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Expression of Growth Form Factors during Morphogenesis in *Candida albicans*

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ABSTRACT

The transition of *Candida albicans* **from unicellular yeast form to filamentous form i.e, pseudohyphae and hyphae is referred to as morphogenesis.** *C.albicans* **has the ability to respond to environmental conditions and accordingly changing its cell morphology. Three main morphological forms of** *C.albicans* **are unicellular yeast, pseudohyphae and hyphae. Environmental factors are important in selectively favouring yeast or hyphal form, most important being the growth medium, incubation temperature and external pH value. Cell morphology in** *C.albicans* **is associated with changes in the expression of specific factors that are expressed exclusively in particular growth form. These growth form factors are an important target in the investigation of morphogenesis in** *C. albicans* **and in the discovery of antifungal drugs that targets the specific growth form of** *C. albicans.*

Keywords: *Candida albicans,* Environmental factors, Growth specific form, Morphogenesis,

INTRODUCTION

Morphogenesis is an essential trait in the pathogenic fungus *C. albicans* and it is clearly required for virulence (Lo *et al*., 1997; Braun *et al*., 2000; Braun *et al*., 2001, Murad *et al*., 2001; Saville *et al*., 2003). Morphogenesis in *C. albicans* is defined as transition from unicellular yeast form to filamentous form (pseudohyphae or hyphae) (Khan *et al*., 2010). It can grow in a variety of morphological forms such as yeast, pseudohyphae and hyphae (Sudbery *et al*., 2004; Merson-Davies *et al*., 1989). *C. albicans* also can form chlamydospores that is to say; thick walled spherical cells or asexual spores which develop over pseudohyphal support cells and appear under unfavourable environmental conditions (Sudbery *et al*., 2004). In pseudohyphae daughter bud elongates and, after septum formation, the daughter cell remains attached to the mother cell. The elongation of buds in pseudohyphae can be so extreme that these filaments can superficially resemble hyphae. 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. (Sudbery *et al*., 2004). However, hyphae are narrower than pseudohyphal cells ("2 mm) and have parallel walls with no obvious constriction at the site of septation (Sudbery *et al*., 2004). Germ tube are the initial projections observed when *C. albicans* switches from yeast form to hyphal growth (Yang, 2003). Growth is polarized in *C. albicans* hypha, with continuous apical growth throughout the cell cycle and parallel cell walls at the septal junctions. In contrast, the growth of pseudohypha and blastophores is only limited to the apical tip during the initial part of the cell cycle (Court *et al*., 2007). Pseudohypha can also be distinguished from true hypha on the basis of their morphological index which quantifies the dimensions of cell compartments. Alternatively they can be distinguished on the basis of the positions of their septal junctions. These lie at the bud neck for pseudohypha, and within the germ tube for emerging hypha (Sudbery *et al*., 2004). Hyphae, pseudohyphae and yeast differ from each other in the rate and order of cell cycle events (Berman, 2006).

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Morphogenesis is interrelated to the pathogenesis of *C. albicans*. Adherence has been shown to play critical role in the pathogenesis of infections. Germ tubes, that are short hyphal elements, are important in the adherence of organism to the host epithelium. (Kimura *et al*., 1978, Lee *et al*., 1938, Sobel *el al*., 1984). Hypha of *C. albicans* are also important for tissue destruction and host invasion (Berman and Sudbery, 2002). The yeast form of *C. albicans* also have virulence attributes and is thought to promote dissemination within the blood stream and establishment of infection at distant sites (Braun *et al*., 2000; Gow, 2002; Sudbery *et al*., 2004; Sundstrom, 2006). Several researchers have investigated that hyphal form is more virulent than yeast form. The principal determinant in the development of disease is the ability of *C. albicans* to switch between yeast and hyphal forms rather than the individual morphologies (Saville *et al*., 2003).

Morphological changes between the yeast and the various filamentous forms occur in response to alterations in the growth conditions. Parameters that promote hyphal development in vitro include a growth temperature above 35 °C, a pH greater than 6.5, nitrogen and/or carbon starvation, nonfermentable carbon sources, low oxygen concentrations, and a wide range of chemicals including N-acetylglucosamine, proline (and other amino acids) and alcohols. Serum is one of the most potent inducer of hyphal development (Brown, 2002). The effect of serum is complex but it is proposed to act, in part, by conferring amino acid starvation. Two known inducers of hyphal formation, N-acetylglucosamine (GlcNAc) and proline, may contribute to the serum effect since they are generated by degradation of serum (glyco-) proteins. It is well established that a pH around neutrality favours hyphal development of *C. albicans* in vitro, while a low pH (pH <6.5) blocks hyphal formation and stimulates growth of the yeast form. Growth of cells in the yeast form is promoted by an inoculums above 10^6 cells/ml, a growth temperature below 35°C, a pH of less than 6.5, glucose and ammonium salts (Odds, 1988). Compared to liquid medium, induction on solid media appears to represent a weaker hypha-inducing condition, because minor defects in filamentation show a defective phenotype on solid but not in liquid media. So the mycelial tendency is stronger in liquid than on solid media (McClary, 1952) and stationary phase cells are most responsive to hyphal and pseudohyphal induction signals (Berman, 2006).

It is generally assumed that changes in cell morphology in *C. albicans* are associated with changes in the expression of specific factors that are expressed exclusively in particular growth form. A total of 5 true hyphae specific genes have been identified. All appear to encode structural proteins, as opposed to regulatory proteins, and four of the five appear to encode cell surface proteins. (Brown, 2002).

ECE1 (extent of cell elongation 1) was the first hyphae specific gene to be identified. ECE1 is probably an intracellular protein; having a predicted molecular size of 28,886 Da. This protein consists of eight degenerate repeats, 34 amino acids in length. ECEI was highly expressed when hyphae were formed, regardless of the induction signal, and ECEI expression occurred soon after the stimulus to form hyphae was given. (Birse *et al*., 1993)

In previous work (Marot-Leblond *et al*., 1995), a *C. albicans* germtube-specific antigen 3D9 antigen was recovered by the use of a MAb. The relative molecular mass ranged from 120 to 220 kDa. Later (Beucher *et al*., 2009) it was concluded that antigen 3D9 is ALS3 with same molecular mass.

ALS3 is a hypha specific protein (Coleman *et al*., 2009) member of one of the major families of *C. albicans* adhesins ALS (agglutinin-like sequence) that encode cell-surface glycoproteins.(Hoyer *et al*., 2008). ALS3 have molecular size of 119,927 Da, it consists of 10 degenerate repeats and 36 amino acids in length (Hoyer *et al*., 1998). ALS3 has been shown to be required for mature-biofilm formation, binding

extra cellular matrix, adhesion to host cells, and internalization of *C. albicans* by endothelial cells (Hoyer *et al*., 2008; Sheppard *et al*., 2004; Zhao *et al*., 2006). ALS3 is activated under serum, N-acetylglucosamine and proline-inducing conditions (Hoyer *et al*., 1998).

ALS8 has been mentioned several times in the literature as an ALS3-like gene (Leng *et al*., 2001; Murad *et al*., 2001) ALS8 gene was also shown to encode a hypha-specific cell surface agglutinin. More detailed analysis confirmed that ALS8 is expressed under serum, N-acetylglucosamine and pH-inducing conditions and hence that ALS8 is a hypha-specific factor (Leng & Brown, unpublished data). ALS8 have a molecular mass of 111,945 Da - UniProtKB (http://www.uniprot.org/uniparc/UPI000006A36A).

HWP1 (hyphal wall protein 1) is expressed during hyphal development (Kim *et al*., 2007; Staab *et al*., 1996). The gene was tentatively called ECE2 (Sharkey *et al*.,1999). It functions as hypha-specific cell surface adhesion (Sharkey *et al*., 1999; Staab *et al*., 1999) Molecular mass of HWP1 is 65,342 Da. Peptide mass obtained from Candida DB (http://genolist.pasteur.fr/CandidaDB/). HWP1 (hyphal wall protein) encodes an outer surface mannoprotein on the hyphal wall; the amino terminal sequences of this adhesin are recognized as mammalian transglutaminase substrate (TGase) and form covalent binding with HBEC (Chaffin *et al*.,1998; Staab *et al*.,1999) Its mRNA being expressed during pH and cell culture medium-inducing conditions (Staabs *et al*.,1996)

HYR1 (hyphally regulated gene) is putative hypha-specific cell surface glycoprotein. The HYR1 sequence revealed a 2,810-bp open reading frame capable of encoding a 937-amino-acid protein with a predicted molecular mass of 94,000 Da. HYR1 is induced specifically during hyphal development during,serum,pH, or N-acetylglucosamine-induction (Bailey *et al*.,1996)

ALS3, ALS8 and HYR1 are all members of multigene families, and a second ECE locus appears to exist in *C. albicans* (CGD). These are genes that are expressed specifically during hyphal development (Swoboda *et al*., 1994).

CONCLUSION

Several experimental approaches have been applied to the investigation of morphogenesis in *C. albicans*. The key purpose of investigation on morphogenesis in *C. albicans* is to define and analyze the expression of growth-form specific factors that determines the cell shape. The growth specific factors are the putative targets in the discovery of antifungal drugs that are aimed at inhibiting hyphae formation in *C. albicans*.

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