PROTEIN SYNTHESIS DURING GERM TUBE FORMATION IN C. ALBICANS
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5.1 Introduction

C. albicans, a dimorphic fungus can exist in yeast or hypha forms depending on the environmental conditions. It is obvious that this yeast to hypha transition is accompanied by qualitative as well as quantitative changes in the pattern of gene expression. Hence an understanding of the regulation of gene expression during yeast to hypha transition would help to elucidate the mechanism of morphogenesis in C. albicans.

Morphogenesis can be regulated by differential gene expression involving switching on and off of specific developmentally regulated genes. Germ tube formation is accompanied by developmentally regulated changes both in protein and mRNA. Developmentally regulated genes can therefore be identified in two ways: 1) By differential hybridization of single stranded labeled probes from germ tubes and buds with replica C. albicans genomic library. 2) By cloning genes coding for morphology specific proteins.

Identification of morphology specific proteins has received much attention as it helps to understand differential gene expression in eukaryotes. These proteins might also be useful as antigens in diagnosis of
candidiasis. A number of groups have examined protein profiles from yeast and hypha forms by both one and two dimensional gel electrophoresis (Ahrens et al., 1983; Finney et al., 1985; Manning and Mitchell, 1980; Dabrowa et al., 1984).

A number of factors are known to regulate yeast to hypha transition; the most important being temperature and pH of the medium (Buffo et al., 1984). In general, cells exist in yeast phase at low temperatures or low pH and in hyphal phase, at high temperature and higher pH. Morphology specific proteins have been identified using either pH or temperature as experimental parameters but never both together to obtain a homogeneous population of yeast or hyphal phenotype. Furthermore, the growth medium used in these studies was complex as it contained glucose, salts and amino acids. This may result in physiological differences that are specific to growth conditions, but not due to differences in phenotype. Hence the use of temperature and pH independently to regulate the phenotype in a simple medium would be ideal to study differential protein synthesis during germ tube formation.

In the following section, changes in the rate of protein synthesis during germ tube formation induced by GlcNAc, proline and glucose plus glutamine are discussed.
Furthermore, using pH and temperature to regulate proline-induced germ tube formation, morphology-specific proteins have been identified.

5.2 Results and Discussion

5.2.1 Germ tube formation is accompanied by a decrease in the rate of protein synthesis.

Germ tube formation in *C. albicans*, induced by GlcNAc was accompanied by a decrease in the rate of protein synthesis (Fig 19A). Factors which inhibited germ tube formation (i.e., presence of TFP (20 μM) or incubation at 25°C) stimulated/increased the rate of protein synthesis. The decrease in the rate of protein synthesis was not due to the depletion of carbon source from the medium as increasing the concentration of GlcNAc in the medium neither increased the rate of protein synthesis nor inhibited germ tube formation (Fig 19B).

Interestingly, germ tube formation induced by proline and glucose plus glutamine was also accompanied by a decrease in the rate of protein synthesis. Factors which inhibited germ tube formation (i.e., lowering the pH to 4.5 or by incubation at 25°C) stimulated the rate of protein synthesis (Fig.20). These results indicated that there was a correlation between cell morphology and the rate of protein synthesis. The decrease in the rate of protein...
Fig. 19. Rate of protein synthesis during germ tube formation induced by N-acetyl-D-glucosamine.

A. Germ tube formation was induced by incubating cells at 37°C in imidazole-MnCl₂ buffer, pH 6.6, with 5 mM GlcNAc as inducer. Germ tube formation was blocked either by adding trifluoperazine (20 μM) or by incubating cells at 25°C. At indicated times 1 ml cells (5x10⁶ cells/ml) were pulse labeled with [³⁵S]methionine (0.6 μCi/ml) for 20 min at 37°C, and hot TCA precipitable counts were estimated. (O), Control (germ tube); (●), Trifluoperazine (20 μM) (yeast phase); (△), incubation at 25°C (yeast phase).

B. Effect of GlcNAc concentration on the rate of protein synthesis. (O), GlcNAc (5mM); (●), GlcNAc (20 mM), (△), GlcNAc (50 mM).
Fig. 20. Rate of protein synthesis during germ tube formation induced by proline and glucose + glutamine. Germ tube formation was induced by incubating cells at 37°C in 20 mM imidazole-MnCl₂ buffer, pH 6.6, with proline (50 mM) or glucose plus glutamine (2.5 mM each). Germ tube formation was inhibited either by lowering the pH of the buffer to 4.5 or by incubating cells at 25°C. At indicated times 0.5 ml cells (5x10⁷ cells/ml) were pulse labeled with [³⁵S]methionine (0.6 μCi/ml) for 20 min at 37°C, and hot TCA precipitable counts were estimated.

(O), pH 6.6 at 37°C (germ tube); (●), pH 4.5 at 37°C (yeast phase); (Δ), pH 6.6 at 25°C (yeast phase).
synthesis, which accompanied germ tube formation could be due to differences in the rate of amino acid uptake or due to difference in the rate of protein turnover. But it has been reported (Torosantucci et al., 1984) that the amino acid pool and the rate of amino acid uptake were comparable in germ tubes and yeast phase cells grown either in imidazole-buffered GlcNAc medium or in amino acid liquid synthetic medium of Lee et al. (1975).

5.2.2 Proline-induced germtube formation is an ideal system to study differential protein synthesis

Germ tube formation in *C. albicans* can be induced by a number of amino acids, of which proline is the most effective one (Holmes and Shepherd, 1987). In the amino acid-salts medium (Lee et al., 1975), which is commonly used for induction of germ tubes, proline is found to be the sole effector of morphogenesis (Holmes and Shepherd, 1987). Proline can also induce germ tube formation in imidazole-MnCl$_2$ buffer (pH 6.6) at 37°C. This can be inhibited either by lowering the pH of the medium to 4.5 (Holmes and Shepherd, 1987) or by incubating cells at 25°C. Thus proline-induced germ tube formation in imidazole-MnCl$_2$ buffer provides an ideal system to study morphogenesis in *C. albicans* as it can be regulated by both pH and temperature. (Table 5).
**TABLE 5: Regulation of proline induced germ tube formation**

<table>
<thead>
<tr>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6</td>
<td>37</td>
<td>Germ tube</td>
</tr>
<tr>
<td>4.5</td>
<td>37</td>
<td>Yeast</td>
</tr>
<tr>
<td>6.6</td>
<td>25</td>
<td>Yeast</td>
</tr>
</tbody>
</table>
5.2.3 Identification of morphology specific proteins: Two dimensional SDS-PAGE analysis of cytoplasmic proteins

As seen in Fig. 21 five proteins (84 kd, 30 kd, 26 kd, 21 kd, and 18 kd) appear to be germ tube specific (marked g). These proteins are either absent or synthesized at low amounts in budding cells grown under two different conditions (incubating at 25°C or lowering the pH of the medium to 4.5). Two proteins (130 kd, 54 kd, and 28 kd) appear to be bud specific (marked b) as these are present only in budding cells. Comparison of three autoradiographs shows some more differences in only reproducible proteins have been marked. The results indicate that the majority of the proteins synthesized during germ tube or bud formation are identical. The fact that certain polypeptides are associated with either germ tube or bud, suggests the involvement of differential gene expression during morphogenesis in \textit{C. albicans}. The changes in the protein synthesis pattern is a reflection of cumulative change in the physiology of cells as they form germ tube or buds and not associated with a single discrete event during germ tube or bud formation.

In all previous reports (Section 5.1) on protein synthesis during morphogenesis of \textit{C. albicans}, either temperature or pH to regulate dimorphism have been used. The number and pattern of polypeptides detected in this
Fig. 21. Two dimensional gel analysis of proteins from C. albicans during proline-induced germ tube formation. Germ tube formation was induced by incubating cells at 37°C in imidazole-MnCl$_2$ buffer, pH 6.6, with 50 mM proline as an inducer. Germ tube formation was inhibited either by lowering the pH of the buffer to 4.5 or by incubating cells at 25°C. Cells were continuously labeled with [35S] methionine (10 μCi/ml) for 3 h. Cell extracts were subjected to two dimensional gel analysis as described (Section 2.6).

(A), pH 6.6 at 37°C (germ tube); (B), pH 4.5 at 37°C (yeast phase); (C), pH 6.6 at 25°C (yeast phase).
study differs from the earlier reports which could be due to differences in strain and growth conditions used. The use of pH and temperature as regulators to study dimorphism, rules out the possibility of morphology specific proteins being either temperature or pH dependent.

At least 120 polypeptides synthesised during germ tube/bud formation were observed. This does not represent all proteins from C. albicans, since extremely acidic and basic polypeptides are poorly resolved under the gel system employed. In addition proteins which contain low levels of methionine would be difficult to detect. Since Candida genome can code for more than 10,000 genes of average size, it is likely that the 120 identifiable polypeptides represented only the most abundant or rapidly synthesised gene products. Unfortunately, the possibility still remains that the regulatory proteins involved in the regulation of dimorphism are either less abundant or synthesized at a low rate.