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Effect of nucleosides and nucleotides and the relationship between cellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and germ tube formation in *Candida albicans*

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Abstract

A yeast-mycelium (Y-M) transition in *Candida albicans* was induced by exogenous yeast extract, adenosine, adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), adenosine 3′:5′ cyclic monophosphate (cAMP) and its analogue N^6, $O^{2'}$-dibutyryl adenosine 3′:5′-cyclic monophosphate (dbcAMP) in defined liquid medium at 25 °C. Adenosine 5′-triphosphate (ATP) was found to delay germ tube formation in yeast cells, whereas the cAMP phosphodiesterase inhibitors, theophylline and caffeine, induced a Y-M transition. Intracellular and extracellular cyclic AMP levels increased during the yeast-mycelium transition and maximum levels of intracellular cyclic AMP coincided with maximum germ tube formation. Of the many inducers and inhibitors of germ tube and mycelium formation in *C. albicans* tested, including incubation at 37 °C or in the presence of 1.5 mM $CaCl_2$, the calmodulin inhibitor calmidazolium (R24571) added together with $CaCl_2$ induced the highest intra- and extracellular cyclic AMP levels. These results confirm the involvement of cyclic AMP in the yeast-mycelium transition of *C. albicans*.

Introduction

Adenosine 3′:5′-cyclic monophosphate (cyclic AMP, cAMP) is implicated in the differentiation of many fungi [1] and dimorphic behaviour has often been linked to intracellular levels of this cyclic nucleotide [2]. There are several examples where an increase in intracellular cAMP accompanies germ tube and subsequent mycelium formation from yeast-like cells in di- and polymorphic organisms, e.g. *Histoplasma capsulatum* [3], *Aureobasidium pullulans* [4], and *Ophiostoma ulmi* [5] or from germinating spores, e.g. those of *Mucor rouxii* and *Phycomyces blakesleeanus* [6–8]. In fungi, as in other eukaryotic cell systems, an increase in cellular cAMP triggers a protein phosphorylation cascade via the action of cAMP-dependent protein kinase(s) [9, 10]. However, although this area of research is now receiving detailed attention, a precise biochemical model of fungal dimorphism which integrates the possible role of cAMP and other postulated second messengers, e.g. Ca^{2+} and inositol lipids, is still unavailable.

Candida albicans is a dimorphic pathogenic yeast which can exist as yeast cells, which reproduce by budding, or a filamentous mycelium, the relative dominance of each phase being affected by a variety of external factors, both physicochemical and nutritional [11–13]. The role of cAMP in morphogenesis of *C. albicans* has received some previous attention, though results obtained have been contradictory. Initial studies described an increase in levels of cellular cAMP on germ tube formation [14, 15] though more recent work has demonstrated that cAMP levels decline during the early stages of germ tube induction but then increase with subsequent germ tube formation [16]. However, maximum cAMP levels at complete germ tube formation were only slightly greater than cAMP levels in cells that did not undergo a yeast-mycelium (Y-M) transition [16].

The objective of this work was to examine the influence of exogenously-supplied nucleosides, nucleotides and yeast extract on the Y-M transition of *C. albicans* and the involvement of cAMP in this process. The effect of inhibitors of cAMP phosphodiesterase, theophylline and caffeine, on the Y-M transition was also examined, and cellular levels of cAMP measured throughout Y-M morphogenesis, induced by a range of environmental and nutritional factors including incubation at 37 °C or the presence of exogenous calcium.

Materials and methods

Organism, media and growth conditions

Candida albicans 3153A (obtained from Dr. N. A. R. Gow, University of Aberdeen) was maintained routinely at 25 °C on MYGP agar of composition (gl^{-1}): malt extract (Lab M), yeast extract (Oxoid) 3; bacteriological peptone (BDH), S; D-glucose, 10; agar (Lab M, No. 2), 15. For liquid cultures, a defined medium, pH 7, was used of composition (gl^{-1}): D-glucose, 12.5; $(NH_4)_2SO_4$, 5.0; $MgSO_4.7H_2O$, 0.2; K_2HPO_4 (anhydrous), 2.5; NaCl, 5.0; D-biotin, 0.001. Adjustment of pH to the desired value was by addition of either 0.1M HCl or 0.1M NaOH. Starter cultures were prepared by loop inoculating 50 ml medium, pH 6.0, which was incubated for 48 h at 25 °C on a rotary incubator (150 cycles min^{-1}). For experiments, 50 ml medium was inoculated from these stationary phase cultures which consisted almost entirely of yeast cells, to an initial cell density of approximately $2 \times 10^6\ ml^{-1}$ and incubated as described previously. When required, filter-sterilized adenosine, AMP, ADP, ATP, cAMP, dibutyryl cAMP or autoclaved solutions of yeast extract (Difco), or caffeine and theophylline were added to the appropriate final concentrations.

Extraction of adenylates

Cell samples (50 µl) were taken at intervals and separated from the medium by microcentrifugation (8000 g; 20 s) through 500 µl of a 'dinonyl' phthalate/silicone fluid mixture (3.2, v/v) into 240 µl of perchloric acid (1.77%). The perchloric acid layer was withdrawn and after the precipitate had been removed by centrifugation (8000 g, 20 s), 100 µl of the supernatant was added to 40 µl 0.5 M-Na_2HPO_4/NaH_2PO_4 buffer, pH 7.2, and 4 µl 5.0 M-Na_2CO_3 for neutralization. Extracts were immediately frozen and maintained at −20 °C.

Cyclic AMP assay

cAMP in extracts was assayed with a cAMP-binding assay kit (Amersham). cAMP in growth medium was estimated by centrifuging culture samples and assaying the supernatant. Radioactivity was determined by transferring the samples to scintillation vials and counting in a scintillation fluid based on toluene and Triton X-100 (2:1, v/v), and containing 4.0 g PPO l^{-1} and 0.1 g POPOP l^{-1}, using a Packard 300 CD scintillation counter. Cellular cAMP levels were expressed as pmol$(10^6$ cells$)^{-1}$ not distinguishing between the relative proportions of yeast cells and germ tubes

present after given incubation times: cAMP in culture supernatants was expressed as pmol(ml culture)$^{-1}$.

Other methods

Cell counts were carried out using a modified Fuchs-Rosenthal haemocytometer after suitable dilution with distilled water: the percentage of yeast cells which possessed a germ tube was assessed at appropriate time periods after incubation. Where required, mycelium was separated from yeast cells by filtration through 63 μm aperture nylon mesh. Dry weights were determined using tared aluminium foil cups dried to constant weight at 105 °C. The pH of culture medium was measured using a Kent/EIL pH meter, Model 7055. All chemicals used were of analytical grade.

Results

Induction of a yeast-mycelium transition in Candida albicans by exogenous adenylates yeast extract

Stationary phase cells of *Candida albicans* underwent a yeast-mycelium transition in the presence of an exogenous supply of yeast extract (Fig. 1). The first germ tubes appeared after approximately 8 h and these subsequently grew as mycelia (Fig. 1). The addition of yeast extract to the culture medium at any concentration tested between 0.05 and 10% (w/v) induced germ tube and mycelium formation but the concentration required for maximum germ tube formation was found to be 0.1% (w/v). The maximum level of germ tube formation was approximately 98% in 0.1% (w/v) yeast extract and this was reached approximately 32 h after initial inoculation (Fig. 1). After 32 h, further germ tube formation did not occur and the mycelium began to produce yeast cells. In unsupplemented medium, growth of *Candida albicans* was predominantly yeast-like with the cells reproducing by budding. Only a small proportion (≥18%) produced germ tubes

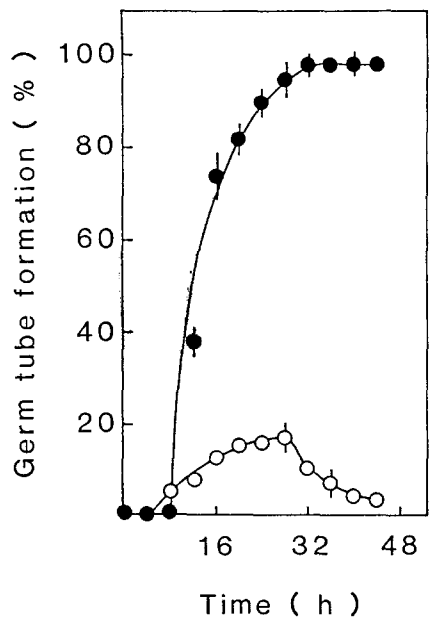

Fig. 1. Germ tube formation by *C. albicans* 3153 A in the absence (○) or presence (●) of 0.1% (w/v) yeast extract. Incubation was in defined liquid medium, pH 7, at 25 °C. Values shown in this, and subsequent Figures, are means of at least 3 replicate determinations; standard errors are shown when they exceed dimensions of the symbols.

and mycelium (Fig. 1). Differential separation of yeast cells and mycelium by filtration through a 63 μm aperture mesh, a method that was found to be ≥ 95% effective, and dry weight determinations showed that there was at least a 7-fold increase in the yield of mycelium in the presence of 0.1% (w/v) yeast extract as compared with the yeast extract-free control, dry weights after 32 h incubation being 7.8 ± 0.2 and 1.1 ± 0.2 mg (ml culture)$^{-1}$ respectively. Total dry weight values after 32 h for control and yeast extract-supplemented medium were 6.3 ± 0.3 and 8.0 ± 0.2 respectively.

Induction of a yeast-mycelium transition in C. albicans by exogenous adenylates and phosphodiesterase inhibitors

When *C. albicans* was grown in the defined liquid medium, pH 6, at 25 °C, growth was predominantly yeast-like whereas by shifting the pH of

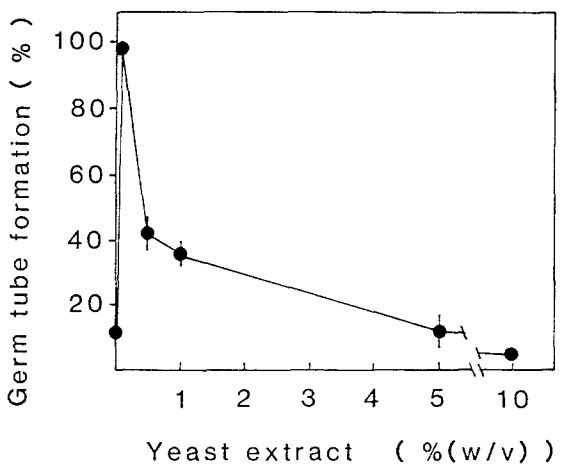

Fig. 2. Germ tube formation by *C. albicans* 3153 A in unsupplemented medium (○) or containing 0.05 mM-adenosine (●); 5 μM-AMP (△); 0.1 mM-ADP (▲); 5 μM-ATP (□); 0.1 mM-cAMP (■); 5 μM-dbcAMP (▽). Incubation was in defined liquid medium, pH 7, at 25 °C. Bars indicate standard errors of the mean (SEM).

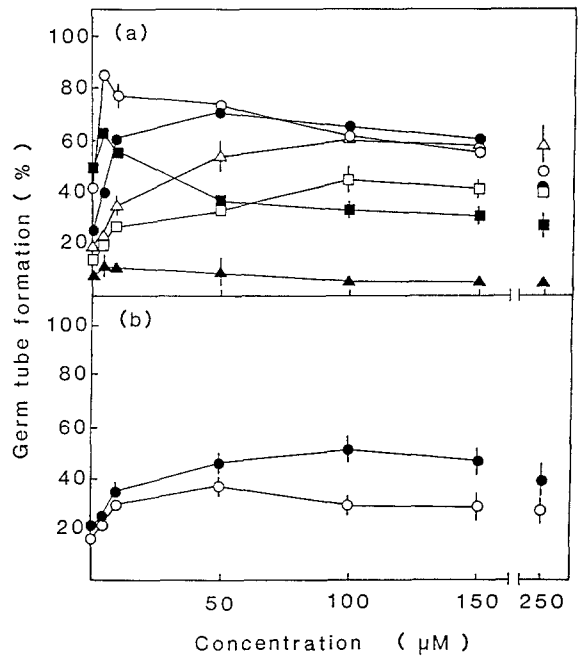

Fig. 3. (a) Effect of different concentrations of (●), adenosine; (○), AMP; (△), ADP; (▲), ATP; (□), cAMP and (■), dbcAMP on germ tube formation by *C. albicans* 3153 A. (b) Effect of different concentrations of (○), theophylline and (●), caffeine on germ tube formation by *C. albicans* 3153A. Incubation was for 24 h in defined liquid medium, pH 7, at 25 °C. Bars indicate SEM.

the medium to 7.0 at the same temperature, a small proportion (≥18%) was induced to form germ tubes (Fig. 2). However, addition of 5 μM AMP to the growth medium of *C. albicans* induced extensive production of germ tubes and mycelium. Morphological changes were detected microscopically after approximately 8 h and after this time, germ tubes grew as mycelia. The maximum level of germ tube formation was approximately 85% and this was reached approximately 24 h after inoculation (Fig. 2). After 24 h, the mycelium began to give rise to more yeast cells. This pattern of morphogenesis was similar to that induced by yeast extract except that the peak of maximum germ tube formation was around 32 h in 0.1% (w/v) yeast extract. Similarly, the presence of either 50 μM adenosine; 100 μM ADP; 100 μM cyclic AMP (cAMP) or its analogue dibutyryl cAMP (dbcAMP) (5 μM) in the medium also induced germ tube formation but to a lesser extent (Fig. 2). The times of onset of germ tube formation and the times of maximum germ tube formation by these inducers showed some slight differences. ATP was found to have little or a slightly inhibitory effect on germ tube and mycelium formation in *C. albicans* (Fig. 2).

The addition of the adenylates used in this study to the culture medium induced germ tube and mycelium formation in *C. albicans* at any concentration tested between 1 and 250 μM. However, optimal initial concentrations for maximum germ tube formation were in the range 5 to 100 μM, and above or below these concentrations, a reduced response was evident (Fig. 3a). The presence of the adenylates at their optimum concentrations for germ tube and mycelium formation in the liquid growth medium was found to increase the yield of biomass. This increase was dependent on the particular adenylate used and it was found that 5 μM dbcAMP produced the best growth yield. Total dry weights (mg ml^{-1}) after 32 h incubation were: unsupplemented medium, 6.0 ± 0.4; 100 μM ADP,

7.9 ± 0.6; 5 μM ATP, 9.7 ± 0.2; 5 μM AMP, 10.0 ± 0.5; 50 μM adenosine, 11.9 ± 0.5; 100 μM cAMP, 12.9 ± 0.4; 5 μM dbcAMP, 14.2 ± 0.5.

The inhibitors of cAMP phosphodiesterase, theophylline and caffeine, when added to the growth medium at concentrations which did not inhibit growth, also induced a yeast-mycelium transition in *C. albicans* at 25 °C. The addition of 50 μM-theophylline and 100 μM-caffeine to the culture medium resulted in a maximum of 37 and 51% germ tube formation respectively after 20 h incubation after which time mycelium began to give rise to yeast cells; germ tube formation in the presence of both inducers began between 8 and 12 h incubation (Fig. 3b). The addition of theophylline or caffeine to the culture medium at all concentrations between 1 and 250 μM induced a Y-M transition. However, optimum concentrations for maximum germ tube formation were 50 μM for theophylline and 100 μM for caffeine (Fig. 3b). The yield of biomass was increased in the presence of theophylline or caffeine with total dry weights (mg ml^{-1}) after 32 h incubation being: unsupplemented medium, 6.1 ± 0.5; 100 μM caffeine, 8.6 ± 0.5; 50 μM theophylline, 9.8 ± 0.6.

Changes in intracellular and extracellular cyclic AMP concentrations during yeast growth and during the yeast-mycelium transition

Increases in intracellular cAMP were directly related to the extent of the yeast-mycelium response. For the adenylates, the highest level of intracellular cAMP occurred during the yeast-mycelium transition induced by 5 μM-AMP, which produced the highest percentage of germ tube formation (≈ 85%) (Fig. 4). Intracellular cAMP increased gradually over the first 20 h, the time of germ tube and mycelium formation, while after this time when the mycelium began to give rise to yeast cells, intracellular cAMP levels began to drop (Fig. 4). In unsupplemented medium at 25 °C (when the percentage of germ tubes was ≥18%), intracellular cAMP increased slightly over the first five hours, then gradually dropped

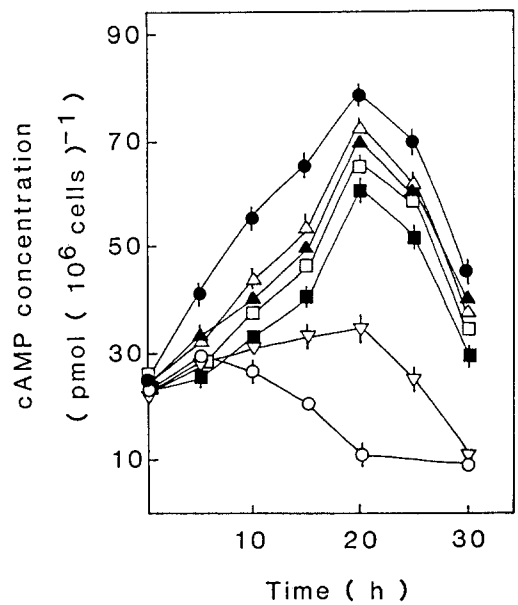

Fig. 4. Intracellular cAMP levels during yeast growth and during the yeast-mycelium transition of *C. albicans* 3153 A. (○), unsupplemented medium; (●), 5 μM-AMP; (△) 0.05 mM-adenosine; (▲), 5 μM-dbcAMP; (□), 0.1 mM-ADP; (■), 0.1 mM-cAMP; (▽), 5 μM-ATP. Incubation was in defined liquid medium, pH 7, at 25 °C. Bars indicate SEM.

(Fig. 4). Intracellular cAMP also increased during the yeast-mycelium transition induced by theophylline and caffeine (Fig. 5). This increase and the maximum levels of intracellular cAMP again coincided with maximum germ tube formation (Fig. 5).

Fig. 6 shows the intracellular cAMP concentration in yeast cells and germ tubes induced by exposure to other inducers or inhibitors of germ tube formation at 25 °C or by shifting the temperature to 37 °C in defined liquid medium. Under our experimental conditions, 10 μM-calmidazolium (R24571) was found to inhibit the yeast-mycelium transition of *C. albicans* even in the presence of a germ tube and mycelium inducer such as 1.5 mM $CaCl_2$ [17]. Intracellular cAMP continuously increased during yeast-like growth of *C. albicans* in the presence of 10 μM R24571 and 1.5 mM CaC12 in the culture medium. In all cells growing in the presence of an inducer of germ tube formation, such as 0.1% (w/v) yeast extract

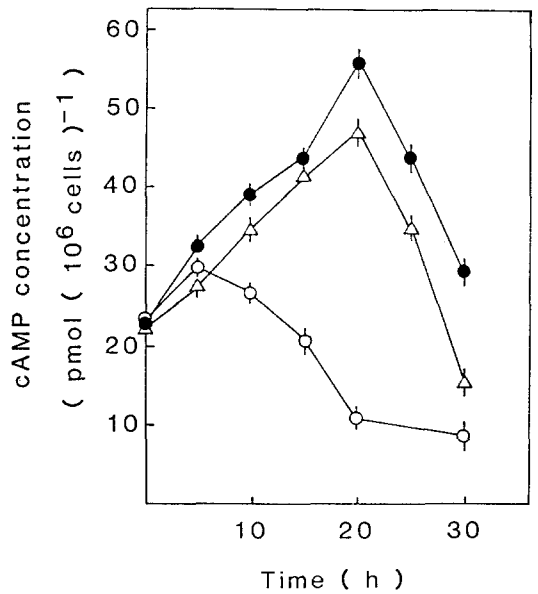

Fig. 5. Intracellular cAMP levels during yeast growth in unsupplemented medium (○) or during the yeast-mycelium transition induced by 0.1 mM caffeine (●) or 0.05 mM theophylline (△) in *C. albicans* 3153 A. Incubation was in defined liquid medium, pH 7, at 25 °C. Bars indicate SEM.

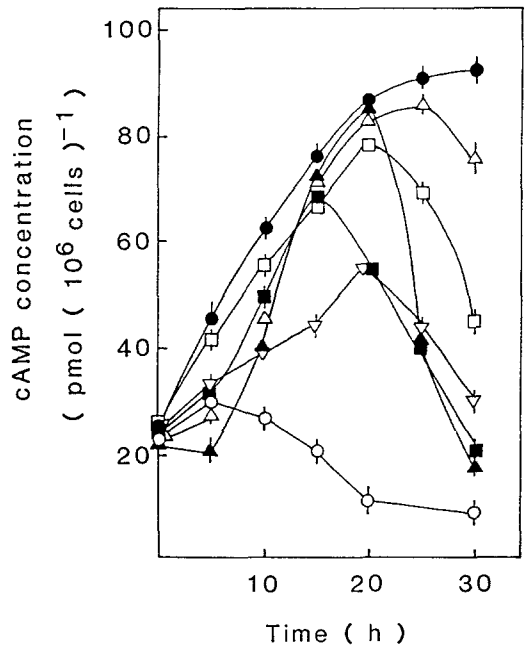

Fig. 6. Intracellular cAMP levels during yeast growth in unsupplemented medium (○) or during the yeast-mycelium transition of *C. albicans* 3153 A induced by 0.1% (w/v) yeast extract, (△); 1.5 mM $CaCl_2 \cdot 2H_2O$, (▲); 5 μM-AMP, (□); 100 μM-caffeine, (▽); unsupplemented medium at 37 °C, (■). (●), incubation with 1.5 mM $CaCl_2 \cdot 2H_2O$ and 10 μM calmidazolium (R24571) (no yeast-mycelium transition). Incubation was in defined liquid medium, pH 7, at 25 °C except where stated. Bars indicate the SEM.

or 1.5mM CaC12 [17], there was an increase in intracellular cAMP (Fig. 6). The increase in intracellular cAMP during the yeast-mycelium transition induced by raising the temperature from 25 °C to 37 °C in defined liquid medium appeared to have the same characteristics as the other treatments mentioned since the highest levels of intracellular cAMP occurred after 15 h incubation (Fig. 6) with the highest percentage of germ tube formation ($\approx 90\%$) occurring after approximately 12 h incubation at 37 °C. The decrease in intracellular cAMP levels after 15 h (Fig. 6) coincided with the mycelium giving rise to further yeast cells.

Extracellular levels of cAMP showed an increase during the yeast- mycelium transition (Fig. 7). The gradual rise in medium cyclic AMP coincided with increases in intracellular cAMP (Figs. 4–6). Extracellular cAMP was greatest in cultures grown in 10 μM-calmidazolium (R24571) with 1.5 mM $CaCl_2$ (Fig. 7).

Discussion

The involvement of cyclic nucleotides, especially cAMP, in dimorphic fungal transitions has received attention from several workers. The potentially pathogenic yeast, *C. albicans*, has been the subject of previous attention in this regard although mechanistic aspects of cAMP involvement remain uncertain. Previous studies have generally used a high temperature (37–40 °C) and a complex medium, often containing amino acids as an induction medium for filamentation. Niimi et al. [14] differentiated unicellular and filamentous cell types by incubation at 30 and 40 °C respectively. These workers found that while levels of cyclic guanosine 3′:5′-monophosphate (cGMP) remained approximately constant, the level of

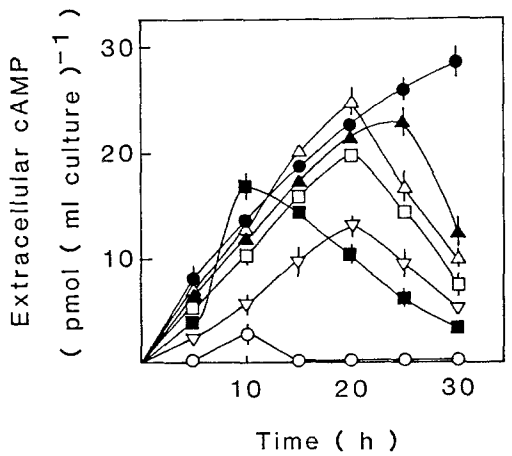

Fig. 7. Extracellular cAMP concentration during yeast growth in unsupplemented medium (○) or during the yeast-mycelium transition of *C. albicans* 3153 A induced by 5 μM-AMP, (△); 0.1% (w/v) yeast extract (▲); 1.5 mM-$CaCl_2 \cdot 2H_2O$, (□); unsupplemented medium at 37 °C, (■); 100 μM-caffeine, (▽). (●), incubation with 1.5 mM $CaCl_2 \cdot 2H_2O$ and 10 μM calmidazolium (R24571). Incubation was in defined liquid medium, pH 7, at 25 °C except where stated. Bars indicate the SEM.

cellular cAMP increased to approximately 1.6× the initial value. The exogenous supply of cAMP failed to induce a yeast-mycelial transition under all the conditions tested while dbcAMP promoted germ tube formation at concentrations ≥ 1 mM at 32–34 °C but not at 30 °C. Chattaway et al. [15] also demonstrated a rise in intracellular cAMP during germ tube formation of *C. albicans* when induced by peptides from seminal plasma or by mixtures of amino acids. In that study, germ tube formation and the accompanying rise in cAMP, which occurred mainly during the first hour of incubation, required a temperature of 37 °C. When 72% or more cells formed germ tubes, the intracellular concentration of cAMP was 2.5× that which occurred in yeast cells. As in the study by Niimi et al. [14], exogenous cAMP failed to induce germination whereas 2 mM dbcAMP induced approximately 20% germ tube formation.

In this study, we have demonstrated that a yeast-mycelium transition can be induced in *C. albicans* at 25 °C by the exogenous supply of yeast extract, nucleosides and nucleotides in simple defined liquid medium. Adenosine 5′-monophosphate (AMP) appeared to be the best inducer although adenosine, ADP, cAMP and dbcAMP were all capable of significant germ tube induction. The order of efficiency as germination inducers was AMP (5 μM) > adenosine (50 μM) > dbcAMP (5 μM) > ADP (100 μM) < cAMP (100 μM). ATP was found to have a slightly inhibitory effect on germ tube formation. The higher efficiency of dbcAMP (64% germ tube formation, 5 μM) as compared with cAMP (50% germ tube formation, 100 μM) is explained by its greater ability to permeate cells and greater resistance to cAMP phosphodiesterase [18]. Consequently, morphogenetic effects induced by this compound in fungi have frequently been observed. The addition of dbcAMP resulted in a germination response in *Ceratocystis multiannulata* [19], *Ceratocystis ulmi* [5] and *C. albicans*, although dbcAMP concentrations needed to be in the millimolar range at 37 °C for the latter organism [14, 15]. In contrast, dbcAMP inhibited a yeast-mycelial transition in several organisms including *Mucor* sp. [20, 21], *Paracoccidioides brasiliensis* [22] and *Sporothrix schenckii* [23] which resulted in a tendency to yeast-like development. It is noteworthy that cAMP at 100 μM does have a significant effect under our conditions of study since exogenous cAMP has previously been reported to have no effect on germination of *C. albicans* at 37 °C [14, 15]. The efficiency of yeast extract as an inducer of a yeast-mycelium transition has previously been noted in *Sarcinomyces crustaceus* (formerly *Cladosporium werneckii*) [24], *Aureobasidium pullulans* [4] and *Ophiostoma* (*Ceratocystis*) *ulmi* [5].

The cAMP phosphodiesterase inhibitors, theophylline and caffeine, induced some germ tube formation in *C. albicans* at 25 °C. A previous study reported that the phosphodiesterase of *C. albicans* was not affected by these inhibitors at high concentrations (1–10 mM) [25] although Chattaway et al. [15] reported germ tube formation in theophylline-exposed cells and a rise in cell

cAMP which clearly demonstrated an inhibitory effect on the phosphodiesterase. Similar results have been recorded for other fungi, including *H. capsulatum* [26], *C. multiannulata* [19], *B. dermatitidis* [27], *A. pullulans* [4] and *O. ulmi* [5]. However, in *S. schenckii* [23] *P. brasiliensis* [22], phosphodiesterase inhibitors were reported to inhibit the Y-M transition.

An increase in intracellular cAMP was observed during the Y-M transition of *C. albicans* whether induced by exogenous adenylates, yeast extract or phosphodiesterase inhibitors at 25 °C, maximum levels of cAMP coinciding with maximum germ tube formation suggesting that cAMP has an important role in the process. Such an increase in cAMP has been observed previously, not only in *C. albicans* [14, 15] but in *A. pullulans* [4], *O. ulmi* [5] and *B. dermatitidis* [27]. Furthermore, transient increases in intracellular cAMP have been detected in germinating spores of *Phycomyces blakesleeanus* [6, 7], *Mucor rouxii* [8, 19] and *M. genevensis* and *M. mucedo* [28]. In contrast to the work detailed above, Egidy et al. [16] have demonstrated a decrease in intracellular cAMP during the early stages of germ tube induction which then increased as germ tube formation proceeded. However, final cAMP levels attained (after 2 h) were not substantially different to those in control cells. These workers also found that germ tube formation was inhibited by cAMP, dbcAMP and theophylline which differs from previous studies on *C. albicans* and several other organisms. These discrepancies may possibly arise from the differing nature of the induction methods used for germ tube formation (in addition to strain differences). Egidy et al. [16] used cells suspended in buffer, with mineral salts, and N-acetyl-D-glucosamine (GlcNAc) as a germ tube inducer at 37 °C. However, it seems that external glucose may be a significant factor in triggering the elevation of intracellular cAMP in germinating fungal systems [6, 7, 29]. Glucose is often present in experimental examinations of germ tube formation, here, as in other studies [4–6, 8] and it is possible that GlcNAc may interact differently with cells. In our study, an increase in cellular cAMP was also observed in cells induced to form germ tubes at 37 °C or by exogenous $CaCl_2$. A Ca^{2+}-calmodulin involvement in dimorphism of *C. albicans* has previously been documented [17, 30]. In mammalian cells, adenylate cyclase activity, and therefore a rise in cAMP, is stimulated by Ca^{2+}, mediated by calmodulin [31] and an increase in cAMP has also been reported in the presence of calmodulin inhibitors [32]. Extracellular cAMP was detected in induction medium supernatants, and this appeared to be related to levels of intracellular cAMP. Liberation of cAMP has also been detected during the Y-M transition of *H. capsulatum* [3].

In eukaryotes, including fungi, cAMP is believed to activate protein kinase(s) which, in turn, phosphorylates and activates cellular proteins critical for phenotypic development [9, 10]. However, the mechanistic relationships of cyclic nucleotides with other external triggers and the biochemical changes which accompany phase transitions are still not fully ascertained. Stewart et al. [33] have demonstrated that filamentation in *C. albicans* is accompanied by alkalinization of the cell cytoplasm which may be due to activation or stimulation of H^+ efflux, mediated by the plasma membrane H^+-ATPase. The activity of cAMP enzymes, adenylate cyclase and phosphodiesterase, is sensitive to pH [34] and a hypothesis for regulation of cAMP by intracellular pH has been proposed [35]. Furthermore, cAMP concentration may also be controlled by the membrane potential, which may modulate adenylate cyclase activity [36] and also the entry of mono- and divalent cations, including Ca^{2+} [37, 38] which is of importance in cellular differentiation of fungi and other eukaryotes [10, 39]. Further work is necessary on these aspects in order to develop a unified biochemical model of dimorphism in *C. albicans*.

Conclusion

Cyclic AMP is involved in the yeast-mycelium transition of *C. albicans*.

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