

The different themes of Morphogenesis of yeasts; regulation & processing

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Abstract: From the outstanding forestry of fungi, thousands of members are pathogenic to humans; among which stands the genus *Candida* by its various species, especially *albicans*, as the most common pathogen. *Candida albicans* may exist, both in vivo & in vitro, in different morphologies including budding yeast, hyphae, & pseudohyphae, thus gaining more ability to adapt to many environmental conditions, in addition to its virulence. Important structural molecules undergo many changes in order to achieve the goal of interchanging morphology & orchestrating the series of events during the yeast, hyphae or pseudohyphae formation. These molecules such as Septins, Actins & Microtubules require further detailed studies to clarify the genetic backgrounds of them & their related ligands. Yeast cell cycle regulation & control either by external regulators, internal or genetic regulators play a pivotal role in the machinery of products along with the morphogenesis of such cell. Further prospective studies are essential to elucidate the signal transduction pathways controlling cell cycle & molecular regulatory mechanisms driving fungal cell growth & consequently its virulence starting from biofilm formation, passing through quorum sensing molecules & reaching the basic evidence of genetic facts beyond the cellular & molecular behaviors.

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Introduction:

A Fungal cell is an eukaryotic cell, with true nucleus, nucleoli, nuclear membrane & a complete cytoplasmic structure with various organelles such as mitochondria, microtubules & ribosomal RNA(80 S). Like other eukaryotes; the fungal cell is surrounded by a cytoplasmic membrane contains sterol & lipoproteins. The cell wall of fungal cells is formed of alternating layers of mannan protiens & glucan located separately or associated with chitin.

Yeast cells represent an important category of fungi that is greatly involved in human fungal infections specially genus *Candida* by its wide spectrum of species. They can grow with many of shapes and forms as they can grow as budding yeast cells where daughter cells physically separate from

the mother cells in a process known as : the budding process. Another form is the pseudohyphal form in which *Candida* cells show distinctive chain formation with apparent constriction between successive cells. The third form is the true hyphal form in which theyeast cells are elongated cells with no apparent constrictions between successive cells. There is no clear sharp demarcations between these different forms as multiple forms may co-exist. The single form prevailing in any fungal population affects its colonial mprphology. This prevalence may change markedly during a phenomenon known as (colony switching)[1,2].It is a fascinating process in which the yeast cell changes its fashion or style every now & then, sometimes it may show the different styles at a time.

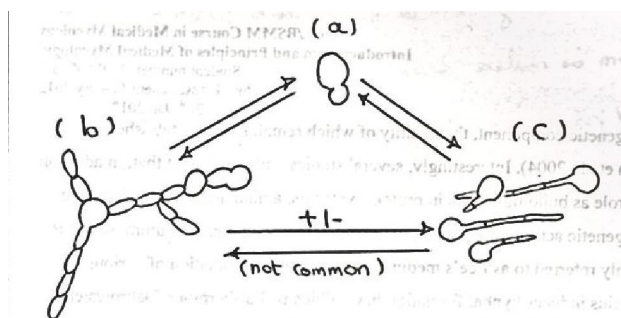


Fig (1): Three different patterns of *C. albicans* cells. (a) Yeast cells can (b) pseudohyphae and (c) true hyphae. The interchanging behavior between these patterns varies in frequency i.e switching between the pseudohyphal and hyphal morphologies is less frequent[3].

Why do yeast cells change their fashion?

In a living host cell, the switching phenomenon affects the property of causing infection. This was proved by genetic manipulations of yeast cells causing their modification to grow in yeast form only. These cells have been proven to be avirulent i.e not primarily causing disease[3,4]. On the other hand, in our labs the cellular forms & shapes are grossly affected by different growth conditions as the temperature, pH and the presence or absence of certain chemical constituents. For example neutral pH and 37°C favor the growth of pseudohyphae, while true hyphae formation requires the presence of serum. The presence of certain substances such as N-acetyl glucosamine in a growing medium favours the hyphal and pseudohyphal formation rather than forming yeast cells [5].

Budding Yeast cells:

In a growing population of yeast cells; there are 2 generation times: short duration of mother cells and long duration of daughter cells, as they need extra time to grow before they can successfully divide. Consequently when measuring a generation time of this population it is finally calculated by the average of both generation times. Such budding process is carefully regulated at several levels; at one level the bud grows initially at distinct points.

**In growing yeast form;* apical growth takes place first followed by isotropic expansion which ends by shut down of apical growth of the daughter cells.

**In mycelial growth;* there is an absolute apical expansion with no multidirectional patterns of expansion.

It can be concluded that the budding patterns depend on cell ploidy:

- Haploid cells: axial budding ; i.e daughter cells are formed from adjacent sites to the original sites to the signal points of budding (previous budding cycle)
- Diploid cells: have two alternative ways:
 - a. A new bud is formed from the opposite side of which it was born. This end over end growth forms a different shaped colony which is the progenitor of filamentous growth of yeast.
 - b. A new bud may also generate from adjacent points to the original area of budding cycle.

Consequently, this bipolar budding is an adaptive form of cells that represents already mated cells capable of continuous growing & gaining nutritive substances.

Pseudohyphal form: the term indicates the untrue hyphal form, it is a distinct form in which yeast cells are variably elongated but constrictions between

adjacent cells are maintained giving a shape similar to a sausage chain. This form includes synchronously dividing cells. It differs in cytoskeleton & genetic regulation [6].

True Hyphal form:

True hyphae are typically formed of elongated cells with no septa and no apparent constrictions between adjacent cells [7]. This modular pattern of organization contributes to the differentiation of hyphae; apical cells are generally engaged in nutrient acquisition and sensing of the local environment, whereas sub-apical cells generate new hyphae by lateral branching. The resulting network of hyphae is known as a mycelium. Hyphal branching appears to serve two general purposes. First, it increases the surface area of the colony, which enhances nutrient assimilation. Second, branches mediate hyphal fusion events that appear to be important for exchange of nutrients and signals between different hyphae in the same colony[8,9]

Many studies revealed that serum is one of the most potent factors causing hyphal formation in *C. albicans*. Such action is mediated via a serum inducing factor which is described as heat stable, non-dialyzable factor. Many serum constituents have been pointed to as the serum inducing factor for germ tube and true hyphal formation, these constituents are; albumin, glucose, amino acids or furthermore the proline amino acid separately.

Studies supporting the hypothesis of serum albumin as the induction factor relied upon the coincident separation of the induction factor and serum albumin in the gel electrophoresis and purification assays for separation of several proteins in a mixture [10,11].

On the other hand, some authors found that a synthetic medium, which is commonly referred to as Lee's medium, containing a combination of various amino acids induces hyphal formation in *C. albicans*. Furthermore, another study showed that proline can induce hyphal differentiation in *C. albicans*[12,13].

On the contrary, the mechanisms by which *C. parapsilosis* yeast cells differentiate into pseudohyphae are not fully studied. In a relevant study, the morphological alterations of a *C. parapsilosis* isolate (CpSH) were examined and grown on various media known to be lacking or having an effect on hyphal differentiation in other filamentous fungi. The CpSH strain formed smooth colonies with no filamentous extensions from the edge of the colonies on minimal (SD) or rich (YPD) medium lacking or supplemented with 10% serum, while the same isolate formed smooth colonies with filamentous extensions on the amino-acid-rich Lee's medium supporting the idea of amino acids as an

effective inducer factor, in the serum, for pseudohyphal form as this organism was proven to form yeast and pseudohyphae only [14].

Factors regulating fungal cell growth:

Several factors affect the fungal cell growth, the following items were described and worked on either individually or simultaneously by some authors, other items were less likely discussed or analysed in the literature. These major factors include: septins, actin, microtubules & the hyphal specific organelle or the Spitzenkörper.

Septin (Neck filaments): wide array of researches have recognized a pivotal role in the process of “bud morphogenesis & cytokinesis” played by an identified family of proteins called Septins. These studies were held on *Saccharomyces cerevisiae* which is highly similar to *C.albicans* but has the advantage of feasibility and easy application of studying.

Septins were observed by transmission electron microscopy and identified as a structural component of a membrane-associated ring of 10 µm filaments. The exact site and modeling of the septin ring varies evidently in hyphae and pseudohyphae. In pseudohyphal form; duplicated rings of septin appear between the mother cell and the daughter cell simultaneously with the appearance of chitin rings. The geometric plane of both rings, i.e septin and chitin, is actually the location at which the process of mitosis takes place [15].

In true hyphal form, the septin ring appears and disappears alternatively prior to mitosis. During evagination of a bud, the septin ring is located at the bud neck, later on it becomes faint or disappear completely. Thereafter, a second septin ring is formed flanking the whole length of the hyphal cell. This new ring is usually brighter than the first septin ring. The process of first mitosis occurs at that plane of second septin ring [16].

On the genetic level, there are four essential genes encoding septins called CDC, they were studied in *C.albicans* and given numbers as CDC3, 10, 11 and 12, besides one non-essential gene called SHS1. Loss of function of any of the essential genes ends in failure of cell division and yields a hyperpolarized state of budding, consequently the resultant buds are very similar morphologically to hyphae and pseudohyphae [17].

Further studies have clarified the intervening roles of other cellular proteins and protein kinases on the septin ring formation. These proteins were demonstrated in septin mutants having a polarized growth pattern. They were titled “morphogenesis check points” for example the Swel protein kinase in *S.cerviciae* and Int1 protein, a homologous protein to the mammalian proteins called Integrins [18].

Studies proved that Int1 deficient mutants (Int1D) are incapable of hyphal growth initiation especially when grown on certain media, whereas over expression of Int1 gene favors the hyphal morphology of a bud and marked disturbance in the discipline of septin ring and its exact site.

Moreover, septin paves the path for other intracellular proteins to operate and perform through the cell as it is mandatory for the action of chitin protein synthetase which is responsible for the localization of chitin ring in the incipient bud zone. Such chitin synthetase is correctly placed by the assembly of septin-chitin complex [19].

Actin: takes different forms inside a fungal cell; either filamentous cables or patches. It plays several roles as it coincides with sites of surface expansion, it allows membrane surface growth against force of cell turgor. On the other hand actin allows invagination leading to endocytosis-clathrin adaptor complex co-localize with patches.

Microtubules: correlate with activities of the nucleus. They are responsible of mitotic spindle formation, nuclear movement & orientation besides their role in various organelles movement.

Fungal cell cycle regulation: as an eukaryocytic cell, fungal cell undergoes the four major stages of cell cycle known as G1, S, G2 and M phases. In the yeast cell the transition phase between G1 and S phases is called: START, at this transition the bud emergence takes place at matching time of DNA replication and the duplication of spindle body. The new yeast cells separate before reaching the size of a mother cell. This new daughter cell enters the consequent cell cycle at a later time than their mothers do, which confirms the idea of “a cell size threshold affects the timing of START” [20]. Pseudohyphal cells exhibit a different pattern regarding the mother and daughter cell synchronization among a single cell cycle. *C.albicans* pseudohyphae take extended time to grow in a polarized manner, thus settle in the G2 phase for a longer period than yeast cells do. Consequently the mother and daughter cells arrive at the transition START when they are approximately equal in size which conveys the synchronicity in entering the next cell cycle [16, 20].

In conditions where hyphae are formed from yeast cells, a basal band formed of septin appear at the mother cell junction with the germ tube, it is a preliminary step before septin rings appear later on, in coordination with other events of START. The nuclei migrate into the germ tube and actually divide within it, this step usually occurs across the plane of a structure called: preseptum i.e the presumptive septum (Fig 2)

A vacuole was demonstrated in some studies during the budding process, the inheritance of such vacuole represents the rate limiting step in hyphal branching. The hyphal linear growth was referred to the quiescent state of subapical cells in the G1 phase for repeated successive cell cycles before branching actually is taking place [20].

The Spitzenkörper: a hyphal-specific organelle:

This structure was demonstrated in *C. albicans* hyphae “as a cap-shaped polarisome” or called tip body. In yeast and pseudohyphal forms,

this organelle regulates the polarized growth simultaneously with other cell cycle events [14].

The motility of spindle threads moves by a mechanism defined as successive sliding of microtubules along the cellular cortex, this also conveys the long distance migration of these threads in hyphal cells. The previous facts elucidated that hyphae of *C. albicans* are muchly resembling the hyphae of filamentous fungi, on the other hand the pseudohyphae of *C. albicans* was proved to be distinctive from its true hyphae[20].

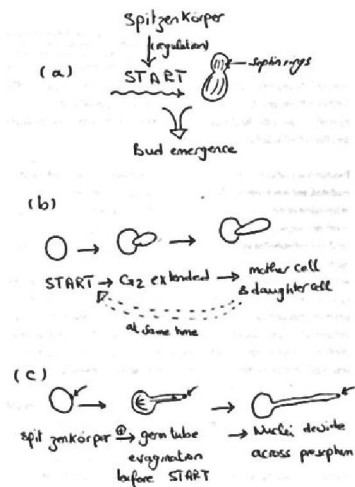


Fig (2): Themes of cell cycle : in yeast, pseudohyphal and hyphal cells. (a) Yeast cells traverse START by forming a septin ring, thus initiating bud emergence directed by the tip body and duplicating the spindle pole body (b) Pseudohyphal cells show a degree of similar features to yeast cells with a few exceptions: the tip body persists longer and cells spend more time in G2 phase, similar in size to mother cells; cells do not separate following cytokinesis. (c) In true hyphal growth from a yeast cell, the Spitzenkörper regulates germ tube evagination, which persists throughout the cell cycle and initiates before START. The polarisome is present at hyphal tips. Nuclei divide across the presumptum, and the septin ring persists into the next cell cycle [21].

Conclusion:

- From the outstanding forestry of fungi, thousands of members are pathogenic to humans; among which stands the genus *Candida* by its various species, especially *albicans*, as the most common pathogen.
- *Candida albicans* may exist, both in vivo & in vitro, in different morphologies including budding yeast, hyphae, & pseudohyphae, thus gaining more ability to adapt to many environmental conditions, in addition to its virulence.
- Important structural molecules undergo many changes in order to achieve the goal of interchanging morphology & orchestrating the series of events during the yeast, hyphae or pseudohyphae formation. These molecules such as Septins, Actins & Microtubules require further detailed studies to clarify the genetic backgrounds of them & their related ligands.
- Yeast cell cycle regulation & control either by external regulators, internal or genetic regulators play a pivotal role in the

machinery of products along with the morphogenesis of such cell

- Further prospective studies are essential to elucidate the signal transduction pathways controlling cell cycle & molecular regulatory mechanisms driving fungal cell growth & consequently its virulence starting from biofilm formation, passing through quorum sensing molecules & reaching the basic evidence of genetic facts beyond the cellular & molecular behaviors.

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