



# The distinct morphogenic states of *Candida albicans*

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**The human fungal pathogen, *Candida albicans* can grow in at least three different morphologies: yeast, pseudohyphae and hyphae. Further morphological forms exist during colony switching, for example, opaque phase cells are oblong, rather than the oval shape of yeast cells. Pseudohyphae and hyphae are both elongated and sometimes there has been little attempt to distinguish between them, as both are 'filamentous forms' of the fungus. We review here the differences between them that suggest that they are distinct morphological states. We argue that studies on 'filamentous forms' should always include a formal analysis to determine whether the cells are hyphae or pseudohyphae and we suggest some simple experimental criteria that can be applied to achieve this.**

Several *Candida* species are normally harmless commensals of the gastrointestinal and genitourinary tract. However, they can also be important pathogens that cause a range of conditions including painful superficial infections, such as vaginitis in otherwise healthy women, severe surface infections of the mouth and esophagus in human immunodeficiency virus (HIV) patients, and life-threatening blood stream infections among vulnerable intensive care patients (especially those undergoing cancer chemotherapy or immunosuppressive therapy following organ or bone marrow transplant procedures) [1]. In the USA, candidemia is the fourth most common cause of hospital-acquired infections [2,3], with annual Medicare costs estimated to exceed \$1 billion [4]. A simple calculation, based on an incidence rate of 8 out of 100 000 per annum [1], 40% mortality [2] and 300 million population size, suggests that in the USA alone there are ~10 000 deaths a year due to *Candida* infections. Clearly, such infections are a major public health concern. Although infections caused by other *Candida* species are increasing in prevalence, the majority of candidiasis is still caused by *Candida albicans*.

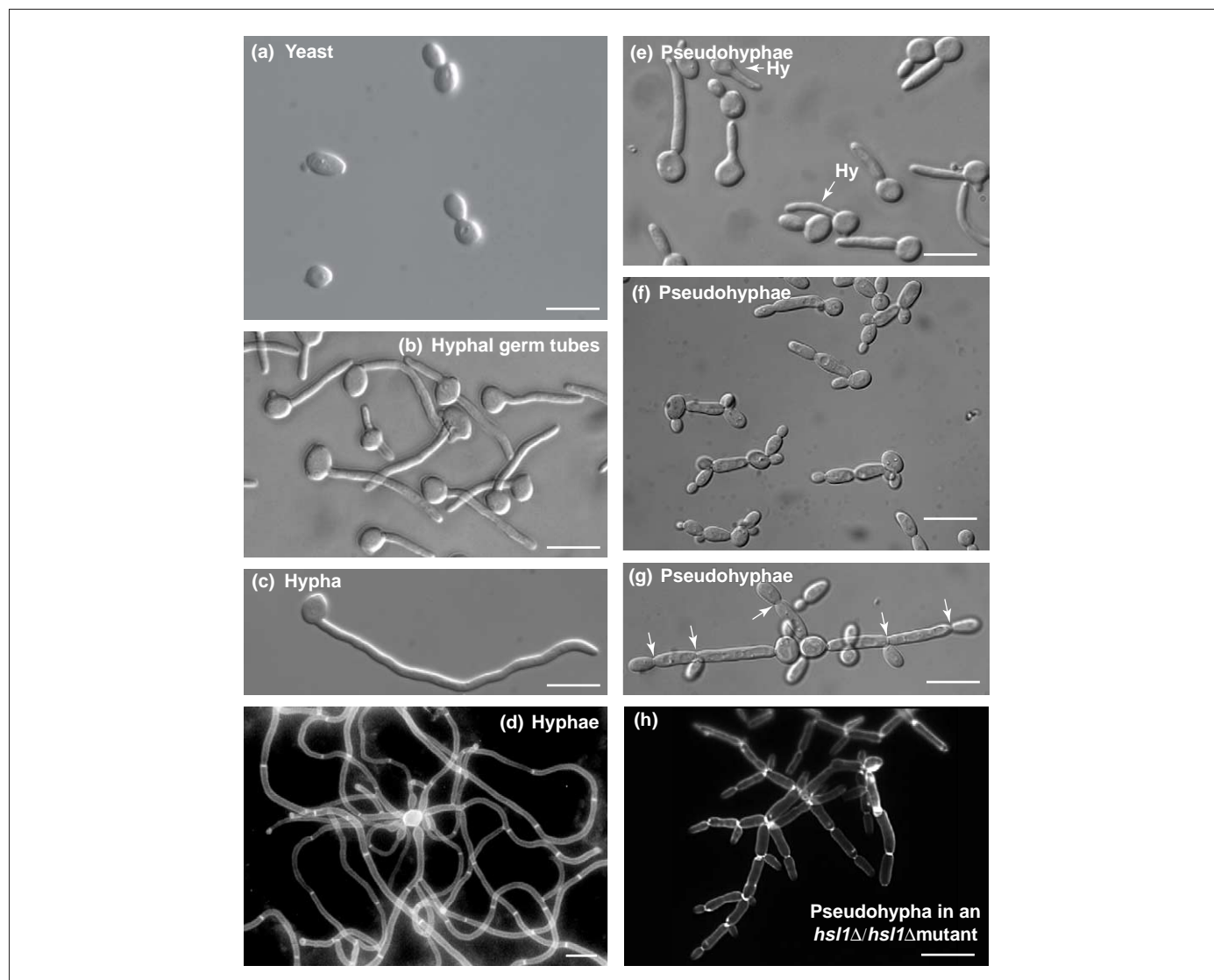
A striking feature of *C. albicans* biology is its ability to grow in a variety of morphological forms. These range from unicellular budding yeast (Figure 1a) to true hyphae with parallel-sided walls (Figure 1b–d) [5–11]. In between these two extremes, the fungus can exhibit a variety of

growth forms that are collectively referred to as pseudohyphae. In these forms, the daughter bud elongates (Figure 1e) and, after septum formation, the daughter cell remains attached to the mother cell. As a result, filaments composed of elongated cells with constrictions at the septa are formed (Figure 1f–h). The elongation of buds in pseudohyphae can be so extreme that these filaments can superficially resemble hyphae (Figure 1c,g). Because of this, it is often useful to be able to refer to pseudohyphae and hyphae collectively and we will use the term 'filamentous' for this purpose.

A change in cellular morphology also occurs during reversible colony switching [12,13]. This is an epigenetic phenomenon; yeast cells normally form smooth, white dome-shaped colonies. However, at low frequency, *C. albicans* strain 3153A can spontaneously and reversibly convert to a variant colony shape (i.e. star, ring, irregular wrinkle, hat, stipple and fuzzy) in which the colonies are composed of a mixture of yeast and filamentous cells. A simpler, biphasic white–opaque switching system, found in the *C. albicans* strain WO-1 and other strains that are homozygous for the mating-type-like locus [14] involves a switch between white domed colonies and opaque flat colonies [15]. White colonies contain oval cells with the normal yeast morphology. Opaque colonies contain oblong-shaped cells that are twice the size of white-phase cells and have pimples on the surface of the cell wall. The importance of the opaque form has increased through the recognition that this form alone is competent to undergo mating [14]. Morphological differences that occur during phenotypic switching represent an important, but under-explored topic. This review will focus on the morphological switch between yeast, pseudohyphae and hyphae, which has been much more intensively investigated.

The ability to switch between yeast, hyphal and pseudohyphal morphologies is often considered to be necessary for virulence, although formal proof remains lacking. Both hyphae and pseudohyphae are invasive (i.e. they invade the agar substratum when they grow in the laboratory). One view is that this property could promote tissue penetration during the early stages of infection, whereas the yeast form might be more suited for dissemination in the bloodstream. The filamentous forms might also be important for the colonization of organs,

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**Figure 1.** Yeast, hyphal and pseudohyphal morphologies. (a) Budding yeast cells appear similar to diploid *Saccharomyces cerevisiae* cells. Shortly after inoculation of unbudded yeast cells, (b) hyphal germ tubes are narrower and more uniform than (e) pseudohyphal buds, which have a constriction at the bud neck. However, it is difficult to obtain a population that consists solely of pseudohyphal cells; in the conditions used, ~25% of the cells are hyphal, examples of these are indicated by an arrow plus 'Hy'. (c) After 180 minutes, hyphae continue to display parallel-sided walls with no constrictions or branches. (d) Mature hyphal mycelia are shown. (e) Pseudohyphae exhibit morphologies ranging from short pseudohyphae that appear to be polarized yeast cells to (g) two long pseudohyphae that superficially resemble hyphae. (h) The mature pseudohyphal mycelium that results from a homozygous *hsl1Δ* mutation is shown. All forms of pseudohyphae superficially resemble hyphae, but have constrictions at the positions of septa (arrows) and show regular branching. Growth conditions were as follows: (a) YEPD pH6.0 at 30°C; (b,c) YEPD pH6 plus 20% serum, grown at 37°C; (d–f) YEPD pH 6.0 at 35°C. The images in (f) and (g) were taken from the same culture. (h) Shown is an *hsl1Δ/hsl1Δ* mutant growing in YEPD at 30°C. The images in (d) and (h) are of cells stained with Calcofluor white, which stains chitin in the cell walls and septa. All scale bars represent 10  $\mu\text{m}$ .

such as the kidney. This controversial topic has been recently reviewed [16]. Phenotypic switching clearly plays a role in virulence [13]. However, it is important to recognize that most dimorphic fungi that are human pathogens exhibit growth by budding into diseased tissues and exist as filamentous mycelial fungi in the external environment [16]. Therefore, filamentous growth is not obligatorily coupled with tissue invasion, and genetic programs associated with growth *in vivo* have no universal association with a specific growth morphology.

Pioneering studies of morphological switching reported detailed descriptions of the different morphologies and the conditions that induced morphological switches. This early work is reviewed extensively [6,7,9–11,16]. Morphological switching from yeast to filamentous forms can be induced by a variety of environmental conditions [6] (Table 1).

Hyphae are reliably induced from unbudded yeast cells by the addition of serum and a growth temperature of 37°C. A culture temperature above 35°C and neutral pH also induces hyphae and pseudohyphae, with small changes in these conditions determining the developmental outcome. Recently, it has been reported that high phosphate (up to 600 mM) concentrations induce uniform pseudohyphal morphology [17]. The signaling pathways that transduce environmental signals into morphological switching have been studied extensively. Interestingly, at least two of these pathways also act in *Saccharomyces cerevisiae* to initiate pseudohyphal growth. First, the signaling pathway based on cAMP and protein kinase A (PKA) targets the CaEfg1 transcription factor. Second, a mitogen-activated protein (MAP) kinase-based module, which in *S. cerevisiae* is also used to transduce the mating

**Table 1. The effect of environmental conditions on morphological forms**

Yeast	Pseudohyphae	Hyphae	Growth conditions that yield filamentous cells that have not been well characterized
Growth below 30°C or pH 4.0 Cell density > 10 <sup>7</sup> cells ml <sup>-1</sup>	pH 6.0, 35°C Nitrogen-limited growth on solid medium (SLAD) High phosphate	Serum > 34°C Lee's medium 37°C pH 7.0, 37°C N-acetylglucosamine	Engulfment by macrophages Spider medium Mouse kidneys Growth in agar matrix Iron deprivation

pheromone response, targets the CaCph1 transcription factor. In addition, a pH response is mediated by a pathway that activates the Rim101 transcription factor and a response to embedding in a solid matrix is mediated by a pathway that activates the Czf1 transcription factor. Lastly, two negative pathways act through the transcriptional regulator Tup1 in association with Nrg1 and Rpg1, respectively. These signaling pathways have been subject to excellent and detailed reviews [18–20] and will not be considered further here.

The early *C. albicans* literature generally ignored the pseudohyphal state or used the terms pseudohyphae and hyphae interchangeably (for examples, see Refs. [5,11]). Moreover, because there appeared to be a continuum of morphologies in *C. albicans*, and because more severe conditions (higher temperature and pH) tend to push the balance toward the hyphal state, pseudohyphae have been considered to represent an intermediate between yeast and true hyphal growth forms [7]. Nevertheless, it was clearly recognised that pseudohyphae were basically yeast cells modified by polarized growth and that do not fully separate after completion of each cell cycle [6]. Recently, evidence has accumulated to show that pseudohyphae exhibit similarities to yeast, and that the apparent superficial similarity to hyphae might be illusory. This poses a considerable problem in the investigation of the role of morphological switching in virulence, because casual observation might be insufficient to characterize cells as hyphae or pseudohyphae. In this paper, we review the differences between hyphae and pseudohyphae and suggest simple tests that could be used to characterize the state to which cells should be assigned.

### Cell shape

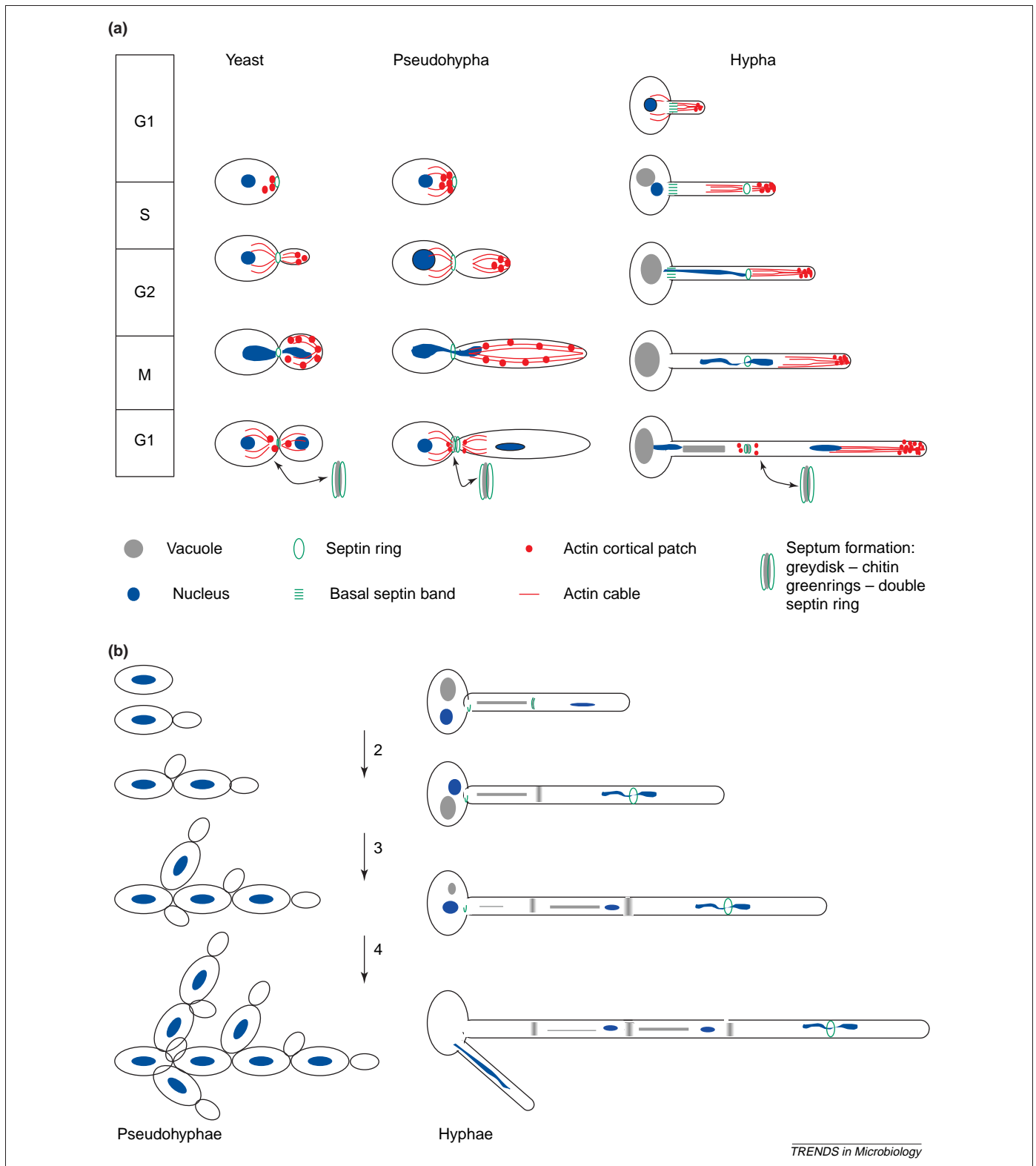
The traditional criterion for distinguishing between different forms is cell shape [5]. Hyphae that develop from an unbudded yeast cell (also termed a blastospore) have no constriction at the neck of the mother cell and have parallel sides along their entire length. The formation of unconfined filaments in response to serum is the basis of the 'germ tube test', which is used in clinical diagnoses to distinguish *C. albicans* from other *Candida* species [21]; although *Candida dubliniensis*, the nearest relative to *Candida albicans*, also forms unconfined hyphae in this test [22]. Pseudohyphal cells have a constriction at the neck of the mother cell and the bud and at every subsequent septal junction. By contrast, both the width and length of a pseudohyphal cell can vary enormously, so that at one extreme they resemble hyphae (Figure 1g) and at the other extreme they can resemble yeast cells with elongated buds (Figure 1f). However, a characteristic feature of pseudohyphae is that the width of the

compartments that make up the filaments is not constant, being wider at the centre than at the two ends [5]. The morphological index (MI) is a metric that distinguishes pseudohyphae from hyphae on the basis of the dimensions of the cellular compartments, which takes into account the cell length and the ratio of the maximum to the minimum cell width [5]. The result is a continuous variable. The difference between hyphae and pseudohyphae is defined by a numerical threshold chosen on the basis of whether cells are separable by sonication. Determination of the MI is quite laborious because each cell requires three individual measurements (length, maximum width and minimum width) and enough cells (usually 200–400) need to be characterized for statistical significance. A simpler alternative is the measurement of width, because even when pseudohyphae most resemble hyphae, the width of pseudohyphal cells is always greater than hyphal cells. Hyphal cells have a width of ~2.0 µm on most media except for N-acetyl glucosamine where hyphae are considerably narrower [23,24]. The minimum width of pseudohyphal cells is ~2.8 µm [5,24]. It is important to note that width determinations can vary according to the microscope calibration, the optics used (i.e. DIC, phase contrast or transmission) and the subjective definition of the cell edge by the investigator. Therefore, if width is being used as a discriminator, the investigator is advised to take control measurements using germ tubes induced by 20% serum at 37°C as a standard.

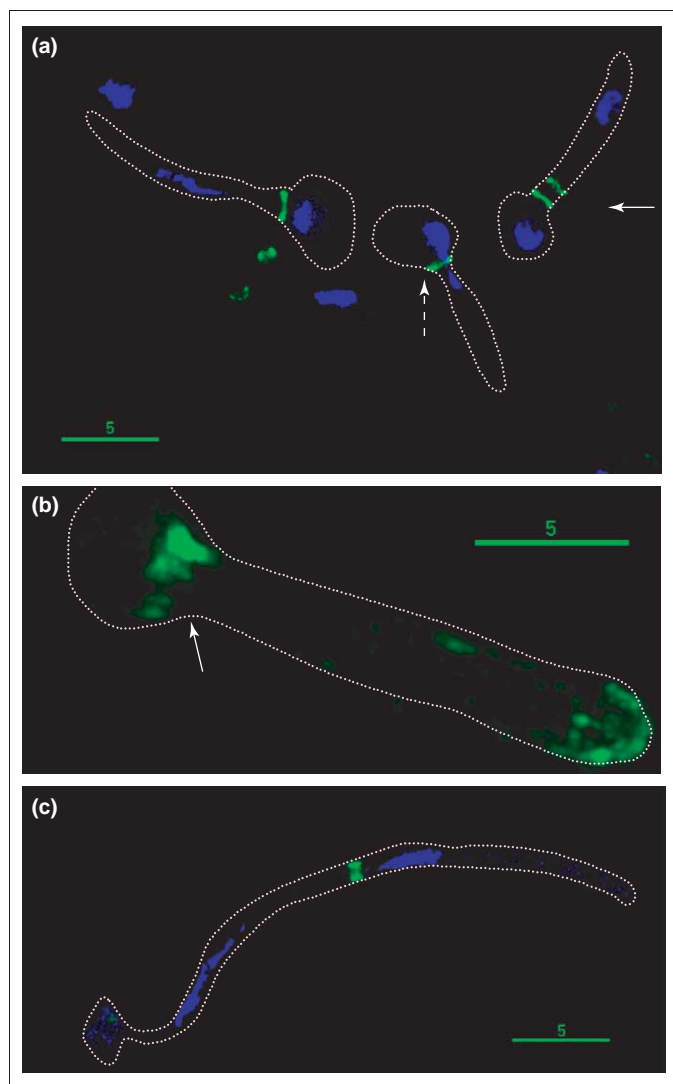
### Cell cycle

The most fundamental differences between hyphae and pseudohyphae relate to the organization of their cell cycles. In the following discussion, we shall consider an unbudded yeast cell that is induced to form hyphae or pseudohyphae by appropriate stimuli as discussed previously, or that is allowed to continue growing in the yeast form. In *C. albicans* the terms hypha and germ tube are often used as synonyms. The hyphal projection that forms in the first cell cycle, before septation, is called a germ tube [6] and should not be used to describe the elongated bud that forms in the development of pseudohyphae. The term hypha refers to all unbranched and branched filaments that have one or more septa and no constrictions at the mother cell neck or at septal junctions [6]. The events that occur in the first cell cycle are depicted in Figure 2a and a comparison between pseudohyphae and hyphae in subsequent cycles is shown in Figure 2b.

In the first pseudohyphal cell cycle, a septin ring appears at the neck between the mother cell and the daughter cell before bud emergence. Mitosis takes place across the plane of this septin ring (Figure 3a). When mitosis is complete, the septin ring separates into two



**Figure 2.** Representations of the cell cycles of yeast, hyphae and pseudohyphae. (a) Cell cycle of yeast and the first cell cycle of hyphae and pseudohyphae induced from unbudded yeast cells. Hyphal germ tubes emerge before the G1/S transition. The information used to depict the behavior of actin in yeast cells and hyphae has been obtained from Refs. [27,38]; the information relating to the behavior of the actin cytoskeleton in pseudohyphae has been obtained from (H. Crampin and P.E. Sudbery, unpublished). (b) Representation of cell cycles 2–4 in hyphae and pseudohyphae after re-inoculation of unbudded yeast cells. Note the synchrony of budding in the pseudohyphal mycelium. The structure shown in cycles 3 and 4 are unlikely to be observed in liquid culture because the shear forces are usually sufficient to fragment longer filaments.



**Figure 3.** Septin ring organization and the nuclear cycle of hyphae and pseudohyphae. The septins (green) are visualized by a Cdc10-GFP fusion and nuclei by DAPI (blue). **(a)** Pseudohyphae are shown undergoing mitosis 90 minutes after reinoculation of unbudded yeast cells. Septin rings have formed at the mother bud neck and mitosis is taking place across the plane of this septin ring in one of the cells (dotted arrow). Note the cell (solid arrow) where septin ring separation has occurred after the completion of mitosis. **(b)** A germ tube is shown at an early stage (50 minutes after re-inoculation) where a basal septin band (arrowed) consists of a series of longitudinal bars arranged around the circumference of the germ tube neck. The septin cap is also clearly visible. **(c)** A hypha that has completed mitosis across the plane of the septin ring is shown, and one nucleus is migrating back towards the mother cell. These images were generated by 3D-restoration microscopy using a Delta Vision Microscope. Pictures are kindly provided by R. Wightman and P.E. Sudbery. All scale bars represent 5  $\mu\text{m}$ .

rings (Figure 3a) and a primary septum composed of chitin is formed between them [24]. As in budding yeast, septum formation involves the appearance and contraction of an actomyosin ring (H. Crampin *et al.*, unpublished). Therefore, the organization of the first pseudohyphal cell cycle is not substantially different from that of a yeast cell, except for bud elongation and failure of cells to fully separate after septum formation.

By contrast, the first hyphal cell cycle shows substantial differences from yeast and pseudohyphal forms of growth, which are summarized in the following:

(i) In the yeast form, budding occurs either next to the bud scar from the previous cell cycle (axial pattern), or at

the opposite end of the cell from which the previous bud formed [25] (bipolar pattern) in a manner that is temperature-dependent [26]. When germ tubes evaginate from yeast cells, ~50% appear at random sites on the mother cell, whereas the remainder evaginate with a bipolar pattern relative to the previous bud site [26].

(ii) Germ tube initiation occurs before the start of the cell cycle marked by spindle pole body duplication and DNA synthesis. In yeast cells, budding depends on the start of the cell cycle [27].

(iii) The first septum forms at the mother-bud neck in yeast and pseudohyphae but within the germ tube in hyphae [23,24,28].

(iv) Germ tubes have a basal band of longitudinal septin bars around the germ tube neck (Figure 3b) [24,29]. This structure is distinct from the true septin rings that appear along the germ tube length that organizes the formation of the first septum (Figure 3b) [24]. The septin ring is equivalent to that forming at the bud neck in yeast and pseudohyphae at the time of bud formation. It is probably the same structure as filament rings observed by electron microscopy at the bud neck in yeast and within the germ tube in hyphae [30]. The formation of the basal septin band, but not the septin ring, is independent of the Nim1 kinase Gin4 [31] and does not contain the Cdc3 septin.

(v) The first nuclear division occurs across the mother bud neck in pseudohyphae (Figure 3a) and yeast, whereas in hyphae the nucleus migrates out of the mother cell and divides within the germ tube (Figure 3c) [24,28,29]. Although mitosis takes place across the plane of the septin rings, these rings are not necessary for nuclear migration or mitosis, because mitosis takes place normally in the absence of rings in a *gin4Δ/gin4Δ* mutant [31].

In hyphae, the location of the septin rings, the first septum and the first mitosis are all strongly influenced by growth medium. On YPD plus serum, which is a rich medium containing glucose, peptone and yeast extract, these landmarks are located 10–15  $\mu\text{m}$  from the germ tube neck [24,29], and the differences between hyphae and yeast or pseudohyphae are unmistakable. However, on defined media the septae can be as close as 2–5  $\mu\text{m}$  [23] to the mother cell and consequently the contrast with yeast is much less noticeable. The use of these defined media in earlier studies might have been responsible for a failure to appreciate the fundamental differences in the first cell cycle of hyphal germ tubes and the buds of hyphae and pseudohyphae. It is now clear that a germ tube cannot be regarded as a modified bud.

Differences between hyphae and pseudohyphae continue to be apparent after the completion of the first cell cycle (Figure 2b). In pseudohyphae, all cells in the filament continue to cycle with a unipolar pattern of budding, where the bud is initiated from the opposite pole from the birth scar [32,33] resulting in a highly branched structure (Figure 1f–h and Figure 2b). As in pseudohyphal growth in *S. cerevisiae*, the G2 phase is extended in *C. albicans* so that the daughter cells and mother cells are similar in size at the completion of the cell cycle (marked by the formation of the septum). As a result, both mother and daughter cells initiate the next cell cycle simultaneously and during the

first few cell cycles synchrony is maintained throughout the pseudohypha [34]. During the first cell cycle in germ tubes, a large vacuole appears in the mother cell [35–37]. During the process of septum formation, most of the cytoplasm is transferred to the apical compartment, which continues to grow and progress through the cell cycle (Figure 2a). After completion of the first cell cycle, the highly vacuolated mother cell remains in the G1 phase. This pattern is repeated so that subapical compartments are also vacuolated and remain in G1. These subapical compartments slowly accumulate cytoplasmic mass at the expense of vacuoles until they have reached a critical size threshold that allows them to re-enter the cell cycle by forming either a branch near an existing septum or a second germ tube from the mother cell (Figure 2b). Therefore, even in rich media, the growth rate of true hyphae is closer to linear [10,11,16,28]. Consequently, the branching patterns of hyphal cells is less regular than that of pseudohyphal cells (Figure 1d,h).

### Polarized growth

In both hyphae and yeast, polarized growth is associated with polarization of the actin cytoskeleton (Figure 2a) [27,38]. As in *S. cerevisiae*, the actin cytoskeleton of *C. albicans* is composed of cortical actin patches and cables. The actin patches are thought to organize endocytosis, whereas actin cables direct the transport of secretory vesicles driven by the Myo2 motor protein. In the yeast form, buds first grow in a polarized fashion until they reach approximately two-thirds of their final size when a switch to isotropic growth occurs, such that growth occurs evenly over the entire surface [39]. By contrast, hyphae grow continuously in a polarized fashion and the switch to isotropic growth does not occur [39]. These patterns of growth are reflected in the pattern of actin polarization. In the hyphal form, actin cortical patches cluster continuously at the growing tip and cables are orientated towards the tip. Whereas, in yeast the actin cortical patches are localized at the tip and cables are orientated to the tip in small- and medium-sized buds, but in large buds the patches become dispersed over the surface of the buds and cables are no longer orientated to the tip. In both yeast and hyphae, actin cortical patches form a ring at the site of septation. However, in hyphae, the majority of actin remains at the growing tip at this time. We are not aware of published observations on the behaviour of the actin cytoskeleton and growth pattern in pseudohyphae. It has been shown that, as in yeast cells, the actin cytoskeleton and the pattern of growth is discontinuous, switching between polarized and isotropic growth (H. Crampin and P. Sudbery, unpublished); however, the period of polarized growth is longer in pseudohyphae.

### Genetic determination of hyphae and pseudohyphae formation

Interestingly, there have been several recent reports of mutations that result in a constitutive pseudohyphal phenotype. These include *tup1Δ/tup1Δ* [40], *nrg1Δ/nrg1Δ* [41], *fkh2Δ/fkh2Δ* [42], *gin4Δ/gin4Δ* and *hsl1Δ/hsl1Δ* [31]. An example of one of these, *hsl1Δ/hsl1Δ* is shown in

Figure 1h. Note how the regular pattern of branching conforms to the theoretical pattern for a mature pseudohypha (Figure 2b). Interestingly, except for *nrg1Δ/nrg1Δ*, none of these mutants was able to respond to serum by producing hyphae (the case of the *hsl1Δ/hsl1Δ* is complicated as the pseudohyphae revert to yeast cell at high densities and these yeast cells can form germ tubes [31]). Therefore, these genes apparently negatively regulate entry into the pseudohyphal state and/or result in a developmental state that precludes entry into the hyphal state. Such specific genetic control is further evidence that pseudohyphae represent a distinct developmental state. One potential caveat should be noted here: in *S. cerevisiae*, activation of the morphogenesis checkpoint in response to defects in bud growth and morphogenesis [43,44] results in elongated cells that resemble pseudohyphae and have been described as ‘pseudo-pseudohyphae’ [45]. The morphogenesis checkpoint is dependent on Swe1, which is responsible for an inhibitory tyrosine phosphorylation of Cdc28 [46]. Therefore, mutations that interfere with bud growth and morphogenesis will mimic pseudohyphal cell shapes. A recent example of this in *S. cerevisiae* was the demonstration that the pseudohyphal phenotype induced by treatment with iso-amyl alcohol was due to the induction of the morphogenesis checkpoint [47]. Because Swe1 is not required for true pseudohyphal growth in *S. cerevisiae* [48], a useful test would be to see whether the pseudohyphal phenotype induced by these mutations in *C. albicans* is Swe1-independent. To date, this has only been done in *gin4Δ/gin4Δ* and *fkh2Δ/fkh2Δ* mutants. In both cases, elongated pseudohyphal cells continued to form when Gin4 was depleted in a *swe1Δ/swe1Δ* mutant [31] or in a *fkh2Δ/fkh2Δ swe1Δ/swe1Δ* strain (E. Bensen and J. Berman, unpublished).

### Experimental guidelines for distinguishing hyphae and pseudohyphae

The literature is replete with descriptions of filamentous growth, which do not distinguish between hyphae or pseudohyphae. Without more rigor in the discrimination of hyphae and pseudohyphae, there is a danger of increasing confusion. This is particularly true with the use of microarray studies that are used to study gene expression during morphological development. In Table 2 several features are listed that distinguish hyphae and pseudohyphae. It would be unrealistic to address all of these characteristics. However, we propose three simple tests for analyzing the filamentous forms of *C. albicans*.

- (i) Cell shape measurements.
  - Are there constrictions at septal junctions?
  - Are the sides of the elongated compartment parallel?
  - Is the width characteristic of hyphae (~2 μm) or pseudohyphae (≥3 μm)?
- (ii) Location of the first septin ring.
  - Is the first septum/septin ring located at the bud neck (pseudohyphae) or within the germ tube (hyphae)?
- (iii) Location of the first mitosis.
  - Does the first mitosis occur across the bud neck (pseudohyphae) or within the germ tube (hyphae)?

**Table 2. Characteristics that distinguish *Candida albicans* pseudohyphal and hyphal cells**

Criterion	Pseudohypha	Hypha	Method
Shape	Sides not parallel Minimum width $\geq 2.8 \mu\text{m}$ Constrictions at mother-bud neck and subsequent septal junctions	Sides parallel Width $\sim 2 \mu\text{m}$ No constrictions at septal junctions	Inspection Measurements in light microscope
Septa Septins	First septum at mother bud neck Septin ring at mother-bud neck	First septum within germ tube Septin ring within germ tube	Calcofluor White staining Cdc3-fluorescent Protein fusions Immunofluorescence with anti-Cdc11 DAPI staining
Nuclear division	Across mother-bud neck	Totally within germ tube	Anti-tubulin anti-tubulin antibodies or Tubulin-fluorescent protein fusions Microscopic appearance
Mature filaments	Highly branched with cell cycle synchrony	Less branched Apical cells continue growth and division, subapical cells are delayed in G1 Large vacuoles in subcellular compartments	Anti-tubulin staining to visualize spindle pole bodies (SPDs) Examination with DIC or staining with FM4-64 or FDC-RDA

### Concluding remarks

We have documented here fundamental differences in the organization of the first and subsequent cell cycles as pseudohyphae and hyphae develop from unbudded yeast cells. Despite these well-documented differences, analysis of morphology is hindered by several inter-related problems. The *C. albicans* morphology is enormously plastic and is very sensitive to different culture regimes, as has been carefully documented [5]. A related problem is the tendency for the morphological composition of *in vitro* cultures to change over time as cell density increases, nutrients become depleted and the medium becomes more acidic. *In vivo*, multiple morphologies might co-exist at a single site of infection. Nevertheless, we do not believe these problems are insurmountable. *In vitro* experiments, such as transcript profiling, should strive to develop conditions that produce homogeneous cultures, which can be fully assessed by the criteria outlined here. Such rigor is more difficult for *in vivo* investigations; nevertheless, the presence or absence of constrictions, cell width and the position of the septum or mitosis where a mother is present, can all be easily used to document the balance of morphologies.

Despite the apparent differences between hyphae and pseudohyphae, it is striking that similar environmental conditions induce both morphologies, with the balance being tipped towards hyphae as the conditions become more extreme (higher temperature and pH). Furthermore, both states apparently have similar genetic requirements, for example, both require the operation of the cAMP-Efg1 pathway. It is intuitively attractive to consider pseudohyphae to be a transitional state between yeast and hyphae. According to such a view, mutations that trap cells in a pseudohyphal state block the developmental pathway leading to the hyphal state. However, it is equally possible that pseudohyphae and hyphae are alternative states induced by conditions that promote infection. Pseudohyphae might have different biological properties from hyphae and serve different roles during infection. The resolution of this conundrum is an attractive target for future research.

### Acknowledgements

P.E. Sudbery and N.A.R. Gow thank the BBSRC and Wellcome Trust for financial support of work relating to this review.

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