Involvement of Adenosine 3':5'-Cyclic Monophosphate in the Germination of Blastospores of *Candida albicans*

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(Received 19 August 1980)

The germination of blastospores of *Candida albicans* is accompanied by a rise in the intracellular concentration of adenosine 3':5'-cyclic monophosphate (cyclic AMP). Germination was induced either by peptides isolated from seminal plasma or by an amino acid mixture, and both germination and the rise in cyclic AMP required a temperature of 37 °C. The rise occurred during the first hour of incubation, but full germination required a temperature of 37 °C for 4 h. Germination and the rise in cyclic AMP, in the presence of suboptimal concentrations of inducers, were stimulated by theophylline. Pre-incubation of cells with dithiothreitol in the absence of inducers inhibited subsequent germination but not the rise in cyclic AMP. Germination and the rise in cyclic AMP were inhibited if dithiothreitol or *N*-succinimidyl-3-(4-hydroxyphenyl)propionate was present during the induction period.

INTRODUCTION

There is evidence for a number of biochemical changes associated with a switch from growth and development by budding of blastospores of *Candida albicans* to germination and growth by the formation of mycelium. Thus, Nickerson & Falcone (1956) related budding development to the activity of a mitochondrial disulphide reductase and suggested that interruption of the normal flow of electrons associated with respiration would give a lowered activity of the enzyme and mycelial development. Land *et al.* (1975*a, b*) showed that filamentation could be induced by proline, histidine or arginine or, in the presence of limiting phosphate, by a high glucose concentration, and that this was accompanied by a brief stimulation of respiration followed by an inhibition which persisted for 2 h and was accompanied by increased activity of the glycolytic pathway. Chattaway *et al.* (1968) found significant changes in the composition of the cell wall during mycelial development, including a threefold increase in the chitin content, changes in the relative proportions of glucan and mannan and changes in the amino acid composition of the protein associated with the alkali-insoluble fraction of the wall. Chattaway *et al.* (1973) showed that changes in enzyme activities occurred during the early stages of germination of blastospores which indicated an increased facility for chitin synthesis from fructose 6-phosphate and a decreased flow of electrons as a result of reduced activity in the hexose monophosphate pathway. Braun & Calderone (1978) found there was a significantly higher activity of chitin synthase in the mycelial form.

There have been numerous claims that environmental factors such as a temperature of 37 °C and the presence of a wide range of chemical agents are required to induce the germination of blastospores, but there is little correlation between their action and the biochemical changes mentioned above. The isolation from seminal plasma of peptides of molecular weights from 2000 to 3000 which will induce germination in low concentration

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(Barlow *et al.*, 1974; Chattaway *et al.*, 1980) and the knowledge that adenosine 3':5'-cyclic monophosphate (cyclic AMP) mediates the effect of external agents, including peptides, on metabolic processes in many organisms suggested a possible role for cyclic AMP in the stimulation of germination in *C. albicans*. Land *et al.* (1975*b*) have shown that cyclic AMP would inhibit respiration of *C. albicans* and induce the germination of 60% blastospores under conditions which normally gave no germination, and Maresca *et al.* (1977) showed that the mycelial form of *Histoplasma capsulatum* contained five times the cyclic AMP concentration of the yeast form. In *Mucor racemosus* growing in air, dibutyryl cyclic AMP induced the development of the Y form, and this form growing in 100% CO₂ had a high cyclic AMP content (Larsen & Sypherd, 1974). However, later work, using variations in the flow rate of N₂ through cultures to produce yeast or hyphal forms (Paznokas & Sypherd, 1975), indicated that the cyclic AMP concentration was approximately equal in the two forms. The present paper presents evidence for a temperature-dependent early rise in cyclic AMP content during germination of blastospores of *C. albicans* and for a correlation between cyclic AMP concentration and morphological form. A preliminary account of some of this work was given in Chattaway *et al.* (1979), and Niimi *et al.* (1980) have independently reported a similar correlation.

METHODS

Organisms. *Candida albicans* strain CA2 was used in all experiments unless otherwise stated. Strains 1324 and 1334 were fresh isolates supplied by E. G. V. Evans.

Growth media. For short-term experiments, cells were grown on Sabouraud glucose agar for 18 h at 30 °C and, after harvesting, were washed three times in distilled water. Neopeptone medium B of Chattaway *et al.* (1968) was used for growth experiments.

Germ-tube production. This was carried out as described by Chattaway *et al.* (1980) except that Mn²⁺ (14 µM) was included in all tests. Germ-tube inducing materials used were peptide fractions prepared from bovine seminal plasma [fractions D1 + D2 of Barlow *et al.* (1974); fractions D/1/3/3 + D/2/2/3 of Chattaway *et al.* (1980)], preparations of bovine and human serum albumin, and the minimal amino acid mixture (MAAM) of Chattaway *et al.* (1976). Results of tests for germination are expressed as a percentage of the germination induced by fractions D1 + D2; the absolute germination induced by these fractions was always >90%.

Preparation of spheroplasts. Cells (500 mg) grown on Sabouraud glucose agar were suspended in 20 ml 0.8 M-mannitol, 12 mM-dithiothreitol, 0.24 % Cytophaga L1 enzyme (BDH), 0.05 M-Tris/HCl buffer pH 7.4 and incubated for 3 h at 37 °C in an atmosphere of N₂.

Cyclic AMP assay. Washed blastospores (10⁶ ml⁻¹) were incubated in the medium used for germ-tube production (33 ml in 100 ml flask). After the required period, they were centrifuged, resuspended in 0.01 M-Tris/HCl buffer pH 7.4 (1 ml) containing isobutylmethylxanthine (1 mM) and heated in a boiling water bath for 3 min. After cooling to 2 °C they were passed four times through a French press. Spheroplasts were prepared as above but with 0.7 M-MgSO₄ as the osmotic stabilizer. After centrifuging, they were treated as for blastospores except that passage through the French press was omitted since after 12 min at 2 °C they were completely lysed. The disrupted cell preparations were centrifuged at 800 g and the supernatants were freeze-dried and resuspended in 80 µl distilled water. Cyclic AMP in the supernatants was assayed by the method of Gilman & Mourad (1974) using a receptor protein prepared from fresh beef adrenal cortices according to Walton & Garren (1970); the results were expressed as pmol (mg dry wt cells)⁻¹.

Labelling of cells with ¹²⁵I. Bolton & Hunter reagent (Bolton & Hunter, 1973) was obtained from The Radiochemical Centre, Amersham. Benzene was removed from a sample containing 100 µCi ¹²⁵I by a stream of N₂. Cells (1 mg dry wt) were washed three times by centrifugation in distilled water, resuspended in 0.1 M-borate buffer pH 8.5 (5 ml) and added to the dry reagent. The mixture was incubated for 10 min at 37 °C with occasional mixing, then diluted to 35 ml with distilled water, centrifuged and washed repeatedly to remove residual radioactivity. Radioactivity of cells and supernatants was determined. In some experiments the unlabelled reagent [i.e. *N*-succinimidyl-3-(4-hydroxyphenyl)propionate] was also added.

RESULTS

Changes in cyclic AMP concentration of blastospores during germination

The concentration of cyclic AMP was determined in blastospores incubated at 37 °C or 30 °C for 4 h in the presence or absence of materials which induce germination (Table 1). When 72% or more of the cells were germinating the cyclic AMP concentration was 2.5

Table 1. *Effect of germination and temperature on cyclic AMP concentrations of blastospores of C. albicans*

Germ-tube tests were carried out as described in Methods with fractions D1 + D2 at 16 µg peptide ml⁻¹, fractions D1/3/3 + D2/2/3 at 4.9 µg peptide ml⁻¹, minimal amino acid mixture (MAAM) at 35.5 µg ml⁻¹, and serum albumin preparations at 10 mg ml⁻¹. Germ-tube development is expressed as a percentage of the development with fractions D1 + D2 at 37 °C.

Temp. (°C)	Germ-tube inducing material	Germ-tube formation at 4 h (%)	Cyclic AMP [pmol (mg dry wt) ⁻¹]
37	None	0	7.2
37	D1 + D2	100	18.0
37	D1/3/3 + D2/2/3	90	17.0
37	MAAM	85	18.0
37	Serum albumin, bovine – essentially fatty acid free (Sigma)	50	11.0
37	Serum albumin, human – Cohn Fraction V (Sigma)	20	7.0
37	Serum albumin, human – Cohn Fraction V (Calbiochem)	70	11.5
30	None	0	7.5
30	D1 + D2	0	7.0
30	MAAM	0	8.0

Table 2. *Effect on germination and cyclic AMP concentration of the pre-incubation of blastospores with germ-tube inducing materials*

Cells were pre-incubated in glucose (0.2%, w/v)/phosphate (10 mM) buffer pH 7.4 with fractions D1 + D2 (16 µg peptide ml⁻¹) and Mn²⁺ (14 µM), and then washed three times followed by incubation in glucose/phosphate buffer alone. Germ-tube development is expressed as a percentage of the development with fractions D1 + D2 in the standard germ-tube test at 37 °C.

Pre-incubation		Incubation		Germ-tube formation at 4 h (%)	Cyclic AMP [pmol (mg dry wt) ⁻¹]	
Temp. (°C)	Time (h)	Temp. (°C)	Time (h)		1 h	4 h
37	1	37	3	92	13	17
37	1	30	3	29	13	12
30	1	37	3	17	7	8
30	1	30	3	3	7	7
37	Standard germ-tube test			100	ND	18
30	Standard germ-tube test			0	ND	8

ND, Not determined.

times that found when no germination occurred. The serum albumin preparations induced only 20 to 70% germination and the cyclic AMP concentration was correspondingly lower. Blastospores incubated at 30 °C in the presence of materials which induce germination at 37 °C but not at 30 °C showed no increase in cyclic AMP. In 17 such experiments where germ-tube production was <5%, the mean cyclic AMP concentration was 7.6 pmol (mg dry wt)⁻¹ (s.d. 2.54), and in 19 experiments where germ-tube production was >70%, the mean cyclic AMP concentration was 17.6 pmol (mg dry wt)⁻¹ (s.d. 5.98). The difference between these mean values was highly significant ($t = 6.09$, $P = 2 \times 10^{-4}$).

To examine the effect of varying the temperature and availability of germ-tube inducing material during the 4 h incubation period, cells were pre-incubated in the presence of fractions D1 + D2 and Mn²⁺ for 1 h at 37 °C or 30 °C, then washed and resuspended in glucose/phosphate buffer and incubated for a further 3 h at either 37 °C or 30 °C. Cyclic AMP concentration was determined in samples taken at 1 h and 4 h and germ-tube production was determined at 4 h (Table 2). The results show that the rise in cyclic AMP

Table 3. *Effect of theophylline on cell cyclic AMP concentration and germination in three strains of C. albicans*

Germ-tube tests were carried out as described in Methods; germ-tube production is expressed as a percentage of that given by optimal amounts of fractions D1 + D2 with strain CA2.

Strain	Germ-tube inducing material ($\mu\text{g ml}^{-1}$)	Theophylline (5 mM)	Germ-tube formation at 4 h (%)	Cyclic AMP [$\mu\text{mol (mg dry wt)}^{-1}$]
CA2	D1 + D2 (16)	—	100	18
	D1 + D2 (3.2)	—	40	10
	D1 + D2 (3.2)	+	85	17
	None —	—	0	7.2
	None —	+	15	11
	MAAM (35.5)	+	92	24
	MAAM (3.6)	—	30	10
	MAAM (3.6)	+	80	15
1324	D1 + D2 (16)	—	95	24
	MAAM (70)	—	90	ND
	MAAM (35.5)	—	55	17
	MAAM (35.5)	+	78	18
	None —	—	0	7
1334	D1 + D2 (48)	—	95	ND
	D1 + D2 (16)	—	25	14
	D1 + D2 (16)	+	58	16
	MAAM (70)	—	94	ND
	MAAM (35.5)	—	34	13
	MAAM (35.5)	+	72	16
	None —	—	0	10

ND, Not determined.

concentration occurred mainly during the first hour and required a temperature of 37 °C; for full germination to be achieved the temperature had to be maintained at 37 °C for the rest of the 4 h period, during which there was a further slight increase in cyclic AMP concentration. When the temperature was decreased to 30 °C after 1 h at 37 °C, only 30% germination occurred although the cyclic AMP concentration remained constant.

Effect of various reagents on cyclic AMP concentration and germination of blastospores

Theophylline. The measured cell concentration of cyclic AMP is the net result of its synthesis through the action of adenylate cyclase and its excretion or hydrolysis by phosphodiesterase. Theophylline is known to inhibit the latter enzyme in a number of species, although Gunasekaran *et al.* (1976) found no inhibition of the enzyme isolated from *C. albicans*. Blastospores were exposed to suboptimal concentrations of germ-tube inducing materials in the presence or absence of theophylline at 37 °C, and the extent of germination and cyclic AMP concentration after 4 h incubation were compared with those found with the minimum concentration of germ-tube inducing material required for full germination and with no germ-tube inducing material present. The results are shown in Table 3 for three strains using the bovine seminal peptides and MAAM for germ-tube induction. Using strain CA2, theophylline, in the presence of suboptimal concentrations of germ-tube inducing material, gave near maximal germ-tube development and the concentration of cyclic AMP increased to that found with optimal amounts of seminal peptide. With theophylline alone there was a small increase in germination and in cyclic AMP concentration. In similar experiments at 30 °C no germination occurred and there was no increase in cyclic AMP concentration. Using concentrations of germ-tube inducers which gave maximum induction in strain CA2, strain 1324 gave near maximal germination with fractions D1 + D2 but only 55% with MAAM; the latter was increased to 78% on the inclusion of theophylline, with a small

accompanying increase in cyclic AMP concentration. Strain 1334 gave only a low percentage of germination with both germ-tube inducers at the optimal concentration for strain CA2; this value was doubled in the presence of theophylline, again with a small increase in cyclic AMP concentration. The extent of germination could be increased in both these strains by increased concentrations of germ-tube inducers; the effect of addition of theophylline on cyclic AMP concentration was not so marked as in strain CA2, the concentration in the presence of germ-tube inducer alone being higher than that found in strain CA2. Isobutylmethylxanthine (1 mM) produced similar but less marked effects to those of theophylline in experiments with strain CA2.

The above results indicate that theophylline may be inhibiting phosphodiesterase and thereby giving an increased cyclic AMP concentration which, in turn, affects germination. Preliminary examination of the effect of theophylline on phosphodiesterase in a cytoplasmic extract of blastospores (unpublished) has confirmed that such inhibition can occur.

Dibutyryl cyclic AMP. The cells of some organisms will give a physiological response to externally supplied dibutyryl cyclic AMP when cyclic AMP itself elicits no or very low response. *Candida albicans* strain CA2 gave no response to cyclic AMP under such conditions but dibutyryl cyclic AMP (2 mM) gave 20% germination in the absence of germ-tube inducers and increased the response to suboptimal concentrations of seminal peptides from 50 to 65% germination. A similar response was obtained with strain 1334, although germination rates were lower. The fact that full germination was not found with this relatively high concentration probably indicates a low uptake by the organisms.

Prostaglandins. Prostaglandins would be expected to be present in seminal plasma, although at room temperature they would probably be labile in the separation stages used to prepare D/1/3/3 and D/2/2/3 (Chattaway *et al.*, 1980). However, in view of the relationship between prostaglandins and cyclic AMP in mammalian tissues (Samuelsson *et al.*, 1978), an ether extract of an aqueous solution of D/1/3/3 and D/2/2/3 combined in a suitable proportion for maximum germination was prepared by the method of Hamberg & Samuelsson (1966): the ethereal phase, which would contain prostaglandins, contained no germ-tube inducing activity, but full germination was recovered in the aqueous phase. Addition of prostaglandin E (Sigma) to germ-tube tests at 0.05 to 50 $\mu\text{g ml}^{-1}$ had no effect on the level of germination in standard germ-tube tests with D/1/3/3 + D/2/2/3 at 0, 1.5 or 4.9 $\mu\text{g ml}^{-1}$.

Dithiothreitol. Inclusion of dithiothreitol (2.4 mM) in a standard germ-tube test resulted in complete inhibition of germ-tube production and the cyclic AMP concentration [9 pmol (mg dry wt) $^{-1}$] was similar to that normally found in blastospores. Pre-incubation of cells in dithiothreitol and then subjecting the washed cells to a normal germ-tube test resulted in some suppression of germ-tube development but the cyclic AMP concentration was that found in a cell suspension of fully germinating cells (Table 4). In addition, the preparation of spheroplasts by the combined action of dithiothreitol and Cytophaga L1 enzyme and incubation of the washed spheroplasts with germ-tube inducing preparations resulted in stimulation of cyclic AMP production although no germination was seen in such a preparation (Table 4). Thus, dithiothreitol treatment can inhibit germ-tube development at a stage subsequent to the increase in cyclic AMP concentration, although if the reagent is actually present during the germ-tube test then this increase is also inhibited. The inhibition of germination by dithiothreitol is not the result of an inability of treated cells to grow: growth at 37 °C after 18 h pre-incubation with dithiothreitol or growth in the presence of dithiothreitol is 87% and 96%, respectively, of that of untreated cells (Table 5).

Bolton & Hunter reagent. This reagent [0.029 $\mu\text{g (mg dry wt cells)}^{-1}$] was used to react with amino groups present in blastospores. Uptake of radioactivity could readily be shown in cells washed free from adhering reagent. These cells showed full germination. The radioactivity was partially released (20%) by incubation in Tris/mannitol buffer, with or without dithiothreitol, and 90% was released under conditions leading to the development of

Table 4. *Effect of dithiothreitol on germination and cyclic AMP concentration of blastospores*

Germ-tube tests and spheroplast production were done as described in Methods. Fractions D1 + D2 were used at $16 \mu\text{g peptide ml}^{-1}$ and MAAM at $35.5 \mu\text{g ml}^{-1}$. Cells and spheroplasts after treatment were washed three times by centrifugation in distilled water or 0.7 M-MgSO_4 , respectively. Incubation of cells with 0.7 M-MgSO_4 in a germ-tube test did not stimulate an increase in cyclic AMP concentration.

Treatment	Germ-tube inducing material					
	None		Fractions D1 + D2		MAAM	
	Cyclic AMP [pmol (mg dry wt) $^{-1}$]	Germ-tube formation at 4 h (%)	Cyclic AMP [pmol (mg dry wt) $^{-1}$]	Germ-tube formation at 4 h (%)	Cyclic AMP [pmol (mg dry wt) $^{-1}$]	Germ-tube formation at 4 h (%)
None	8	2	16	100	—	—
Dithiothreitol (2.4 mM), 3 h	7	4	22	41	—	—
Dithiothreitol (2.4 mM), 24 h	10	2	21	10	—	—
Spheroplast preparation	15	0	29	0	25	0

Table 5. *Growth of cells treated with reagents which inhibit germination*

Cells were treated with dithiothreitol [10^8 cells in 20 ml 0.8 M-mannitol , 50 mM-Tris/HCl buffer pH 7.4 and $2.4 \text{ mM-dithiothreitol}$ were incubated at 37°C for 24 h] or *N*-succinimidyl-3-(4-hydroxy-phenyl)propionate [10^8 cells in 10 ml 50 mM-phosphate buffer pH 8.5 were added to $15 \text{ mg dry reagent}$; after 10 min at 37°C with intermittent mixing, the precipitate was allowed to settle and the cell suspension was removed with a Pasteur pipette]. Treated cells were washed three times in buffer. Cells (0.5 mg dry wt) were then suspended in $50 \text{ ml neopeptone medium}$ and incubated for 18 h at 37°C .

Cell treatment	Increase in cell dry wt (%)	Germ-tube formation at 18 h (%)
None	1230	25
None; 2.4 mM-dithiothreitol present in growth medium	1180	0
Dithiothreitol	1070	0.8
<i>N</i> -Succinimidyl-3-(4-hydroxy- phenyl)propionate	980	1.0

spheroplasts. Treatment as above could be carried out with the addition of unlabelled reagent to give higher concentrations. At $56 \mu\text{g (mg dry wt cells)}^{-1}$ full germination still occurred, but with $\geq 560 \mu\text{g (mg dry wt cells)}^{-1}$ germination was completely inhibited and such cells showed no rise in cyclic AMP concentration in a standard germ-tube test whether seminal peptides or amino acids were used as stimulants for germination; they also showed 90% release of radioactivity when protoplasts were prepared. It is concluded that the reagent reacts primarily with cell wall components. Cells treated with the unlabelled reagent showed 80% of the growth of untreated cells when incubated at 37°C for 18 h in growth medium (Table 5).

DISCUSSION

There is general agreement that germination of blastospores of *C. albicans* requires a temperature of 37°C . Soll & Bedell (1978) have shown that this temperature alone is sufficient for the germination of stationary phase cells. Mattia & Cassone (1979) have shown that cells at other stages of the cell cycle can germinate if inducers are present in addition to a

temperature of 37 °C and have drawn attention to the need to elucidate the early changes in metabolism which regulate the morphological development. Evans *et al.* (1975) demonstrated that cells incubated under germ-tube inducing conditions become committed to germination early in the incubation period. Soll & Bedell (1978) and Mattia & Cassone (1979) found that extensive germination occurred within 1 h of incubation under germ-tube inducing conditions. The present paper shows a highly significant rise in cell cyclic AMP concentration when germination occurs. This rise occurs within 1 h and is dependent upon an incubation temperature of 37 °C. Full germination can be achieved even if the cells are removed from the inducer after 1 h provided that the temperature is maintained at 37 °C (Chattaway *et al.*, 1980), which suggests that there are temperature-dependent changes during germination in addition to the increase in cyclic AMP concentration. Dibutyryl cyclic AMP will induce a moderate degree of germination, its effect probably being limited by inadequate penetration (cyclic AMP supplied externally does not induce germination). By analogy with other organisms the cyclic AMP concentration will be regulated by excretion from the cells and through the action of adenylate cyclase and phosphodiesterase. Theophylline, a known inhibitor of the latter enzyme, enhances the germination of cells incubated with suboptimal concentrations of inducers and these cells show an enhanced cyclic AMP concentration. There is, therefore, appreciable evidence to support a correlation between cyclic AMP concentration and germ-tube development.

It is well established that the presence of thiol compounds such as dithiothreitol inhibits germination and it has now been shown that in such circumstances the cyclic AMP concentration remains at a low level. However, pre-treatment of cells with dithiothreitol and then exposure of the washed cells to inducers results in a marked inhibition of germination but a cyclic AMP concentration at or above that found in normal germinating cells. A similar result is seen with spheroplasts prepared by the action of dithiothreitol and Cytophaga L1 enzyme and then incubated with inducers, where no normal germination occurs. Thus dithiothreitol is inhibiting a reaction(s) subsequent to the production of cyclic AMP in the changes leading to germination.

On the other hand, *N*-succinimidyl-3-(4-hydroxyphenyl)propionate has been shown to inhibit germination and the increase in cyclic AMP concentration, and evidence is presented that it exerts its action by reacting with free amino groups of cell wall components. This reagent and dithiothreitol show some inhibition of growth but their inhibitory effect on germination is appreciably greater and thus they appear to be specifically affecting reactions concerned with germination.

Thus germination of blastospores can be effected by peptide or amino acid inducers and is accompanied by an increase in cyclic AMP; whether the latter is effected by the activation of adenylate cyclase through association of inducers with cell surface receptors remains to be determined. Work is in progress to study the properties and cell distribution of this enzyme and phosphodiesterase.

We wish to thank the Medical Research Council for a grant to P. R. Wheeler, and Dr A. J. Turner and Miss Jane Stockton for a gift of bovine cyclic AMP binding protein.

REFERENCES

- BARLOW, A. J. E., ALDERSLEY, T. & CHATTAWAY, F. W. (1974). Factors present in serum and seminal plasma which promote germ-tube formation and mycelial growth of *Candida albicans*. *Journal of General Microbiology* **82**, 261–272.
- BOLTON, A. E. & HUNTER, W. M. (1973). The labelling of proteins to high specific radioactivities by conjugation to a ¹²⁵I-containing acylating agent. *Biochemical Journal* **133**, 529–539.
- BRAUN, P. C. & CALDERONE, R. A. (1978). Chitin synthesis in *Candida albicans*: comparison of yeast and hyphal forms. *Journal of Bacteriology* **133**, 1472–1477.
- CHATTAWAY, F. W., HOLMES, M. R. & BARLOW, A. J. E. (1968). Cell wall composition of the mycelial and blastospore forms of *Candida albicans*. *Journal of General Microbiology* **51**, 367–376.
- CHATTAWAY, F. W., BISHOP, R., HOLMES, M. R.,

- ODDS, F. C. & BARLOW, A. J. E. (1973). Enzyme activities associated with carbohydrate synthesis and breakdown in the yeast and mycelial forms of *Candida albicans*. *Journal of General Microbiology* **75**, 97–109.
- CHATTAWAY, F. W., O'REILLY, J., BARLOW, A. J. E. & ALDERSLEY, T. (1976). Induction of the mycelial form of *Candida albicans* by hydrolysates of peptides from seminal plasma. *Journal of General Microbiology* **96**, 317–322.
- CHATTAWAY, F. W., O'REILLY, J., WHEELER, P. & BARLOW, A. J. E. (1979). *Abstracts of VIIth Congress of the International Society for Human and Animal Mycology*, Israel, p. 7.
- CHATTAWAY, F. W., WHEELER, P. R. & O'REILLY, J. (1980). Purification and properties of peptides which induce germination of blastospores of *Candida albicans*. *Journal of General Microbiology* **120**, 431–437.
- EVANS, E. G. V., ODDS, F. C. & HOLLAND, K. T. (1975). Resistance of the *Candida albicans* filamentous cycle to environmental change. *Sabouraudia* **13**, 231–238.
- GILMAN, A. G. & MOURAD, F. (1974). Assay of cyclic nucleotides by receptor protein binding displacement. *Methods in Enzymology* **38**, 49–57.
- GUNASEKARAN, M., HUGHES, W. T. & MAGUIRE, W. J. (1976). Cyclic 3',5'-nucleotide phosphodiesterase of *Candida albicans*. *Microbios Letters* **3**, 47–53.
- HAMBERG, M. & SAMUELSSON, B. (1966). Prostaglandins in human seminal plasma. Prostaglandins and related factors 46. *Journal of Biological Chemistry* **241**, 257–263.
- LAND, G. A., McDONALD, W. C., STJERNHOLM, R. L. & FRIEDMAN, L. (1975*a*). Factors affecting filamentation in *Candida albicans*. Relationship of the uptake and distribution of proline to morphogenesis. *Infection and Immunity* **11**, 1014–1023.
- LAND, G. A., McDONALD, W. C., STJERNHOLM, R. L. & FRIEDMAN, L. (1975*b*). Factors affecting filamentation in *Candida albicans*. Changes in respiratory activity of *Candida albicans* during filamentation. *Infection and Immunity* **12**, 119–127.
- LARSEN, A. & SYPHERD, P. S. (1974). Cyclic adenosine 3',5'-monophosphate and morphogenesis in *Mucor racemosus*. *Journal of Bacteriology* **117**, 432–438.
- MARESCA, B., MEDOFF, G., SCHLESSINGER, D. & KOBAYASHI, G. S. (1977). Regulation of dimorphism in the pathogenic fungus *Histoplasma capsulatum*. *Nature, London* **266**, 447–448.
- MATTIA, E. & CASSONE, A. (1979). Inducibility of germ-tube formation in *Candida albicans* at different phases of yeast growth. *Journal of General Microbiology* **113**, 439–442.
- NICKERSON, W. J. & FALCONE, G. (1956). Identification of protein disulphide reductase as a cellular division enzyme in yeasts. *Science* **124**, 722.
- NIIMI, M., NIIMI, K., TOKUNAGA, J. & NAKAYAMA, H. (1980). Changes in cyclic nucleotide levels and dimorphic transitions in *Candida albicans*. *Journal of Bacteriology* **142**, 1010–1014.
- PAZNOKAS, J. L. & SYPHERD, P. S. (1975). Respiratory capacity, cyclic adenosine 3',5'-monophosphate and morphogenesis of *Mucor racemosus*. *Journal of Bacteriology* **124**, 134–139.
- SAMUELSSON, B., GOLDYNE, M., GRANSTRÖM, E., HAMBERG, M., HAMMERSTRÖM, S. & MALMSTEN, C. (1978). Prostaglandins and thromboxanes. *Annual Review of Biochemistry* **47**, 997–1029.
- SOLL, D. R. & BEDELL, G. W. (1978). Bud formation and the inducibility of pseudo-mycelium outgrowth during release from stationary phase in *Candida albicans*. *Journal of General Microbiology* **108**, 173–180.
- WALTON, G. M. & GARREN, L. D. (1970). An assay for adenosine 3'-5'-cyclic monophosphate based on the association of the nucleotide with partially purified binding protein. *Biochemistry* **9**, 4223–4229.