae*. Ccn1p (formerly termed Cln1p [33]) has similarity to the ScCln3p (*S. cerevisiae* Cln3p) cyclin box and was isolated because of its dominant-negative effect on *S. cerevisiae* pheromone responses [34]. It is expressed in G<sub>1</sub> and early S phase [11,25] and is required for the maintenance of polarized growth but not for its initiation [35].

Hgc1p (formerly named Cln21p) is most similar to ScCln1p and ScCln2p. It associates with the Cdc28 cyclin-dependent kinase and weakly complements START activity in *S. cerevisiae*. Importantly, it is expressed in hyphae and not in yeast cells and is co-regulated with other hyphal specific genes [36] (B Zirbes, M Steinbach, V Kumar and J Berman, unpublished). Hgc1p is necessary, but not sufficient, for hyphal growth. It promotes the maintenance of actin and Spa2p, a polarisome component, at hyphal tips [36]. In addition, Hgc1p is required to inhibit the localization of Cdc14p at the septum [28<sup>•</sup>]. In yeast and pseudohyphae (but not in hyphae) Cdc14p initiates a cascade of events leading to cell separation [28<sup>•</sup>]. Thus, Cdc14p might be a (direct or indirect) target of the Hgc1–Cdc28 CDK [28<sup>•</sup>].

Cln3p (formerly Cln2p), the only essential G<sub>1</sub> cyclin, is most similar to ScCln3p and complements *S. cerevisiae* lacking G<sub>1</sub> cyclins [33]. Loss of Cln3p also affects morphogenesis: depletion of Cln3p in yeast cells causes cells to first increase in diameter and then to form hyphae that continue to grow and divide [30<sup>•</sup>,31<sup>•</sup>]. Thus, Cln3p is essential for yeast growth and might be important for size control at G<sub>1</sub>. The timing of the transition to hyphal growth appears to depend upon the amount of Cln3p in the cell (because of the degree of *pMET3–CLN3* repression) and thus, the rate of cell growth before the transition [30<sup>•</sup>,31<sup>•</sup>]. This also implies that a size or volume threshold must be crossed in order to induce this transition to hyphal growth. Interestingly, the levels of Cln3p are reduced in the presence of farnesol, which inhibits hyphal growth, suggesting that Cln3p might modulate cell cycle progression in both yeast and hyphal cells.

Pcl2p is a cyclin homolog that is expressed preferentially in yeast cells [23<sup>•</sup>,26<sup>••</sup>] and that is required for morphogenesis in *S. cerevisiae* [37]. Accordingly, its levels are increased in the presence of farnesol [23<sup>•</sup>] and decreased in Cln3p-depleted cells that have started forming hyphal-

like extensions [30<sup>•</sup>]. Given the opposite patterns of Pcl2p and Hgc1p expression, it is tempting to speculate that they have complementary roles in yeast and hyphal cells. Alternatively, they might each execute very different processes in the two cell types, given that Hgc1p associates with Cdc28 CDK [36] and Pcl2p is predicted to associate with the Pho85 CDK.

*C. albicans* has only two B-cyclins (homologs of ScClb2p and ScClb4p), one of which (Clb2p, formerly termed Cyb1p) is essential [29<sup>•</sup>]. Both B-cyclins negatively regulate polarized growth, albeit to different degrees and with very different morphological phenotypes: cells lacking Clb4p (formerly termed Cyb99) grow slowly with a constitutively pseudohyphal morphology; Clb2p-depleted strains arrest in late anaphase with highly elongated cells and divided nuclei connected by long mitotic spindles; they elongate without completing a cell cycle and eventually die [29<sup>•</sup>]. A similar phenotype is seen with cells depleted of Cdc28p, the CDK1 homolog [38]. This implies that, like *S. pombe*, *C. albicans* has one major mitotic cyclin, Clb2p, that associates with Cdc28p to mediate cell cycle progression.

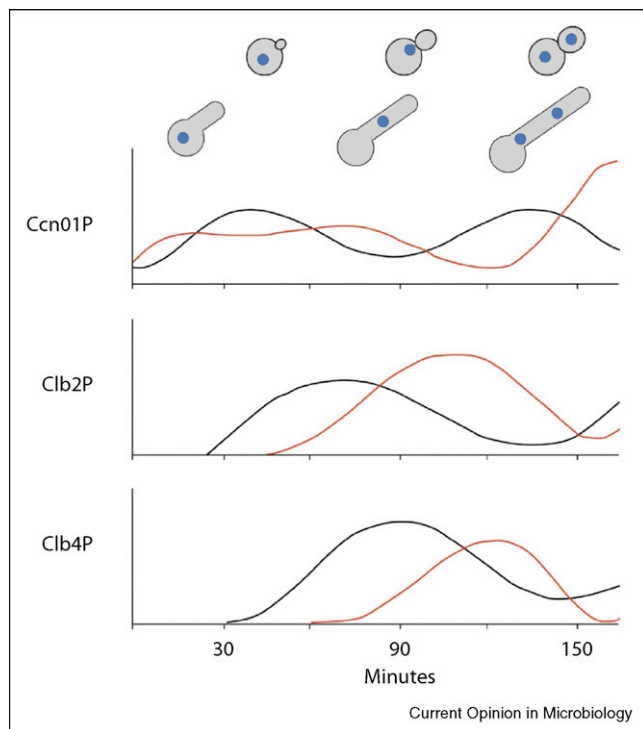
In yeast cells, Ccn1p levels are high in G<sub>1</sub> phase and decline in early G<sub>2</sub>/M phase just as Clb2p levels peak (Figure 3) [28<sup>•</sup>,29<sup>•</sup>]. Clb4p levels peak ~15 min later in mid G<sub>2</sub>/M phase and levels of both B-cyclins decline at M phase when nuclei divide. Interestingly, in hyphae, Ccn1p accumulates earlier and persists longer than Clb2p and Clb4p, which appear at later times that correspond with M phase, and then disappear during the exit from mitosis. Thus, the cell cycle is significantly delayed in hyphal cells, especially when one considers that hyphae form at higher temperatures than yeast cells. This delay in the cell cycle also indicates that a G<sub>1</sub> cyclin is present for a larger portion of the hyphal cell cycle than for the yeast cell cycle and suggests that the cyclins might have slightly modified roles in hyphae relative to yeast.

There is no obvious difference in the phosphorylation state of Cdc28 Tyr19 phosphorylation between yeast and hyphal cells [11]. This implies that phosphorylation of Cdc28 Tyr19 might not be important for polarized growth in *C. albicans*, and that Swe1p (the ortholog of ScSwe1p) and *S. pombe* Wee1p (a checkpoint kinase that phosphorylates Tyr19 on Cdc28/cdc2) is not required for hyphal growth. Indeed, although *swe1Δ/Δ* yeast cells are slightly rounder in shape than wild type cells, they form normal pseudohyphae and hyphae [3].

### Morphogenesis during cell cycle arrest or delay

Conditions that arrest cell cycle progression often result in a polarized growth phenotype (Table 1) [11,39–41]. For example, treatment of cells with hydroxyurea (also known as HU), which depletes ribonucleotides and thus impedes DNA replication elongation and S phase, or with

Figure 3



Cell cycle progression and cyclin levels differ in yeast and hyphae. G<sub>1</sub> phase yeast daughter cells were synchronized by elutriation and then released into yeast (30°C) or hyphal (37°C, 5% serum) growth conditions. Cell morphology and levels of G<sub>1</sub> cyclin Ccn1p, and B-cyclins Clb2p and Clb4p were followed using epitope-tagged proteins. Ccn1p levels persisted longer and B-cyclins appeared later in hyphae, relative to yeast. Adapted from [29\*].

nocodazole (known as NZ), which depolymerizes microtubules and locks cells in mitosis, give rise to cells that continue to elongate despite their inability to divide [40,42\*\*]. These cells have some features that are pseudo-hyphal-like (they are constricted at the neck and >2 μm

in width) and others that are hyphal-like (they elongate continuously, nuclei move into the elongating bud and eventually they express some hyphal specific genes) [42\*\*]; however, unlike either cell type, they do not divide and they eventually die. Thus, they represent a terminal phenotype different from either pseudohyphae or true hyphae.

Whereas the morphology of arrested cells is similar in cells treated with hydroxyurea or depleted for Cdc5p [39], the gene expression patterns of the arrested cells have significant differences that reflect the cell cycle stage at which they are arrested [42\*\*]. They exhibit common expression of a few genes encoding cell wall proteins and virulence factors (e.g. *CSA2*, *PHR1* and *DDR48*) that are also expressed in elongating hyphal cells. Because pseudohyphae express low levels of hyphal specific genes (P Amornrattanapan, C Ketel, KR Finley, PE Sudbery and J Berman, unpublished), this expression pattern is not diagnostic of a specific cell type.

In general, arrest of the cell cycle triggers cell cycle checkpoints: in nocodazole the polarized growth response requires the Mad2p (for mitotic arrest defective) spindle assembly checkpoint [40] and in Cdc5-depleted cells the polarized growth response requires Bub2p, the mitotic spindle checkpoint [42\*\*]. The Swe1p morphogenesis checkpoint partially affects the elongation of hydroxyurea-treated cells (KR Finley, K Bouchonville, A Quick and J Berman, submitted) and *rad52Δ/Δ* cells [43\*]. Whereas *S. cerevisiae* Rad53p and Mec1p are required for the elongation of hydroxyurea-arrested cells [44], the orthologous *C. albicans* genes have not been tested.

Interestingly, Ras1p is required for polarized growth in response to hydroxyurea, possibly by a mechanism that is independent from its role in hyphal signaling [42\*\*]. An intriguing question is whether Ras1p has a role in the S phase checkpoint. In summary, different cell cycle arrest conditions result in different gene expression patterns

Table 1

## List of cell cycle conditions and mutants that cause changes in morphogenesis.

Gene or condition	Cell cycle arrest <sup>a</sup> /delay stage	Altered morphology	Refs
Cln3-depletion	G <sub>1</sub> <sup>a</sup>	Large round, then hyphal	[30*,31*]
<i>cdc4Δ/Δ</i>	G <sub>1</sub>	Constitutive hyphal	[26**]
Hydroxyurea treatment	S <sup>a</sup>	Polarized growth	[42**,46]
<i>rad52Δ/Δ</i>	S/G <sub>2</sub>	Polarized growth	[43*]
<i>grr1Δ/Δ</i>	G <sub>2</sub> /M?	Constitutive pseudohyphal	[45]
<i>fkh2Δ/Δ</i>	G <sub>2</sub> /M	Constitutive pseudohyphal	[24]
<i>clb4Δ/Δ</i>	G <sub>2</sub> /M, spindle assembly	Constitutive pseudohyphal	[29*]
Cdc5p-depletion	M <sup>a</sup>	Polarized growth	[39]
Nocodazole treatment	M <sup>a</sup>	Polarized growth	[40]
Clb2p-depletion	Anaphase <sup>a</sup>	Highly polarized tubes	[29*]
<i>SOL1</i> overexpression	Late mitosis	Highly polarized growth	[26**]
<i>cdc14Δ/Δ</i>	Mitotic exit	Cell separation defects, hyphal growth defective	[27*]

<sup>a</sup> Essential genes that are terminally arrested. Genes or conditions are ordered by approximate cell cycle stage at which arrest or delay occurs. G<sub>1</sub> arrested cells tend to be more hyphal-like, S or G<sub>2</sub> and M arrests tend to be polarized pseudohyphal-like.

and trigger different checkpoints. Nonetheless, several arrest conditions result in similar morphologic outcomes. Perhaps the different checkpoints activate a common pathway (related to a pathway that operates in normal hyphal cells) that uncouples polarized growth from other cell cycle events.

Although several types of cell cycle arrest and/or checkpoint activation result in a similar polarized growth phenotype, this is not always the case. Most notably, depletion of Cln3p results in production of large round cells that later form hyphal-like tubes [30<sup>\*</sup>,31<sup>\*</sup>], suggesting that arrest in late G<sub>1</sub> has a different morphological outcome than does arrest in the S, G<sub>2</sub> or M phases of the cell cycle.

Polarized growth phenotypes are also observed in strains lacking genes that are not essential (*CDC4*, *CLB4*, *CLB14*, *FKH2*, *GRR1*, *RAD52* and *SOL1*; Table 1) [25,27<sup>\*</sup>–29<sup>\*</sup>,43<sup>\*</sup>,45]. The shape of cells lacking these genes might be related to the length of the cell cycle delay, and thus to a delay in the switch to isotropic growth. In cases where it has been tested, this polarized growth does not require the Efg1p and Cph1p transcription factors necessary for normal hyphal growth, suggesting that it affects processes downstream of the signaling pathways that modulate Efg1p and Cph1p [29<sup>\*</sup>].

Importantly, the Mad2p spindle assembly checkpoint is required for virulence and polarized growth in the systemic mouse model of candidemia [40]. This suggests that *C. albicans* cells undergo cell cycle arrest during growth in the animal host and that the response to this arrest is required for survival and successful colonization and/or invasion of host niches. Other cell cycle checkpoint genes have some effect on virulence: deletion of *SWE1* attenuated virulence in a mouse model of candidemia (CA Gale, personal communication). It will be important to determine if other cell cycle checkpoint proteins, such as Bub2p and potentially Rad53p, have an effect on virulence.

## Conclusion

Hyphae, pseudohyphae and yeast differ from each other in the rate and order of cell cycle events. A major difference is the uncoupling of elongation from other cell cycle events both in hyphal cells as well as under conditions that arrest or delay cell cycle progression. Polarized growth in yeast and pseudohyphae appears to resemble that in *S. cerevisiae*, whereas polarization in hyphae requires a Spitzenkörper and is more analogous to hyphal growth in filamentous fungi. Regulation of morphogenesis involves cyclins, some of which function specifically in yeast or hyphal cells. The functions of, and relationships between, the different cyclins also appear to have diverged substantially from those of *S. cerevisiae*. Future work is likely to reveal how *C. albicans* cell type specific cyclins participate in morphogenesis and how activation of different cell cycle checkpoints influence morphogenesis.

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- of special interest
- of outstanding interest

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