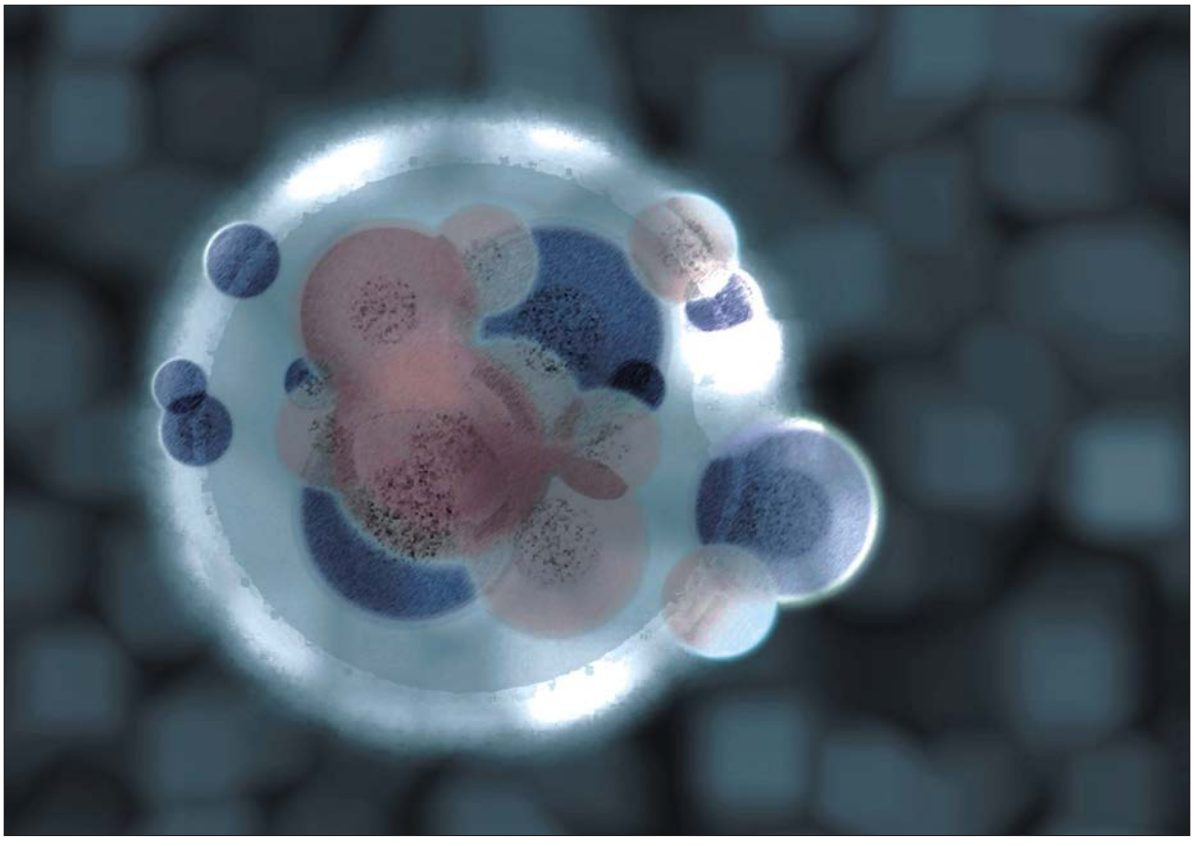

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# Nuclear Proteins Associated with Hyphen Growth in *Candida albicans*

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**Abstract:** *Candida albicans* is an opportunistic fungus and the most prevalent among human pathogenic yeasts. The *Candida* spp. are dimorphic fungi with mycelium (M) and budding yeast (B) growth phases. Dimorphism of *C. albicans* is believed to be a critical component of pathogenesis, to ensure whether the yeast form or the hyphal form is primary responsible for pathogenicity. Growth-form-specific transcripts of *C. albicans* were characterized using ddRT-PCR to ascertain their fundamental differentiation process. The isolated transcript gene (cam3) was identified when it's being expressed during morphogenesis utilizing North blot technique. Differentially expressed mRNAs from both budding and mycelia cultures were characterized for sequence and time of expression during growth phases. One cDNA was identified for transcripts which is apparently unique to hyphal cells. It appears to encode protein homologous to known nuclear proteins. The cDNA (cam3) encodes a polypeptide which shows intriguing similarities to two proteins involved in gene and cell cycle regulation. Portions of the gene align with a protein which interacts with Sin3, a general transcriptional regulator in *S. cerevisiae*. Other portions appear homologous to proteins involved in uracil anabolism.

**Key words:** *Candida albicans*, dimorphism, differential display, cDNA, morphogenesis, pathogenesis

## INTRODUCTION

Dimorphism is the ability of certain fungi to grow as mycelium (M) (hyphae and pseudohyphae) and budding yeast (B) phases. Dimorphism is a process in which under certain conditions, a blastospore cell gives rise to hyphae. A blastospore is defined as a unicellular form of a fungus and is often used interchangeably with the term yeast cell (Brown and Gow, 1999; Ernst, 2000). A hyphae is a thin filamentous cylindrical outgrowth that is derived from blastospore. A single hyphae filament is comprised of cell units separated by septa. Collectively, the hyphae, branches and lateral buds are referred to as mycelium (Soll, 1990). Dimorphism is a fundamental process in *Candida albicans* and is believed to be a critical component of pathogenesis process, although controversy exists as to whether the yeast form or the hyphae form is primarily responsible for pathogenicity, but yeast cells and filaments (mycelium) are found at infection sites. So, it is reasonable that both contribute to pathogenesis and play a prominent role in tissue invasion (Sawyer, 1989; Julie *et al*., 2006). It is also interesting that *C. albicans* differs from other dimorphic pathogenic fungi in that the various dimorphic forms can coexist under certain conditions (Odds, 1985).

Evolutionary theory holds that many pathogenic organisms developed the ability to exist dimorphically in order to adopt varied forms that may colonized and disseminate progeny within a broader spectrum of host tissues. The widespread use of broad spectrum antibiotics has reduced the normal flora making patients susceptible to fungi super infection in patients with intact immune system, so incidence of infections produced by *Candida* spp. has increased significantly in the past few decades (St. Georgiev, 1988; Richards *et al*., 1999; Vincent *et al*., 1995).

Many antifungal drugs used in treating systemic *Candida* infections have undesirable side effects in addition both the limited spectrum of antifungal drugs currently in clinical use and the emergence of resistances make necessary the development of new effective antifungal drugs with minimal side effects; however, such a research is limited by the small number of specific target sites identified to date (Gozalbo *et al*., 2004). Currently, my research is seeking to characterize the processes inherent on cam3-, isolated from transcripts. This gene is involved in dimorphic growth. In addition, It was attempted to identify when this transcripts is being expressed during morphogenesis by utilization of the Northern blot technique so through molecular analysis of growth, we will be able to identify biochemical molecular steps in the fungus growth cycle that can be targeted for the developments of novel antifungal therapies thereby reducing the tissue invasion and immune-evasive properties of this organism.

Finally the collecting sequence data for the genomic clones that have been hybridized with the cDNAs. Cam3

is a 1.6 Kb cDNAs, derived from a hypha specific mRNA, In addition to several genomic clones have been isolated from lambda ZAP and have been expressed as a genomic library using standard plaque preparation techniques. Complete sequence for this gene (cDNA) is already completed and lay the groundwork for the complete sequencing of the genomic clones.

## MATERIALS AND METHODS

Isolation of budding and mycelial RNA was made using *Candida albicans* strain 207 grown in a defined medium (pH 6.8) at 25°C for (B) budding yeast cells and 37°C for mycelial (M). RNA was extracted using acidified phenol and freeze thaw lyses (Schimitt *et al*., 1990). cDNA was reverse transcribed using a poly-T primer and MMLV reverse transcriptase (Gibco). PCR was carried out using standard elaborative temperature profiles (Liang and Pardee, 1992). A $(T)_{12}$ V primer was paired with a specific random sequence upstream primer and PCR products were separated on denatured acrylamide gels. Differential bands were excised, cloned into pGEM-T (Promega), sequenced and used to probe northern blots for time profiles of mRNA expression. Probes were prepared using PCR labeling with $\alpha^{32}$PdATP.

Photomicrography was done using an immersion 40x Nikkor objective, phase contrast and a 400 line S-video camera. Images were captured to computer using Radius compression.

## RESULTS

Differentially expressed RNAs were identified by using ddRT-PCR (Fig. 1), one mycelium-specific clone was identified by using a single anchor and many upstream primers. Pcam3 is a 1.6 kbp cDNA hybridized with RNA extracted from mycelial cells but does not hybridized with RNA from budding cells (Fig. 2). The hyphal specific RNA of approximately the same size was detected at one hour post-induction but does not detected at 3 h (Fig. 3). This clone is not a germ tube specific but is clearly mycelium-specific. This gene of the isolated transcript is involved in dimorphic growth and is being expressed during morphogenesis by utilizing northern blot technique. The sequence data was collected for this genomic clone that has been hybridized with cDNA. Cam3 is represented as a 1.6 Kb from a hypha specific mRNA as it has been isolated from lambda ZAP and expressed as a genomic library by using standard plaque (Fig. 4 and 5), respectively.

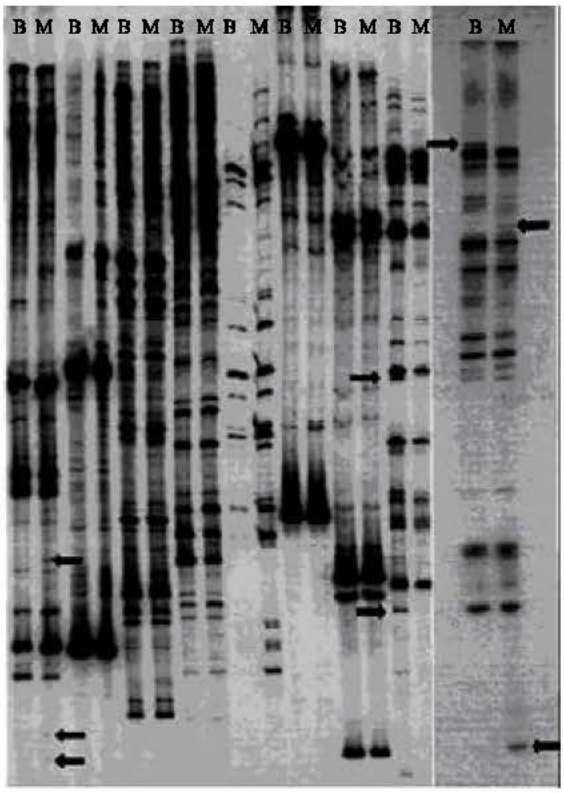


Fig. 1: Differential display result using 1 anchor primer (5′T12 C 3′) and 9 different 5 arbitrary primers. Arrows point to fragments representing cDNAs differentially expressed in budding or mycelial cultures

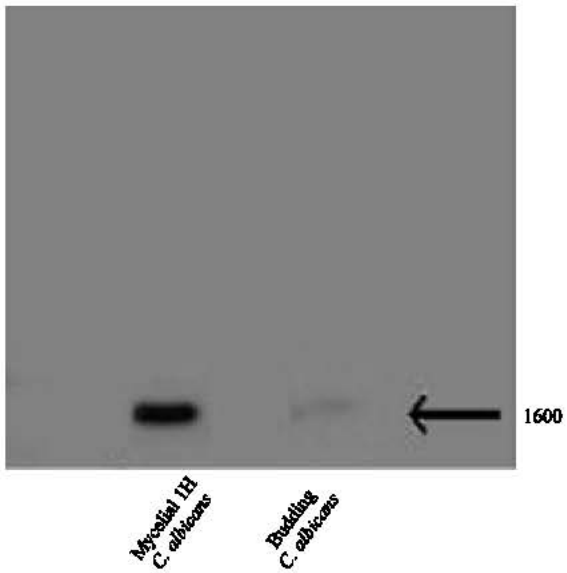


Fig. 2: Pcam3 is a 1.6 kbp cDNA hybridized with RNA extracted from mycelial cells but not with RNA from budding cells

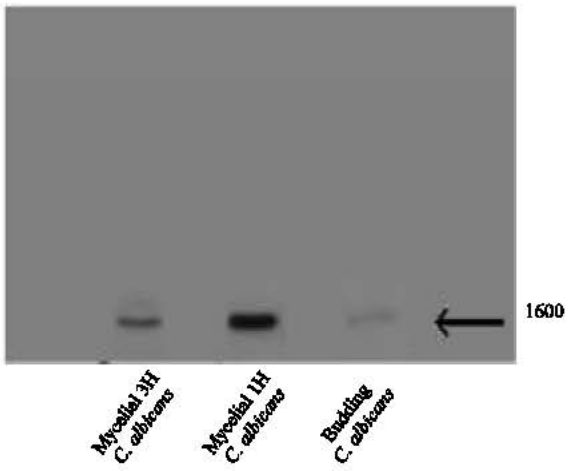


Fig. 3: Pcam3 is a 1.6 kbp cDNA hybridized with RNA extracted from mycelial cells detected at 1 h post-induction (but not at 3 h)

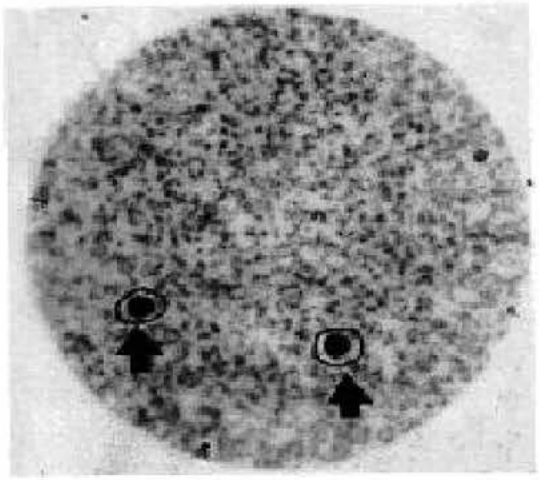

Fig. 4: Standard Plaque shows hybridization of genomic fragment with 1.6 kbp Pcam3 cDNA

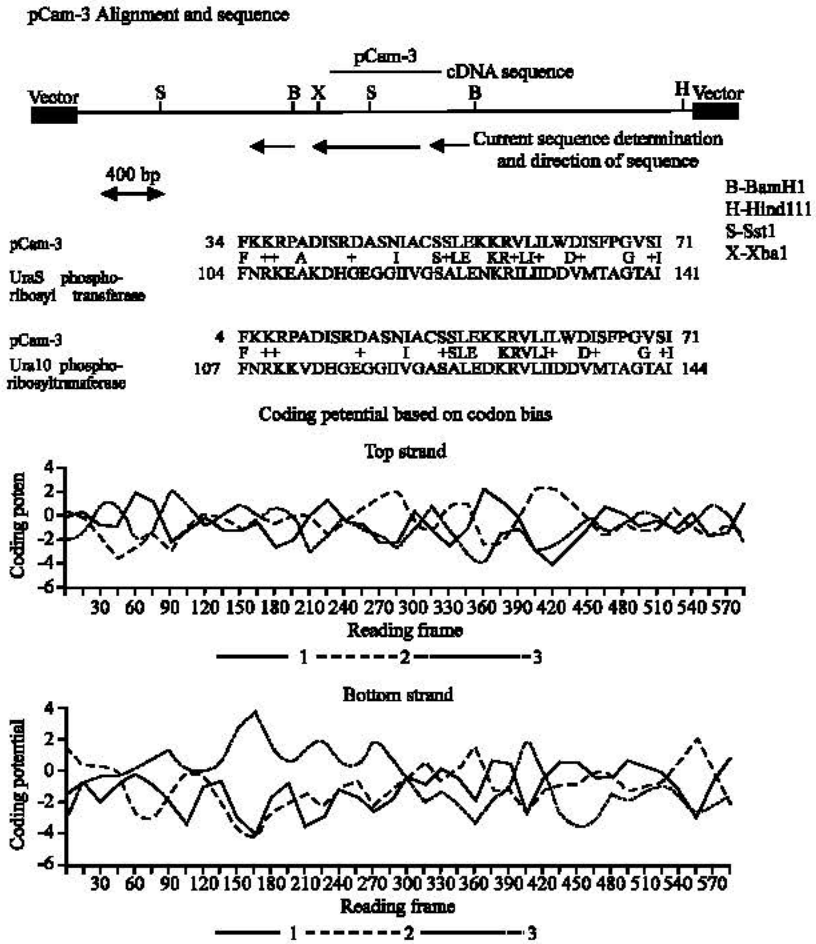


Fig. 5: Partial output from a search of the *Saccaromyces* Genome database with a potential translation product from the sequence of cam3. The translation product is for the region indicated as having significant coding potential on the bottom strand

## DISCUSSION

Studies show that certain events inside the cells as well as the environmental conditions cause gross morphological changes in *Candida albicans* dimorphic growth. It is believed that these internal changes are responses to environmental changes in the extracellular environment (Gow and Goody, 1995). Dimorphism in *Candida albicans* is seem to be due to differentially expressed genes between budding and mycelium form as shown by Liu *et al.* (1994), Srikantha *et al.* (1995) and Saporito *et al.* (1995). Their research describes and characterizes genes associated with direct enhancement of particular aspect of dimorphic growth in *Candida albicans*. These genes were cloned using differential hybridization to cDNA and include: PEP1-an encoder for an extracellular protease that is specifically induced in opaque cells (Morrow *et al.*, 1993); ECE1-an encoder for mycelia that codes a protein of 8 degenerate 34 amino acid repeats (Birse *et al.*, 1993); Cph1-an encoder of STE 12 homologous, a hypha formation suppressor (Liu *et al.*, 1994); PHD1-pseudohyphal growth inducer (Liu *et al.*, 1994); and PHR1 and ECE1-genes demonstrated to be critical for dimorphic growth. Both genes have been isolated and disrupted by knocking out both copies of these genes in the diploid genome. PHR1 has a profound effect in determining cell morphogenesis due to its response to extracellular ions (Saporito *et al.*, 1995). On the other hand, ECE1 is capable of being dispensed for hypha formation (Birse *et al.*, 1993).

## CONCLUSION

Dimorphism growth of *Candida albicans* is controlled by certain genes, one of them is cam3 which is 1.6 Kb cDNAs, derived from a hypha specific mRNA. Complete sequence for this gene (cDNA) is already completed and lay the groundwork for the complete sequencing of the genomic clones.

## ACKNOWLEDGMENTS

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