The Role of Glucose in the pH Regulation of Germ-tube Formation in *Candida albicans*

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It has been reported that *Candida albicans* can form germ-tubes only in the narrow pH range of 6–8, and that by changing only the pH one can regulate germ-tube formation. We found that the pH minimum for germ-tube formation could be dramatically lowered by eliminating the glucose present in many induction solutions. Lee's medium lacking glucose, ethanol, *N*-acetyl-D-glucosamine, and proline induced germ-tubes at pH values as low as 3 under most conditions. The presence of as little as 1 mM-glucose in these induction solutions was sufficient to cause the cells to grow either as yeasts with multiple buds or as pseudohyphae when the pH was 3.7. However, when *C. albicans* was grown in any of the above induction solutions (with the exception of ethanol), containing 200 mM-glucose buffered at pH 5.8, not only were germ-tubes formed, but their rate of formation and length were also increased. Preincubation of the cells in a solution buffered at pH 3.7 and containing 200 mM-glucose, before exposure to induction solutions lacking glucose at pH 3.7 or at pH 5.8, did not inhibit germ-tube formation. Likewise, addition of glucose after 45 min exposure to an induction solution was without effect. Theophylline and dibutyl cAMP did not counteract the action of glucose. Other sugars which suppressed germ-tube formation at low pH were fructose, galactose, mannose, xylose, gluconic acid and the nonmetabolizable sugar 3-*O*-methylglucose. These results indicate that pH does not directly regulate dimorphism in *C. albicans*, and that glucose or its metabolites may play an important role.

INTRODUCTION

A number of factors are known to regulate the dimorphic transformation of *Candida albicans* from budding yeast cells to elongating hyphae, the most important being the growth medium and temperature. Specifically, when *C. albicans* yeast cells are transferred to a medium containing either certain amino acids (such as proline), *N*-acetyl-D-glucosamine (GlcNAc) or ethanol, and incubated at a temperature between 33 and 42 °C, they are induced to grow by forming germ-tubes which elongate to form hyphae.

Although many groups have reported that germ-tube formation is restricted to the narrow pH range of 6–8 (Odds, 1979), only recently has pH been advanced as an equally important 'regulator' of *C. albicans* dimorphism. Buffo *et al.* (1984) presented evidence that as long as the pH of a medium allowing germ-tube formation is kept above 6, *C. albicans* grows in the hyphal form. Should the pH drop below 6, the cells revert to growing as a budding yeast. Using ethanol as an inducer of germ-tube formation (Pollack & Hashimoto, 1985), we have shown that germ-tubes could form even when the pH was as low as 3.0. This led us to look for an explanation for the differences between our findings and those of Soll and co-workers (Mitchell & Soll, 1979; Buffo *et al.*, 1984).

Abbreviations: LAA, Lee's amino acids; LM, Lee's medium; SDA, Sabouraud dextrose agar; SDB, Sabouraud dextrose broth; TAPS, tris(hydroxymethyl)methylaminopropanesulphonic acid.

This paper attempts to show that it is glucose, present in many media including that of Lee *et al.* (1975) used by Soll and co-workers (Mitchell & Soll, 1979; Buffo *et al.*, 1984), which suppresses germ-tube formation when the pH is below 5.8 and that, therefore, pH can at most be considered an 'indirect' regulator of germ-tube formation.

METHODS

Organisms. *C. albicans* ATCC 58716 was routinely used for the experiments described in this paper. In some experiments *C. albicans* ATCC 10261 was used for comparative purposes. Cells were maintained on Sabouraud dextrose agar (SDA, Difco) at 20 °C.

Growth and preparation of organism. Yeast-phase cells were prepared by growing the cells on SDA for 24 h at 37 °C. In some experiments, cells were also prepared by growing them for 24 h at 37 °C in Sabouraud dextrose broth (SDB) containing 0.1% yeast extract, or by growth in the medium of Lee *et al.* (1975) (referred to as 'Lee's medium', LM) for 48–72 h at 23 °C as described by Buffo *et al.* (1984). The cells were collected and washed ten times with glass-distilled, deionized water by vacuum filtration on a Millipore filtration apparatus, the cells being resuspended in water by repeated suction and forcible ejection through a Pasteur pipette before each washing.

Induction of germ-tube formation. Washed *C. albicans* yeast-phase cells (final concn 3×10^6 cells ml⁻¹) were inoculated into a buffered solution (final volume, 0.5 ml), containing an inducer and the following salts: FeSO₄ (0.1 mg l⁻¹); KCl (400 mg l⁻¹); MgSO₄ · H₂O (200 mg l⁻¹); NaH₂PO₄ · H₂O (125 mg l⁻¹); NaCl (6400 mg l⁻¹); and NaHCO₃ (750 mg l⁻¹). The buffers used were citrate phosphate (pH 2.5–6.5), HEPES (pH 7.0–8.0), MES (pH 5.5–6.5), succinate (pH 4.0–5.0), Tris/maleate (pH 5.0–9.0), transaconitate (2.5–6.0), and tris(hydroxymethyl)methylaminopropanesulphonic acid (TAPS, pH 7.5–9.5). With the exception of citrate phosphate buffer, all buffers were adjusted to the desired pH with NaOH. The concentration of the buffer salt was 0.05 M. The inducers used were GlcNAc, L-proline (4 mM), glucose plus NH₄Cl (each 5 mM), ethanol (17.1 mM, 0.1% v/v), and a solution containing the amino acids found in LM: L-alanine, 5.6 mM; L-leucine, 9.9 mM; L-lysine, 5.5 mM; L-methionine, 0.7 mM; L-phenylalanine, 3.0 mM; L-proline, 4.3 mM; and L-threonine, 4.2 mM (referred to as 'Lee's amino acids', LAA). The cells were incubated at 37 °C in a water bath for 4 h. The pH of each solution was determined at the beginning and end of each incubation with a digital Ionalyzer (Orion Research, model 701A) and a semi-micro, combination pH/reference electrode (no. 476050, Corning Glass Works, Medfield, Mass., USA). Preliminary experiments showed that pH did not vary by more than 0.3 units during the 4 h incubation period. The percentage of cells forming germ-tubes or multiple buds was measured by counting 100–200 cells (or cell clusters) in each sample with a phase contrast microscope (Nikon). A cell was considered multi-budded if two or more round or ellipsoidal (pseudohyphal) buds were attached to the mother cell: a cell was considered to have formed a germ-tube when a narrow tube, at least 2 µm long and without a constriction at its base, extended from the mother cell. Each cluster of cells was considered as one unit so that 50 multi-budded clusters in a population containing an additional 50 single cells would mean that 50% of the population was multi-budded. Although, generally, germ-tubes were formed from single cells, in those instances when they were formed from budded cells they were counted as one 'germ-tube unit' regardless of whether both the mother and daughter or only one of them had a germ-tube. Germ-tube lengths were measured using an ocular micrometer.

Chemicals. Most of the sugars used in this study were obtained either from Nutritional Biochemicals or from Sigma. The exceptions were GlcNAc, glucosamine, sodium glucoheptonate and gulono-1,4-lactone (Pfanstiel), glucose and glycerol (Mallickrodt) and lactose (J. T. Baker). HEPES, MES, TAPS, amino acids and dibutyl cAMP were obtained from Sigma, theophylline from Calbiochem, and the salts used in the buffered salts solution from J. T. Baker. All chemicals were used without further purification.

RESULTS

Influence of pH on induction of germ-tubes by different inducers

The range of pH values which allowed germ-tube formation was tested using a number of different inducers. The results shown in Fig. 1 demonstrate that germ-tubes were induced in *C. albicans* by proline when the pH was between 3 and 9. Almost identical results were obtained when proline was replaced by LAA or GlcNAc. If ethanol replaced proline, a similar result was obtained with the exception that the maximum proportion of cells forming germ-tubes was approximately 85% (see Pollack & Hashimoto, 1985). Decreasing the concentration of inducer 100-fold resulted in only a partial decrease in the percentage of cells forming germ-tubes at the pH extremes. The pH range at which germ-tube formation occurred was not altered by increasing the concentration of proline or GlcNAc 100-fold either. In other words, there

Fig. 1

Fig. 2

Fig. 1. Influence of pH on germ-tube formation. *C. albicans* yeast cells were incubated for 4 h at 37 °C in buffered salts solution (●) or buffered salts solution containing 4 mM-proline (○). For buffers used to control the pH, see Methods. Values are the means of at least four independent experiments. Bars represent SD.

Fig. 2. Influence of glucose on germ-tube and multiple bud formation at different pH values. *C. albicans* yeast cells were incubated for 4 h at 37 °C in buffered salts solution containing LAA in the presence (●, ■) or absence (○) of 200 mM-glucose. Similar curves were obtained if LAA was replaced by proline or GlcNAc. A curve similar to (●) was also obtained if the cells were induced by the combination of glucose and NH_4Cl , each at 5 mM. ○, ●, Cells forming germ-tubes; ■, cells forming multiple buds. The values are the means of four independent experiments. Bars represent SD.

Table 1. *Suppression by glucose at low pH of germ-tube formation induced by different compounds*

Cells were incubated with each individual inducer at pH 3.7 (except for ethanol, at pH 4.3) in the presence or absence of 200 mM-glucose. Values represent the means of four to seven independent experiments $\pm$ SD.

Inducer	Percentage of cells forming germ-tubes	
	Glucose concn (mM)	
	0	200
LAA	92.3 $\pm$ 6.9	12.0 $\pm$ 8.0
Proline	88.2 $\pm$ 5.8	10.7 $\pm$ 7.1
GlcNAc	93.5 $\pm$ 4.6	21.2 $\pm$ 7.2
Ethanol	66.6 $\pm$ 9.5	8.8 $\pm$ 8.0

apparently is no sharply defined pH optimum for germ-tube formation. The addition of 200 mM-glucose to LAA (Fig. 2) or the other inducers (Table 1) suppressed germ-tube formation when the pH was less than 5.8. The addition of glucose to ethanol-induced cells, however, suppressed germ-tube formation even above pH 5.8. Up to 90% of the cells which normally formed germ-tubes at pH 3.7 were inhibited from doing so by the presence of glucose (Table 1). These results were not unique to strain ATCC 58716, but were also confirmed using strain ATCC 10261. In addition, since the cells used by Buffo *et al.* (1984) were grown initially in LM, yeast cells were prepared as they reported and by growth in SDB containing 0.1% yeast extract for 24 h. These yeast cells were also tested for their ability to form germ-tubes at different pH values. The results (not shown) were essentially identical to those obtained using yeast cells grown on SDA.

Concentration of glucose necessary for suppression of germ-tube formation

The concentration of glucose in LM is fairly high (70 mM). We therefore tested the cells in the different induction media for the minimum concentration of glucose required to suppress germ-tube formation (Fig. 3). Even 1 mM-glucose suppressed germ-tube formation at pH 3.7. Increasing the concentration of glucose resulted in enhanced suppression of germ-tube

Fig. 3. Effect of increasing the glucose concentration on germ-tube and multiple bud formation in *C. albicans* yeast cells induced to form germ-tubes at pH 3.7. Cells were incubated for 4 h at 37 °C in buffered salts solution containing LAA (●, ■) or GlcNAc (○). Curves essentially identical to ● and ■ were obtained when LAA was replaced by proline. ○, ●, Cells forming germ tubes; ■, cells forming multiple buds. Values are the means of four independent experiments. Bars represent SD.

formation; however, even at the highest concentration of glucose tested (400 mM), germ-tube formation was never completely inhibited, approximately 10% of the cells still being capable of forming germ-tubes. Suppression was considerably greater (at all concentrations of glucose tested, except 400 mM) when the cells were induced by LAA or proline than when they were induced by GlcNAc. While germ-tube formation induced by LAA was suppressed approximately 67% and 84% by 5 mM- and 200 mM-glucose respectively at pH 3.7, it was only suppressed 18% and 76% respectively when induced by GlcNAc. Increasing the pH to 5.0 required greater amounts of glucose to obtain suppression than at pH 3.7 (data not shown).

Appearance of cells grown at low pH in the presence of glucose

Yeast cells grown in non-germ-tube-inducing conditions (i.e. on SDA or in LM at room temperature) generally formed single buds, which separated from the mother cell before another bud was formed. Occasionally two buds could be seen attached to the mother cell. In contrast, cells grown in glucose at low pH tended to grow as pseudohyphae or as chains of yeast cells with multiple buds, which did not detach from the mother cell. As many as 12 cells could be seen attached together by the end of the 4 h period in which they were observed (Fig. 4). The number of cells forming multiple buds was also dependent on the pH and the concentration of glucose (Figs 2 and 3). The lower the pH and the higher the concentration of glucose, the more cells formed multiple buds. At pH 3.7, approximately 60% of the cells induced by LAA formed multiple buds when exposed to 200 mM-glucose, while only 15% of those exposed to 5 mM-glucose did so (Fig. 3). At pH 5.0, only 25% of the cells exposed to 200 mM-glucose formed multiple buds (Fig. 2). Cells induced by GlcNAc formed fewer multiple buds than those induced by either LAA or proline. Only 39% of the cells induced by GlcNAc at pH 3.7 in the presence of 200 mM-glucose formed multiple buds (data not shown).

Time of exposure of cells to glucose necessary for suppression of germ-tube formation

Yeast cells were exposed to 200 mM-glucose at various times after induction by proline, to determine whether the cells always retain their susceptibility to suppression by glucose. The cells rapidly lost their ability to have germ-tube formation suppressed by glucose. When glucose was added 10 min after initiation of induction, 48% of the cells subsequently formed germ-tubes, while 73% of the cells did so when glucose was added 30 min after induction. By 45 min, 91% of the cells were committed to hyphal growth and further addition of glucose (up to 120 min) did not cause the hyphal cells to revert to budding during the subsequent 4 h incubation period (data not shown). Furthermore, glucose suppressed germ-tube formation only as long as it remained in the medium. Yeast cells were preincubated for up to 4 h in the salts solution buffered at pH 3.7, containing LAA and 200 mM-glucose, before being washed by filtration. When the washed cells

Fig. 4. Phase-contrast micrographs of *C. albicans* yeast cells (a) grown for 4 h in the presence of LAA and 200 mM-glucose at pH 3.7, and (b) induced to form germ-tubes by LAA in the absence of glucose at pH 3.7 for 4 h. Bars, 10 μ m.

Table 2. *Relief of glucose suppression of germ-tube formation after removal of glucose*

Cells were incubated for the indicated time either in glucose (200 mM) plus salts solution or in LAA containing glucose (200 mM) and salts, washed by filtration and returned to LAA at the indicated pH. Values are the means of two or three independent experiments.

Time glucose removed (min)	At pH . . .	Percentage of cells forming germ-tubes when returned to induction solution after exposure to:			
		200 mM-glucose		200 mM-glucose in LAA	
		3.7	5.8	3.7	5.8
0		92	100	92	100
15		87	93	96	98
30		86	98	90	98
45		82	95	91	98
60		83	96	79	95
90		ND	92	96	93
120		62	68	82	87
150		ND	ND	64	88
180		33	77	66	81
240		4	62	69	73

ND, No data.

were reinoculated into the LAA induction solution, lacking glucose, at either pH 3.7 or 5.8, no less than 70% of the cells still formed germ-tubes. When preincubated for 4 h in the buffered salts and glucose only, 62% of the cells formed germ-tubes when returned to the induction solution buffered at 5.8. Only if these cells were returned to the induction solution buffered at 3.7 was germ-tube formation inhibited. However, even here, if these cells were preincubated for only 2 h, 62% of the cells formed germ-tubes (Table 2). These results may have been affected by budding of the cells in the preincubation solution, and possible cell cycle changes.

Fig. 5. Kinetics of germ-tube formation in the presence and absence of glucose at pH 5.8. *C. albicans* yeast cells were induced to form germ-tubes at pH 5.8 and 37 °C by 4 mM-proline in buffered salts solution in the presence (●) or absence (○) of 200 mM-glucose. The average length of 50 germ-tubes formed at each interval is indicated in the inset. Values are the result of a representative experiment. Bars represent SD.

Table 3. *Suppression of germ-tube formation by other sugars*

Cells were induced with LAA, to which 200 mM (except where indicated otherwise) of the compounds listed was added. The values are the means $\pm$ SD of three or more independent experiments. The following were inhibitory either to growth or to germ-tube formation at both pH values: 2-deoxyglucose (1 mM), 2-deoxygalactose (10 mM), galactono-1,4-lactone, glucono-1,5-lactone, glucuronic acid and L-sorbose. The following had no effect on germ-tube formation at either pH value: glyceric acid, glycerol, erythritol, ribose, L-arabinose, D-arabinose, dulcitol, sorbitol, α -methylglucose, gulono-1,4-lactone, mannono-1,4-lactone, glucose 1-phosphate, glucosaminic acid, 6-phosphogluconic acid, glucoheptonic acid, mannoheptulose, cellobiose, lactose, melibiose, sucrose, trehalose, melezitose and raffinose.

Compound	At pH . . .	Percentage of cells forming germ-tubes	
		3.7	5.8
Galactose		27.3 $\pm$ 12.9	90.0 $\pm$ 9.6
Mannose		10.0 $\pm$ 1.7	97.0 $\pm$ 2.6
Fructose		22.0 $\pm$ 5.6	97.7 $\pm$ 2.5
Xylose		9.3 $\pm$ 5.5	79.3 $\pm$ 6.8
Gluconic acid		7.3 $\pm$ 4.5	91.3 $\pm$ 7.5
Glucosamine		17.3 $\pm$ 3.2	7.8 $\pm$ 4.3
3-O-Methylglucose		5.6 $\pm$ 3.4	94.6 $\pm$ 3.4
L-Fucose (80 mM)		82.7 $\pm$ 3.2	93.0 $\pm$ 2.0
L-Rhamnose		94.6 $\pm$ 4.7	93.3 $\pm$ 4.9

Suppression of germ-tube formation by other sugars and related compounds

Galactose, mannose, fructose, xylose and gluconic acid also suppressed germ-tube formation at pH values below 5.8, but not above that value. Glucosamine inhibited germ-tube formation even when the pH value was greater than 5.8 if its concentration was above 20 mM. However, at a concentration of 4 mM, glucosamine induced about 60% of the cells to form germ-tubes (data not shown). The non-metabolizable glucose analogue 3-O-methylglucose also suppressed germ-tube formation at pH 3.7. However, L-fucose, and L-rhamnose, which are also thought not to be metabolized by the cell, did not affect germ-tube formation (Table 3).

Suppression by glucose of other growth parameters at pH 5.8

Although glucose was shown not to affect germ-tube formation at pH values above 5.8, we wished to determine whether it had any suppressive effects on growth at the higher pH values. We examined the kinetics of germ-tube formation and germ-tube length in the presence and absence of glucose at pH 5.8 (Fig. 5). In both instances, glucose was stimulatory. In the presence

of glucose, germ-tube formation could be observed approximately 15 min earlier and the length of germ-tube after 4 h was more than twice that of those formed in the absence of glucose (35 μ m versus 14 μ m).

Effect of cAMP on suppression of germ-tube formation

Since the effect of glucose appeared to be similar to that of catabolite repression in bacteria, we examined whether cAMP or theophylline had any effect on the suppression of germ-tube formation. Neither dibutyryl cAMP (4 mM), nor theophylline (5 mM), had any effect on germ-tube formation in the presence or absence of 200 mM-glucose (data not shown).

DISCUSSION

Both glucose and pH have been reported individually to influence the ability of *C. albicans* to form germ-tubes. To our knowledge, this is the first report which demonstrates a relationship between the effects of pH and glucose.

The effect of glucose on germ-tube formation in *C. albicans* has been a matter of dispute. Nickerson & Mankowski (1953) first reported that glucose inhibited filamentation in *C. albicans*. Widra (1964) reported that cells grown on neopeptone agar containing potassium phosphate, but lacking glucose, formed mycelia, while those grown on the same agar containing 0.25% glucose grew in the yeast-phase. Dabrowa *et al.* (1976) found that glucose concentrations as low as 1 mM suppressed germ-tube formation induced by proline at pH 7.0. Odds *et al.* (1978) reported that while peptones containing 0.2% glucose were equally effective in inducing germ-tubes, Mycophil broth, which contained a preformulated glucose concentration of 4%, did not support germ-tube production above the control levels. Hrmová & Drobnica (1981) observed that exposure to an initial glucose concentration greater than 10 mM resulted in suppression of germ-tube formation at 28 °C, pH 6.5, in cells induced by glucose plus $(\text{NH}_4)_2\text{SO}_4$. Nolting *et al.* (1982) also reported that glucose concentrations above 1 mg ml⁻¹ suppressed germ-tube formation. Conversely, Nishioka & Silva-Hutner (1974) found that 2% glucose did not suppress germ-tubes induced by glutamate, while Evans *et al.* (1975*b*) found that even 20% glucose had no effect on germ-tube formation induced by peptone at pH 7.4. Barlow *et al.* (1974) also reported that glucose (10 mM) was required to maintain filamentation induced by serum, and Land *et al.* (1975) reported that 1 M-glucose was necessary to obtain moderate filamentation in the presence of NH_4Cl .

Similarly, differences have been reported as to the effect of pH on germ-tube formation. Most workers have reported a pH optimum for germ-tube formation which varies from approximately 6.6 to 7.4 (Evans *et al.*, 1975*b*; Simonetti *et al.*, 1974; Buffo *et al.*, 1984). There are only a few reports of germ-tube formation occurring below pH 6.0 (McClary, 1952; Shepherd & Sullivan, 1976), and only one previous report of germ-tube formation occurring as low as pH 3.0 (Pollack & Hashimoto, 1985). This is despite the fact that *C. albicans* is known to grow at very low pH values (Odds & Abbott, 1980) and that the pH of the human vagina, a common site for *Candida* infections, is between 4 and 5 (Riley, 1986).

The results presented in this paper suggest that the dearth of reports showing the ability of *C. albicans* to form germ-tubes at low pH values may, in part, be due to the presence of glucose in the induction solutions used by many groups. Unlike the solutions used here, many induction solutions are poorly buffered, so that even if the initial pH would have allowed germ-tube formation to occur in the presence of glucose, a quick drop in pH could suppress germ-tube formation. In fact, just increasing the pH of a non-inducing solution, such as SDB, to 7.5 was sufficient to allow germ-tube formation to occur (Evans *et al.*, 1974). We also found that buffering SDB at pH 5.8 resulted in germ-tube formation (data not shown). This is not to say that other factors do not affect the ability of *C. albicans* to form germ-tubes at non-optimal pH values. Shepherd *et al.* (1980) showed that the buffer used may also affect the ability of the cells to form germ-tubes. We have found that the concentration of NaCl created by adjusting the pH of some buffers such as Tris/maleate with NaOH may also inhibit germ-tube formation under certain conditions. Thus, we found that few germ-tubes were formed above pH 7.5 when cells were

induced by a low concentration of proline or GlcNAc (0.08 mM) if Tris/maleate was used as the buffer, but almost all cells produced germ-tubes if HEPES or TAPS were used (data not shown). Others have also suggested that inorganic phosphate affects the ability of *C. albicans* to form germ-tubes (Widra, 1964; Dabrowa *et al.*, 1975; Odds *et al.*, 1978).

The results presented here do not agree with the recently proposed pH regulation of germ-tube formation in *C. albicans* (Mitchell & Soll, 1979; Buffo *et al.*, 1984). The proposal of Soll and co-workers is based on the assumption that germ-tube or bud formation can be controlled solely by regulating the pH of the inducing solution, similar to the more common temperature or nutritional regulation of germ-tube formation. Their proposal is tempered by the statement that there are variant strains which are capable of forming germ-tubes at pH values below 4.0, and that their transition points are 'rigidly defined only for the environmental conditions and growth history of the cells' induced under their experimental regimen (Buffo *et al.*, 1984). However, we were able to reproduce their results with different strains and under different growth conditions. Since, by only removing glucose from several induction media, we were able to obtain germ-tubes at pH values as low as 3.0, we believe that pH cannot be considered a sole determinant of germ-tube formation. At most, pH can be considered an 'indirect' regulator or coregulator of dimorphism in *C. albicans*.

When grown in the presence of glucose at low pH values, the budding cells, also, apparently did not separate, which resulted in the formation of large clusters of multiple-budded cells or pseudohyphae. Similar modes of growth have been reported previously in response to glucose at lower temperatures (Soll & Bedell, 1978; Dujardin *et al.*, 1980). It might be useful to use this phenomenon to distinguish between growing and non-growing cells at low pH or low temperatures.

The results presented here differ from those of others also with respect to commitment to germ-tube formation. We found that by 45 min germ-tube formation could no longer be suppressed by the addition of glucose, while in most situations tested, removal of glucose resulted in cessation of suppression. It would seem, therefore, that *C. albicans* is committed to hyphal formation very early, but is never committed to the budding form of growth. The time for commitment to germ-tube formation compares favourably with those reported by Evans *et al.* (1975a) and Chaffin & Wheeler (1981). However, those studies and that of Mitchell & Soll (1979) also found that at some point the cells were committed to budding. Besides using different criteria (temperature or pH shift) to determine commitment, it is possible that the previously mentioned experimenters did not allow sufficient time after switching the growth conditions to properly assess the pluripotency of the cells to form germ-tubes. The cells in the present study were incubated for 4 h post-transfer, while cells used by both Mitchell & Soll (1979) and Chaffin & Wheeler (1981) were incubated for a total of only 4 h, including the time before the switch in growth conditions. Furthermore, the previous authors claimed that *C. albicans* yeast cells must be in stationary phase in order for germ-tube formation to proceed. This claim has been questioned by others (Mattia & Cassone, 1979; Ahrens *et al.*, 1983; Soll & Herman, 1983). However, it is possible that the extensive washing that the cells received before their transfer to the new growth conditions was equivalent to the starvation period which Soll & Herman (1983) found sufficient to activate the cells, although it was for a much shorter period (4–5 min).

Suppression by glucose of differentiation in other organisms has been reported. Bartnicki-Garcia (1968) reported that glucose inhibited initiation of filamentation, but not hyphal elongation, in *Mucor rouxii*. Redshaw *et al.* (1976) observed that some *Streptomyces* species were inhibited from forming aerial mycelia in the presence of glucose only when the pH was between 5 and 5.5, but not when the pH value was higher. These reports, and those on glucose repression in *C. albicans*, suggest that the action of glucose is mediated by catabolite repression or inactivation. The data presented here are inconclusive in regard to whether a catabolic product of glucose is mediating the effect since, in addition to the other sugars, a supposedly non-metabolizable analogue of glucose also suppressed germ-tube formation. However, cAMP did not seem to mediate the effect. This was unexpected, since cAMP has been reported to affect dimorphism in *C. albicans* (Niimi *et al.*, 1980; Chattaway *et al.*, 1981). On the other hand, it is not clear what role cAMP plays in catabolite repression in yeasts (Eraso & Gancedo, 1984,

1985). The mode of action of glucose remains to be determined. Some interesting possibilities suggested by others are: increasing the rate of proteolytic degradation of the nutrient inducer transport system (Horák & Říhová, 1982); the suppression of production of some key enzyme such as chitinase (Monreal & Reese, 1969); or the repression of the catabolic pathway for the inducer (Singh & Datta, 1978).

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