INTRODUCTION
1 INTRODUCTION

Morphogenesis can be defined as the development of an organism to its specific form. This implies differentiation of a cell into a form differing in its shape, size, structure and chemical composition. Morphogenesis in fungi is mainly concerned with the yeast \( \rightarrow \) hypha transition which is commonly referred to as dimorphic transition or dimorphism.

1.1 Fungal dimorphism

Dimorphism has been known to microbiologists since Pasteur's time, when transition between yeast and mycelia was used as a basis for the theory of transmutation (the conversion of one species to another). It was Pasteur who showed that the morphological forms of *Mucor* sp. were dependent on environmental conditions under which the organism was grown. More recently, the phenomenon of dimorphism has attracted particular attention for two reasons. Dimorphism is a useful model to understand the molecular basis of cell differentiation in eukaryotes. Further, the understanding of this phenomenon may provide clues for the control of fungal diseases as many pathogenic fungi are dimorphic (Table 1).
Table 1: Dimorphic pathogenic fungi

<table>
<thead>
<tr>
<th>Organism</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Ajellomyces dermatitidis</em></td>
<td>Blastomycosis</td>
</tr>
<tr>
<td></td>
<td>(Skin disease)</td>
</tr>
<tr>
<td>2. <em>Histoplasma capsulatum</em></td>
<td>Histoplasmosis</td>
</tr>
<tr>
<td>3. <em>Paracoccidioides brasiliensis</em></td>
<td>South American blastomycosis</td>
</tr>
<tr>
<td>4. <em>Sporothrix schenckii</em></td>
<td>Sporotrichosis</td>
</tr>
<tr>
<td>5. <em>Exophiala werneckii</em></td>
<td>Skin disease</td>
</tr>
<tr>
<td>6. <em>Wangiella dermatitidis</em></td>
<td>tinea nigra</td>
</tr>
<tr>
<td>7. <em>Emmonsia parva</em></td>
<td>Phaeohyphomycosis</td>
</tr>
<tr>
<td>8. <em>Candida albicans</em></td>
<td>Adiaspiromycosis</td>
</tr>
</tbody>
</table>

Candidiasis
Dimorphism in fungi has been reviewed extensively (Cutler and Hazen, 1983; Howard, 1983; Maresca and Kobayashi, 1989; San Blas and San Blas, 1984; Stewart and Rogers, 1983). Very few fungi have been studied systematically in order to obtain an insight into molecular events regulating dimorphism. These include Candida albicans, Sporothrix schenckii, Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidiodioides brasiliensis and several species of Mucor. These fungi have been studied at length from various point of views. The yeast to hyphal transition of these fungi is regulated by a wide variety of environmental factors which include type of carbon source and their concentration, CO₂:O₂ ratio, C:N ratio, pH, temperature, metal ions, amino acids and other growth factors. Various ultrastructural and biochemical changes accompanying yeast to hypha transition have also been reported. These include levels of various metabolic enzymes, cell wall structure and biosynthesis, intracellular levels of cAMP and cGMP, rates of macromolecular (DNA, RNA and proteins) synthesis, RNA polymerase activities and the morphology specific proteins. The absence of a well defined genetic systems among dimorphic fungi has significantly hampered the morphogenetic studies in these organisms. Thus, a thorough understanding of the molecular basis of morphogenesis is lacking.
1.2 Morphogenesis in *C. albicans*

*Candida albicans*, a pathogenic, dimorphic yeast can grow as yeast or hypha depending on the environmental conditions. It is frequently found as a commensal on the oral, gastrointestinal and vaginal mucosae in human and other warm blooded animals. It causes a variety of clinical forms of illness ranging from localised cutaneous candidiasis in healthy individuals to life threatening systemic candidiasis in immunocompromised hosts. In the last two decades, there has been an increase in the incidence of candidiasis, which is attributed to the widespread use of antibiotics and immunosuppressive drugs. Increase in number of AIDS cases is also responsible for higher incidence of candidiasis. *C. albicans*, is therefore considered as the most common opportunistic human pathogen. Morphogenesis of *C. albicans* has attracted particular attention, since it is relevant to the virulence of the organism (Odds, 1988). It also provides a good model system to study eukaryotic cell differentiation. In the last few years, several reviews have been published on the biology, genetics, pathogenicity and morphogenesis of *C. albicans* (Odds, 1985; Shepherd et al., 1985; Odds, 1988; Datta et al., 1989).
1.2.1 *C. albicans* - a polymorphic fungus

*C. albicans* is classified as fungi imperfecti because it lacks a known sexual stage in its life cycle. It exists in different cellular morphologies. The most commonly encountered growth form of *C. albicans* is budding yeast (Fig. 1). It develops through mitotic life cycle, $G_1 \rightarrow S \rightarrow G_2 \rightarrow M$, in the same way as in other yeasts, but it has proved extremely difficult to define these phases clearly in *C. albicans* (Odds, 1988).

*C. albicans* is polymorphic and grows as a multipolarly budding yeast, as pseudohyphae or as a true septate hyphae. Both true hypha and pseudohypha may regenerate yeast phase by producing clusters of yeast-like blastoconidia. The polymorphic nature of *C. albicans* is further augmented by the production of thick walled chlamydospores. Mycelium is the entire fungal cellular aggregate including hyphae, pseudohyphae, buds and chlamydospores. Yeast cells are easily distinguished from the other morphologies, but the recognition of hyphae as true hyphae/pseudohyphae is more difficult. True hyphae grow continuously by apical extension. Mitotic division occurs and nonconstricted septa are formed. Pseudohyphae are elongated yeast cells, formed by polar budding, constricted at cell junction and usually joined in chains/clusters. Germ tube, an intermediate stage
Fig. 1. Scanning electron micrographs of C. albicans. A, Hypha (arrow), pseudohypha (double arrow) and blastoconidium (triple arrow). Bar represents 2 μM. B, Germ tube. Bar represents 1 μM. C, Hypha with intermediate septum (S). Bar represents 1 μM. (From Szaniszlo et al., 1983).
In yeast to hypha transition, is a newly evaginating hypha upto the time of formation of first septum. Chlamydospores are thick walled, short lived asexual spores, which are believed to be 'dormant growth forms and arise in the condition of nutrient depletion.

Another interesting feature of \textit{C. albicans} is its ability to switch colony morphology. It switches heritably, reversibly and at a high frequency ($10^{-2}$ to $10^{-4}$) between at least seven colony phenotypes (Slutsky et al., 1985) or between white and opaque colour phenotypes (Slutsky et al., 1987). Colony morphology switching may cause variation in susceptibility to drugs and hence in pathogenicity of the organism.

1.2.2 Pathogenesis

\textit{C. albicans} is a normal commensal in human being but can cause life threatening candidiasis when host resistance breaks down. The factors which contribute to the virulence of the organism are the ability to change colony and cell morphology (Odds FC, 1988), adherence to mucous membrane (Caldrone et al., 1985) and production of hydrolytic enzymes (Macdonald and Odds, 1980). The yeast, hyphal and pseudohyphal morphologies are all associated with host lesions. Yeast morphology is considered by many as a
saprophytic phase, which colonizes body surfaces, whereas conversion to hyphal form is required for tissue invasion. But it appears that all morphological forms are required for the establishment and maintainence of the disease (Odds, 1988). However, it has been reported that germ tube forming strains are more virulent than the non-germ tube forming strains (Richardson and Smith, 1981).

_C. albicans_ mostly infects amino sugar-rich mucous membranes and the mycelial form is thought to be responsible for its pathogenocity. Hence the focus of a number of studies has been on germ tube induction by N-acetyl-D-glucosamine (GlcNAc), an amino sugar (Simonetti et al., 1974, Shepherd et al., 1980; Natarajan et al., 1984). It has been shown in our laboratory that only pathogenic yeast like _C. albicans_ can utilize amino sugars, like GlcNAc, as a sole carbon source (Singh and Datta 1979). It is believed that GlcNAc metabolism is related to pathogenesis of the organism.

### 1.2.3 Environmental factors affecting yeast to hypha transition

Dimorphism in _C. albicans_ is regulated by a wide variety of environmental factors which include temperature, pH and nutrients (Shepherd et al., 1985; Odds, 1988; Datta et al.,
A temperature range of 33°C to 42°C and a pH range from 6 to 8 are critical for germ tube formation. However, it has been shown that under certain conditions, cells can form germ tubes at 25°C (Sabie and Gadd, 1988), and at a pH as low as 3.0 (Pollack and Hashimoto, 1985). Furthermore, germ tube formation in defined media is also influenced by yeast growth phase and by strain variation (Odds, 1988; Datta et al., 1989).

Yeast to hypha transition in C. albicans is induced by a variety of complex exogenous substances which include serum, cerebrospinal fluid, plasma and egg white. Other substances which can also induce yeast to hypha transition in C. albicans are N-acetyl-D-glucosamine, amino acids, alcohols and glucose (reviewed by Odds, 1988). But there is not a single 'morphogen' which alone under all conditions can induce cells to grow in one morphological form.

1.2.4 Ultrastructural and biochemical changes accompanying morphogenesis

Several experimental approaches have been used to the identification of biochemical and molecular events responsible for morphogenesis (Odds, 1988; Shepherd et al., 1985; Datta et al., 1989). Still, the underlying mechanism is not clearly known.
The formation of germ tube is visibly an elongation of cell wall. Hence it is thought that factors controlling the structure and biosynthesis of the cell wall are of importance in dimorphism. This led to studies on cell wall constituents and their biosynthetic pathways. Yeast and hyphal cell walls vary in the amount of protein, lipids and glucosamines. Compared to yeast forms, hyphal forms have higher chitin (Chattaway et al., 1968; Chiew et al., 1980) and higher chitin synthase activity (Braun and Calderone, 1978). Studies with inhibitors have shown that chitin synthase can play a regulatory role in morphogenesis of C. albicans (Chiew et al., 1982).

Cell biological aspects of morphogenesis in C. albicans have been investigated in detail (Reviewed by Odds, 1988). It has been shown that the major differences between yeast and hypha are brought about by the changes in timings, location and dimensions of various components of developmental process. The timing of events such as evagination, the volumes of cellular outgrowths, and differences in sites of cell wall synthesis combine to regulate cell wall structure and thus the cell shape. Temporal and spatial differences in cell wall expansion during bud and hypha formation have been studied (Staebell and Soll, 1985). It has been suggested that the yeast to
The hypha transition could be due to exclusive synthesis of the primary cell wall at the apical tip in hyphae. Ultrastructural changes during morphogenesis have also been reported (Gow and Gooday, 1982, 1984).

Some investigators have concluded that if chemical inhibitors of RNA or protein synthesis also inhibit hypha formation then this proves that the regulation of morphogenesis is at the transcriptional or translational level of gene expression. However, this interpretation is inaccurate as these inhibitors also inhibit the growth of the organism. Hence their effect is not specific to morphological development (Odds, 1988).

Attempts have been made to identify proteins specific to either yeast or hyphal phase using both one and two dimensional SDS-polyacrylamide gel electrophoresis (section 5.1). In the most careful of these studies (Finney et al., 1985) only one protein has been identified as specific to the two morphological forms.

Actin granules also appear to be associated with the growth zones of yeast and hypha of C. albicans (Anderson and Soll, 1986). There is a difference in actin localization during formation of buds and hyphae. In budding cells, actin granules are distributed throughout the cytoplasmic
cortex, while during hyphal growth, majority of the actin granules are clustered at the apex.

Unfortunately, the media and growth conditions used in these studies are often highly complex and very different. Furthermore \textit{C. albicans} strains display a great deal of heterogeneity in germ tube/mycelium formation (reviewed by Datta et al., 1989). In addition, the diversity of environmental factors which regulate morphogenesis has made it difficult to identify a unifying explanation for morphogenesis in \textit{C. albicans}.

\section*{1.3 Aim and Scope}

The aim of this work has been to study the biochemical events which regulate morphogenesis in \textit{C. albicans}. Morphogenesis in \textit{C. albicans} has attracted particular attention as it is relevant to the virulence of the organism. It also provides a good model system to study eukaryotic cell differentiation.

A study of factors which block yeast to hypha transition can give an indication as to which processes are involved in morphogenesis. It was first time reported from our laboratory that calmodulin inhibitor, trifluoperazine, can block germ tube formation in \textit{C. albicans}. As calcium
and calmodulin are known to be involved in growth and differentiation of many fungi, we were interested in studying their role in the morphogenesis of C. albicans.

An attempt to elucidate the effect of Ca\(^{2+}\)-calmodulin leads to a study of protein kinases. Protein kinases, which catalyze the phosphorylation of key proteins of cellular reactions, are known to be an important group of enzymes. Modifications of cellular proteins by phosphorylation influence several important cellular processes. Hence we followed up our initial studies with an analysis of phospho-protein profiles from yeast and germ tube forming cells in an attempt to identify the type of protein kinases involved in this process.

An alternative approach in understanding the role of calmodulin would be to study the expression of calmodulin gene during morphogenesis. For this cloned genes are necessary. If cloned genes are available, mutant strains can be constructed by in vitro gene disruption and site directed mutagenesis. Cloned genes can then be overexpressed in morphological mutants to study its effect.

Two different approaches could be tried to clone calmodulin.
1. As calmodulin is a conserved protein, a heterologous probe can be used to screen a genomic library to pick up calmodulin gene.

2. If antibodies against calmodulin are available, they can be used to screen a λgt 11 expression library of C. albicans, which was earlier made in our laboratory. This initiated us on the problem of purifying and characterizing calmodulin to raise antibodies against it.

Morphogenesis in C. albicans is expected to be accompanied by differential gene expression. This led us to study protein synthesis accompanying germ tube formation. Differential gene expression can be studied by identifying differentially expressed genes or gene products. This led us to the identification of morphology specific protein in C. albicans. Furthermore, these proteins might also be useful as specific markers to diagnose candidiasis.