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Cytoplasmic Alkalinization during Germ Tube Formation in *Candida albicans*

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Weak acids were used to measure the internal pH of yeast cells of *Candida albicans* that had been induced to form buds or germ tubes. Under conditions that supported germ tube formation the internal pH rose from around 6·8 to over 8·0 after 30 min in two different induction media. Internal pH measured by ^{31}P NMR confirmed this pattern and also showed that the internal pH fell to around 7·0 prior to the outgrowth of germ tubes. Conditions which led to budding induced less cytoplasmic alkalinization. This alkalinization was brought about when cells were inoculated into media of neutral pH and at an increased temperature. Increasing the temperature of the medium augmented the alkalinization of the cytoplasm induced by raising the external pH. Strains of *C. albicans* defective in the ability to produce germ tubes did not show this dramatic cytoplasmic alkalinization under conditions which normally supported filamentous growth. The raising of internal pH may be due to the activation of the plasma membrane proton-pumping ATPase since diethylstilboestrol inhibited the cytoplasmic alkalinization and germ tube formation without causing irreversible loss of cell viability. The results show that the induction of the dimorphic transition in this organism is accompanied by a steep rise in internal pH. It is not known whether these changes are the cause or consequence of morphogenesis.

INTRODUCTION

The role of the environment in controlling the morphology of *Candida albicans* has been studied extensively, yet remains elusive. A wide range of nutritional factors have been shown to be important in selectively encouraging growth by hyphal extension or by budding (Odds, 1979). However, no single environmental factor is responsible for inducing the dimorphic shift. Filamentous growth is favoured by conditions of neutral external pH and a temperature of around 37 °C (Odds, 1985). The inherent sensitivity of *C. albicans* to alterations in external pH is exploited in experimental protocols in which external pH alone is used to control structural dimorphism (Lee *et al.*, 1975; Buffo *et al.*, 1984). In these protocols a batch culture of yeast cells is grown at 25 °C in a synthetic medium of pH 4·5 until the cells reach stationary phase. Inoculation of these cells into fresh medium induces growth by germ tube formation when the medium is at pH 6·5 and 37 °C and by budding in the same medium at pH 4·5 and a temperature of 25 or 37 °C (Buffo *et al.*, 1984). This method is therefore ideal for investigations of environmentally induced dimorphism. There has been much less work investigating the physiological consequences of those conditions which select for one growth form over the other although there is detailed information regarding the changes occurring in cell shape, ultrastructure and wall biosynthesis and metabolism as a consequence of dimorphic transitions (Soll, 1985, 1986; Gow & Gooday, 1984; Cassone *et al.*, 1985; Shepherd *et al.*, 1985). The rationale for this study was to investigate the effects of external pH, temperature and medium composition on internal pH.

Abbreviation: DMO, 5,5-dimethyloxazolidine-2,4-dione.

Changes in internal pH have been implicated in the control of cellular differentiation, metabolism and cell cycle events in a wide range of organisms including the activation of sea urchin and *Xenopus* eggs (Johnson *et al.*, 1976; Winkler & Grainger, 1978; Charbonneau & Webb, 1987), stalk cell differentiation in *Dictyostelium discoideum* (Gross *et al.*, 1983), germination of yeast ascospores (Barton *et al.*, 1980) and the control of starvation, refeeding and catabolite derepression in *Saccharomyces cerevisiae* (den Hollander *et al.*, 1981). For a review see Busa & Nuccitelli (1984). Internal pH transitions responsible for these events almost invariably involve a rise in internal pH which varies in magnitude between 0·1 and 1·6 pH units (Busa & Nuccitelli, 1984).

In this paper we report that there is dramatic alkalinization of the cell cytoplasm of *C. albicans* prior to the emergence of the germ tube and that this alkalinization does not occur in those strains which do not form germ tubes. We also present preliminary evidence that alkalinization may be due to the activation of proton-pumping by the cytoplasmic membrane ATPase under conditions that promote filamentous growth.

METHODS

Organisms and culture conditions. Candida albicans (Robin) Berkhout strain 3153 was obtained from the London Mycological Reference Laboratory. The non-inducible strain CA_2 and germ-tube-minus mutant 100192′ were gifts from Antonio Cassone (Istituto di Microbiologia, University of Rome, Italy) and César Nombela (Departmento de Microbiología, Universidad Complutense, Madrid, Spain), respectively. Cells were grown in liquid media in 250 ml glass Erlenmeyer shake-flasks. Germ tube or budding growth was induced from stationary phase yeast cultures using an amino acids/salts medium or medium containing *N*-acetylglucosamine according to the methods of Buffo *et al.* (1984) and Gopal *et al.* (1982) respectively. Cells were inoculated into media inducing germ tubes or buds at a concentration of 5×10^7 yeast cells ml^{-1} and flasks were stirred to ensure mixing of added radiochemicals and aeration of the culture. Control experiments showed that 5×10^7 cells ml^{-1} was the maximum density that could be achieved without reducing the proportion of cells that formed germ tubes. Induction using amino acids/salts medium was under conditions of zinc sufficiency (Anderson & Soll, 1984). *Saccharomyces cerevisiae* 106 was from the University of Aberdeen culture collection.

Measurement of internal pH using weak acids. The internal pH of cells was measured by determining the distribution of the radiolabelled weak acids [7-^{14}C]benzoate and 5,5-dimethyl[2-^{14}C]oxazolidine-2,4-dione (DMO) according to the method of Booth *et al.* (1979). These weak acids were used at final concentrations of 0·6 μM [19·3 Ci mol^{-1} (714 GBq mol^{-1})] and 0·5 μM [16·2 Ci mol^{-1} (600 GBq mol^{-1})], respectively, and were added with ^3H_2O [1 μCi ml^{-1} (37 kBq ml^{-1}) final concentration] as a total water marker. Control experiments showed that these compounds equilibrated within 2 min. Triplicate 1 ml samples were taken at intervals and centrifuged in Eppendorf tubes for 30 s. A 100 μl volume of the supernatant was removed from each tube and transferred to Eppendorf tubes containing pellets of equal numbers of cells which had not been exposed to radiochemicals. This enabled the degree of quenching caused by the cells to be accounted for when comparing radioactivity in pellets and supernatants. The ^3H_2O count enabled the total volume of pellets to be determined and the amount of extracellular (trapped) ^{14}C in pellets to be calculated. Pellets were resuspended in 100 μl fresh medium and 80 μl of this was added to 2 ml scintillation fluid (Optiphase X, LKB). The radioactivity in samples of supernatant and pellet was counted using a Packard Tricarb 300C scintillation counter with the ^{3}H and ^{14}C window settings at 0–12 keV and 12–156 keV respectively. The ^{14}C spillover into the ^{3}H channel was estimated using identical samples containing ^{14}C only. From a knowledge of the external pH and the pK of the weak acid the internal pH was calculated (Booth *et al.*, 1979).

Measurement of internal pH using ^{31}P NMR. Measurements of internal pH were made in cells in stationary phase and at various times after the induction of germ tube formation in amino acids/salts medium at pH 6·5 and 37 °C. In order to obtain a working signal the cells had to be prepared at a concentration of $2·5 \times 10^9$ cells ml^{-1} – a density at which the cells failed to form germ tubes. Thus each measurement was made separately on a sample of cells prepared for a particular time point. The cells were induced in media at a concentration of 5×10^7 cells ml^{-1} and then concentrated to the working concentration. D_2O (2%, v/v) was added as an internal standard to 2·5 ml of cell supension. Spectra were also referenced to 85% (w/v) H_3PO_4 in a separate NMR tube. Spectra were recorded on Bruker WM250 or General Electric GN500 NMR spectrometers tuned at 101·256 MHz and 202·443 MHz, respectively. The pulse angle was at 57°, 75° or 90° (optimum 90°) with 20–26 μs pulses and a relaxation delay of 100 ms. Typically 2000 scans were made over a period of 8·3 min (acquisition time 128 ms). The chemical shifts were calibrated using solutions of 10 mM-KH_2PO_4 at a range of pH values. Since some spectra were recorded at 37 °C and others at 25 °C calibration measurements were corrected for temperature where necessary. The amino acids/salts medium contained appreciable amounts of phosphate and gave a strong signal but the internal pH was

found to be in all cases more alkaline than the external pH and so the internal phosphate signal was readily identifiable. Bubbling of air into sample tubes had no effect on the values of the internal pH that were obtained. Spectra had a higher background noise when samples were aerated and so most spectra were made without aerating the cultures. The concentrated cell suspensions noticeably acidified the sample growth medium. The external pH fell by 1 pH unit (from 6·5 to 5·5) within the time it took to make the 2000 scans.

Cell volume determinations. Attempts to measure the extracellular water space using [^{3}H]inulin, carboxymethyl-inulin and D-lactose were unsuccessful. Inulin and carboxymethyl-inulin bound to the cell surface while lactose permeated the cells (data not shown). Instead, yeast cells were stained with 5 μg Calcofluor white ml^{-1} (American Cyanamid) and the volume was calculated from the dimensions of the ellipsoid axes as measured at a magnification of 1000× in an Olympus fluorescence microscope. Calcofluor stained cells had a clearly defined boundary which facilitated these measurements. The mean cell volume of stationary phase cells of strain 3153 was 45·1 ± 1·1 μm^3 ($n = 100$). This figure is in close agreement with figures published elsewhere for the same strains and cultural conditions (Herman & Soll, 1984). The mean cell volume of cells after 30 min in amino acids/salts medium, pH 6·5, 37 °C was 44·5 μm^3. Mean volumes of stationary phase cells of *C. albicans* strains 1001 92′ and CA_2 were 43·4 and 46·1 μm^3 respectively.

Analysis of germ tube and budding growth. Small volumes of induced cultures were removed, fixed in buffered formalin solution and the percentage of cells showing any visible germ tube was recorded for 100 specimens. Cell densities were determined by counting in an Improved Neubauer haemocytometer.

Sources of chemicals. Radiolabelled DMO and benzoate were from Amersham and New England Nuclear, respectively. Diethylstilboestrol, procaine, nicotine and benzylamine were from Sigma.

RESULTS

Use of weak acids to measure internal pH in C. albicans

Internal pH in cells forming buds and germ tubes was followed for the first 30 min after the induction of growth. The changes in internal pH therefore reflected events preceding the emergence of buds or germ tubes which occurred after 30 min in the amino acids/salts medium. Germ tubes started to form after 60 min but maximum germ tube formation occurred only after 360 min. Unlike Herman & Soll (1984) we found that the mean volume of the yeast cells did not increase significantly during the first 30 min in fresh growth medium. Measurement of internal pH after evagination of buds or germ tubes was complicated by the difficulty in making accurate cell volume determinations for mixed populations of evaginating and non-evaginating cells and by the knowledge that the acidic vacuolar space increases dramatically at the onset of filamentous growth (Gow & Gooday, 1982; Gow *et al.*, 1986). These difficulties were overcome by monitoring the initial changes which occurred prior to the formation of buds or germ tubes – during which time the cell volume and vacuolar volume were constant. Moreover, since we were interested in those events which might control dimorphism these would necessarily precede morphogenesis. Experiments using weak acids were therefore restricted to those events which preceded the morphological changes associated with dimorphism.

Control experiments showed that benzoate and DMO equilibrated within 2 min and gave closely comparable values for the internal pH, although under conditions where internal pH was most alkaline, benzoate gave values up to 0·4 units higher than those obtained with DMO. This may be due to the lower pK of benzoate (4·2 compared to 6·32 for DMO) and the accumulation of appreciable amounts of this acid in the acidic vacuoles. *C. albicans* was not able to use benzoate or DMO as sole carbon source for growth on agarose plates containing nitrate, ammonium and inorganic nutrients. Cells were loaded with DMO or benzoate and then broken open in a Braun MSK glass-bead homogenizer and the homogenate run on TLC plates using a solvent of chloroform/cyclohexane/acetic acid (80:20:10, by vol.). No detectable breakdown of the intracellular radiolabelled acid could be observed on autoradiograms of the plates indicating that the cells did not metabolize the acids. Cells loaded with benzoate or DMO showed free efflux of the radiolabel when placed in non-labelled (weak-acid-free) medium indicating that the acids were not bound inside the cells. Also, the addition of 1% (v/v) toluene or n-butanol made the membrane permeable and prevented the acids from accumulating above extracellular concentrations. In some experiments internal pH was checked by concentrating the cells, breaking them open in non-buffered medium in a Braun homogenizer and measuring the pH of the homogenate. This crude technique produced values that were within 0·3 pH units of those

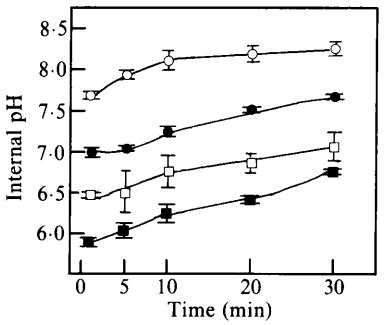

Fig. 1

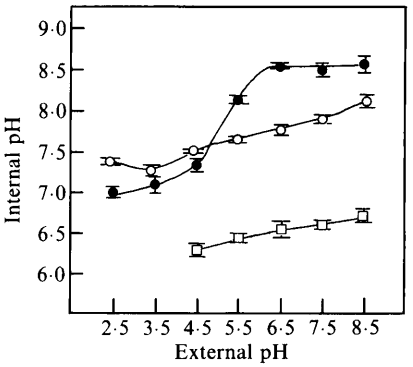

Fig. 2

Fig. 1. Changes in internal pH after stationary phase cells of *C. albicans* were inoculated into growth medium at 37 °C, pH 6·5 (○), 37 °C, pH 4·5 (●), 25 °C, pH 6·5 (□) or 25 °C, pH 4·5 (■). Germ tube formation occurred only at 37 °C, pH 6·5. Error bars are SD values based on nine determinations from three separate experiments. Internal pH was determined using DMO.

Fig. 2. Effect of external pH on internal pH in yeast cells of *C. albicans* (○, ●) or *S. cerevisiae* (□). Values for *C. albicans* were for stationary phase cells at 37 °C (○) or for cells inoculated into fresh growth medium and incubated for 30 min at 37 °C (●). The values for *S. cerevisiae* were for cells 30 min after inoculation into the same medium but at 30 °C. Error bars are SD values based on nine determinations from three separate experiments using benzoate to determine internal pH.

measured using DMO. The use of newborn calf serum medium was found unsuitable for use in experiments for determinations of internal pH. The radiolabels were bound extracellularly in appreciable quantities in the presence of 20% (v/v) serum. Data obtained with ^{31}P NMR were comparable with those obtained using weak acids (see below).

Effect of external pH and temperature on internal pH

Stationary phase yeast cells of *C. albicans* were inoculated into fresh medium at pH 4·5 at 25 or 37 °C or at pH 6·5 at 37 °C according to the method of Buffo *et al.* (1984). Germ tubes were formed with the latter set of conditions while growth was by bud formation when the medium was at the lower pH and/or temperature. The internal pH of the stationary phase cells at 25 °C was 6·8 ± 0·15 as measured using benzoate or DMO. The internal pH was also estimated at 6·63 by recording the pH of homogenates of these cells. There was a rapid rise in internal pH within the time taken to make the first measurement after inoculation into fresh medium followed by a gradual increase in internal pH over the next 30 min (Fig. 1). The increase was largest under those conditions leading to eventual germ tube formation where internal pH rose from 6·8 to 8·2 in 30 min. The pH of a homogenate of cells at this stage of growth was 7·92. This cytoplasmic alkalinization was less pronounced when the growth medium was at the lower temperature or pH (Fig. 1). Replicate experiments using benzoate to measure internal pH gave values that were higher. The difference was greatest when cytoplasmic pH was most alkaline (see Fig. 2). Cells of *C. albicans* and *S. cerevisiae* were cultured in fresh medium of identical composition but varying pH; internal pH was then measured 30 min after inoculation (Fig. 2). *C. albicans* had a more alkaline and less constant cytoplasmic pH than *S. cerevisiae*. The internal pH of non-growing stationary phase yeast cells of *C. albicans* varied less over the same range of external pH (Fig. 2). The inoculation of stationary phase cells of *S. cerevisiae* into fresh growth medium at pH of either 4·5 or 6·5 led to only a slight rise in internal pH in comparison with that observed with *C. albicans* (data not shown). The dramatic increase in internal pH seen with *C. albicans* under similar conditions is therefore a feature of the physiology of this organism rather than a general effect of the culture conditions used.

Changes in internal pH occurred in response to alterations in external pH and in temperature. An increase in temperature alone was found to increase internal pH (Fig. 3). Decreasing the

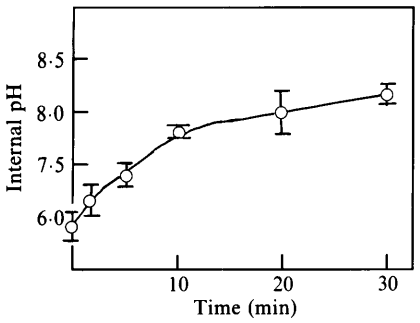

Fig. 3

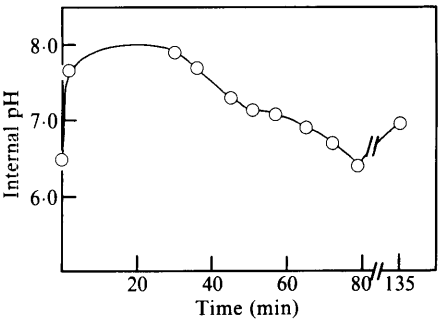

Fig. 4

Fig. 3. Effect of an increase in temperature on internal pH. Cells were preincubated for 63 min at 25 °C in nutrient depleted (stationary phase) medium at pH 4·5 before the temperature was increased to 37 °C at time = 0. Error bars are SD values of triplicate measurements using benzoate to determine internal pH.

Fig. 4. Changes in internal pH during the induction of germ tubes in media at 37 °C and pH 6·5. Internal pH measurements were made by ^{31}P NMR; each point was determined from a single experiment as described in Methods.

temperature to 4 °C decreased the value obtained for internal pH by over 1 pH unit. Changes in temperature therefore potentiate internal pH changes brought about by altering external pH. Alterations in temperature and pH of the medium both contribute to an alkalinization of the cell cytoplasm. Increasing the cell density of cultures from 5×10^7 to $2{\cdot}5 \times 10^9$ ml^{-1} had the effect of reducing equivalent internal pH values by around half a pH unit. This may be due to the concentrated cells actively acidifying the medium.

Internal pH changes measured by ^{31}P NMR

Samples were prepared as described in Methods and the internal pH determined from the chemical shift measured for the internal phosphate peak (Fig. 4). The spectra were recorded at the same temperature that was used in the experiments. The internal pH of stationary phase cells was determined as 6·5 – lower than that determined using weak acids (6·8). After induction of germ tube formation the internal pH rose to 7·8 or above before falling to around 7·0 at 135 min. Since around 10% of cells had formed germ tubes by this time it was clear that the internal pH of growing cells was around neutrality.

Internal pH changes in medium containing N-acetylglucosamine

In order to establish whether the cytoplasmic alkalinization accompanying germ tube formation in amino acids/salts medium (Buffo *et al.*, 1984) was a general feature of the dimorphic transition, we followed internal pH in cells induced in medium containing *N*-acetylglucosamine (Gopal *et al.*, 1982). When the pH and temperature of this medium was 4·5 and 25 °C, respectively, cells failed to form germ tubes and internal pH rose to 7·7 after 30 min (data not shown). At 37 °C and an external pH of 6·5 cells formed germ tubes and again the internal pH rose to above 8·0. The difference in internal pH of germ-tube-forming and bud-forming cells was not therefore as great as in amino acids/salts medium.

Internal pH in germ-tube-less strains of C. albicans

Two strains defective in their ability to produce germ tubes under conditions that normally support filamentous growth were used in studies of internal pH. Strain CA_2 is a 'low responder' (Mattia *et al.*, 1982) strain, and produced only 4% germ tubes after 6 h in amino acids/salts medium. Strain 100192′, a germ-tube-less mutant, produced no germ tubes under the same conditions. The internal pH of these germ-tube-less strains did not rise as much as in strain 3153 during the first 30 min after inoculation into media that promotes hyphal growth (data not

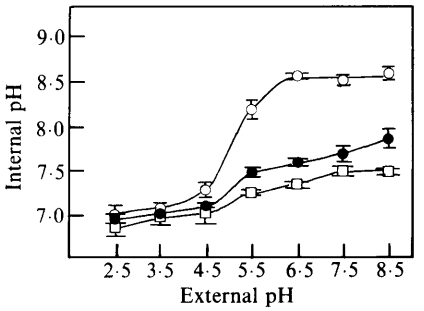

Fig. 5. Effect of external pH on internal pH of *C. albicans* strains 3153 (○), CA_2 (●) and 100192′ (□) 30 min after inoculation of cells from stationary phase cultures into growth medium at 37 °C and pH 6·5. Error bars are SD values of nine measurements from three experiments using benzoate to determine internal pH.

shown). In CA_2 and 100192′ the internal pH rose to 7·85 and 7·70, respectively, after 30 min in fresh medium at 37 °C and pH 6·5. The internal pH of each strain reached 7·10 and 6·95 after the same period in fresh medium at 25 °C and pH 4·5. These strains were therefore better able to regulate their internal pH compared to the wild-type strain 3153 (Fig. 5).

Effect of diethylstilboestrol on internal alkalinization

We reasoned that one way in which a rapid alkalinization of the cytoplasm could be achieved would be through the activation of the cytoplasmic membrane ATPase. This proton pump expels protons electrogenically and its activity can be inhibited relatively specifically by diethylstilboestrol (Goffeau & Slayman, 1981; Hubbard *et al.*, 1985). We therefore studied the effect of this drug on internal pH and on germ tube formation. Diethylstilboestrol (dissolved in ethanol) was used at a final concentration of 0·1 mM (final ethanol concentration 1%). When added at the beginning of a germ tube induction experiment diethylstilboestrol prevented internal pH from rising above 7·5 and when added 10 min after induction it prevented any subsequent alkalinization and led to a slight decrease in internal pH. In neither case did germ tube formation occur. This was not due to the killing of the cells since the treated cells could be washed free of diethylstilboestrol after 30 min in its presence and be shown to have no decreased viability as assessed by a viable numbers count. Washed cells formed germ tubes readily (92% germ tube formation) if they were resuspended in fresh medium for 6 h. Controls using 1% ethanol alone had no effect on internal pH or germ tube formation.

Induction of germ tube formation using weak bases

We wondered whether treatments that increased internal pH would lead to the induction of germ tube formation. Weak bases such as procaine, nicotine and benzylamine can be used to raise the internal pH of fungi (Sanders & Slayman, 1982). In order to do this the pK_a of the base ought to be higher than the internal pH and the external pH ought to be sufficiently high to allow significant quantities of the free base to be generated externally. The pK_a values of procaine, nicotine and benzylamine are 9·0, 8·2 and 9·33, respectively, and so experiments cannot readily be done under conditions that are physiological yet allow appreciable quantities of base to enter the cells. We used these weak bases at a rather low external pH (4·5) yet were able to show that they could partially induce germ tube formation under conditions that would normally support growth by budding. In fresh growth medium containing 1 mM-procaine at 25 °C and titrated to pH 4·5, the internal pH of cells rose to 7·81 and 20% of the cells formed germ tubes by 6 h. Nicotine at the same concentration increased internal pH to 8·07 and increased the proportion of cells with germ tubes to 31%. Benzylamine (1 mM) failed to increase internal pH above 7·0 and did not induce germ tube formation. Concentrations of procaine and nicotine at or above 50 mM caused clumping and some lysis of the cells. Increasing the concentration of the weak

bases did not increase further the degree of germ tube formation. Similar results were obtained when the experiments were repeated at 37 °C and an external pH of 4·5. Benzylamine appeared to be able to permeate the cells and bring about cytoplasmic alkalinization and germ tube formation at the higher temperature. Because of the extremely low external pH used in these experiments the internal concentration of these bases must be assumed to be vanishingly small ($< 0{\cdot}1$ μM) and therefore incapable of increasing internal pH directly. We must conclude that the weak bases acted by activating processes which led to cytoplasmic alkalinization and not by increasing cytoplasmic pH directly.

DISCUSSION

We have shown that conditions that lead to filamentous growth in *C. albicans* also lead to alkalinization of the cell cytoplasm to pH 8·0 or above. This pH change was larger than that observed when cells resumed growth by budding and was not seen under identical cultural conditions in strains unable to undergo dimorphism. The finding that temperature and pH both influence internal pH is of interest since these have been recognized as key environmental parameters in the control of dimorphism (Odds, 1979, 1985; Buffo *et al.*, 1984). When the external pH was at or below 4·5 cells did not undergo the increasing cytoplasmic alkalinization that was evident when the external pH was raised above this value. This is of interest since Buffo *et al.* (1984) reported a similar transition for the effect of external pH on the induction of either budding or germ tube development. An inhibitor of the plasma membrane ATPase prevented cytoplasmic alkalinization and inhibited dimorphism. The increase in internal pH may therefore be induced by the activation of proton-pumping by the ATPase. Culture conditions used to induce germ tube formation in *C. albicans* had little effect on the internal pH of *S. cerevisiae* and in general it appeared that *C. albicans* did not regulate its internal pH as tightly as *S. cerevisiae*.

The values reported here for internal pH of *S. cerevisiae* are in close agreement with those published elsewhere (Krebs *et al.*, 1983; Borst-Pauwels, 1981). We show that the internal pH of *S. cerevisiae* varies by less than 0·4 pH units over a range of external pH values that generate a difference of more than 1·2 pH units in *C. albicans*. The internal pH of exponential phase yeast cells and stationary phase cells of *C. albicans* was between 6·8 and 7·0 when the external pH was between 4·5 and 2·5. Internal pH of stationary phase cells rose to 7·8 and 8·1 at 25 and 37 °C respectively when the medium pH was titrated to 8·5. These data are in general agreement with those of Prasad & Hofer (1987) who used [^{14}C] propionate to measure internal pH of late exponential phase cells at between 7·3 and 8·1 when external pH was varied from 3·5 to 8·5. Cassone *et al.* (1983) used ^{31}P NMR to measure internal pH in yeast cells, germ tubes and hyphae of *C. albicans*. Germ tube pH was estimated at 6·4 in packed cell suspensions at 4 °C, 90 min after induction. We have shown that cooling and concentrating cells both led to a reduction in the value of the internal pH that was obtained. We repeated experiments measuring internal pH using benzoate or DMO at 4 °C and obtained a value of 6·7 for germ tubes after 30 min growth. As reported previously (Borst-Pauwels, 1981) values for internal pH obtained by NMR tended to be lower than those obtained by other methods. We also used ^{31}P NMR at physiological temperatures to provide corroborative evidence for the results obtained using weak acids.

A wide range of biochemical factors have been implicated in a variety of dimorphic fungi as being central to the control of yeast/mould dimorphism (San-Blas & San-Blas, 1984; Odds, 1985). Of these, two molecules which have received much attention are the calcium binding protein calmodulin (Hubbard *et al.*, 1982; Muthukumar & Nickerson, 1984; Muthukumar *et al.*, 1987) and cAMP (Maresca *et al.*, 1977; Niimi *et al.*, 1980; Chattaway *et al.*, 1981). The activity and levels of these molecules may well be responsive to changes in cytoplasmic pH. For example, calmodulin has a titratable histidyl residue at position 107; its activity may therefore be pH dependent. Tkachuk & Men'Shikov (1981) showed that the amount of Ca^{2+} complexed to calmodulin varied by a factor of ten over a pH range between 6·5 and 7·5. If calmodulin is active in processing regulatory information during the dimorphic transition it may serve both as a

proton and calcium sensor and thereby initiate its effects in response to alterations in internal pH.

Modulations in the levels of cyclic nucleotides such as cAMP are known to be central to the regulation of cell metabolism and many morphogenetic systems. The pH activity profiles of adenylate cyclase and phosphodiesterase enzymes which make and destroy cAMP have been studied in a limited number of systems. In most cases it is clear that adenylate cyclase activity is often particularly sensitive to pH (see discussion of Busa & Nuccitelli, 1984). Again it is possible to invoke mechanisms which incorporate changes in internal pH with modulations of those biochemical activities which are corporately responsible for the translation of an environmental signal into a switch from a budding to a mycelial growth habit. Whatever the details of the mechanism it is clear that changes in internal pH accompany structural dimorphism in *C. albicans* and may play a role in its control.

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REFERENCES

ANDERSON, J. M. & SOLL, D. R. (1984). Effects of zinc on stationary-phase phenotype and macromolecular synthesis accompanying outgrowth of *Candida albicans*. *Infection and Immunity* **46**, 13–21.

BARTON, J. K., DEN HOLLANDER, J. A., LEE, T. M., MACLAUGHLIN, A. & SHULMAN, R. G. (1980). Measurement of the internal pH of yeast spores by ^{31}P nuclear magnetic resonance. *Proceedings of the National Academy of Sciences of the United States of America* **77**, 2470–2473.

BOOTH, I. R., MITCHELL, W. J. & HAMILTON, W. A. (1979). Quantitative analysis of proton linked transport systems: the lactose permease of *Escherichia coli*. *Biochemical Journal* **182**, 687–696.

BORST-PAUWELS, G. W. F. H. (1981). Ion transport in yeast. *Biochimica et biophysica acta* **650**, 88–127.

BUFFO, J., HERMAN, M. A. & SOLL, D. R. (1984). A characterisation of pH-regulated dimorphism in *Candida albicans*. *Mycopathologia* **85**, 21–30.

BUSA, W. B. & NUCCITELLI, R. (1984). Metabolic regulation via intracellular pH. *American Journal of Physiology* **246**, R409–R438.

CASSONE, A., CARPINELLI, G., ANGIOLELLA, L., MADDALUNO, G. & PODO, F. (1983). ^{31}P Nuclear magnetic resonance study of growth and dimorphic transition in *Candida albicans*. *Journal of General Microbiology* **129**, 1569–1575.

CASSONE, A., SULLIVAN, P. A. & SHEPHERD, M. G. (1985). *N*-Acetyl-D-glucosamine-induced morphogenesis in *Candida albicans*. *Microbiologica* **8**, 85–99.

CHARBONNEAU, M. & WEBB, D. J. (1987). Weak bases partially activate *Xenopus* eggs and permit changes in membrane conductance whilst inhibiting cortical granule exocytosis. *Journal of Cell Science* **87**, 205–220.

CHATTAWAY, F. W., WHEELER, P. R. & O'REILLY, J. (1981). Involvement of adenosine 3′:5′-cyclic monophosphate in the germination of blastospores of *Candida albicans*. *Journal of General Microbiology* **123**, 233–240.

GOFFEAU, A. & SLAYMAN, C. W. (1981). The proton-translocating ATPase of the fungal plasma membrane. *Biochimica et biophysica acta* **639**, 197–223.

GOPAL, P., SULLIVAN, P. A. & SHEPHERD, M. G. (1982). Enzymes of *N*-acetyl-D-glucosamine metabolism during germ-tube formation in *Candida albicans*. *Journal of General Microbiology* **128**, 2319–2326.

GOW, N. A. R. & GOODAY, G. W. (1982). Vacuolation, branch production and linear growth of germ tubes of *Candida albicans*. *Journal of General Microbiology* **128**, 2195–2198.

GOW, N. A. R. & GOODAY, G. W. (1984). A model for the germ tube formation and mycelial growth form of *Candida albicans*. *Sabouraudia* **22**, 137–143.

GOW, N. A. R., HENDERSON, G. & GOODAY, G. W. (1986). Cytological interrelationships between the cell cycle and duplication cycle of *Candida albicans*. *Microbios* **47**, 97–105.

GROSS, J. D., BRADBURY, J., KAY, R. R. & PEACEY, M. J. (1983). Intracellular pH and the control of cell differentiation in *Dictyostelium discoideum*. *Nature, London* **303**, 244–245.

HERMAN, M. A. & SOLL, D. R. (1984). A comparison of volume growth during bud and mycelium formation in *Candida albicans*: a single cell analysis. *Journal of General Microbiology* **130**, 2219–2228.

DEN HOLLANDER, A., UGURBIL, K., BROWN, T. R. & SHULMAN, R. G. (1981). Phosphorus-31 nuclear magnetic resonance studies of the effect of oxygen upon glycolysis in yeast. *Biochemistry* **20**, 5871–5880.

HUBBARD, M. J., BRADLEY, M., SULLIVAN, P. A., SHEPHERD, M. G. & FORRESTER, I. (1982). Evidence for the occurrence of calmodulin in the yeasts *Candida albicans* and *Saccharomyces cerevisiae*. *FEBS Letters* **137**, 85–88.

HUBBARD, M. J., SULLIVAN, P. A. & SHEPHERD, M. G. (1985). The kinetics and divalent cation inhibition of the plasma membrane ATPase in the yeast *Candida albicans*. *Journal of Biological Chemistry* **260**, 6782–6787.

JOHNSON, J. D., EPEL, D. & PAUL, M. (1976). Intracellular pH and activation of sea urchin eggs after fertilisation. *Nature, London* **262**, 661–664.

KREBS, H. A., WIGGINS, D., STUBBS, M., SOLS, A. & BEDOYA, F. (1983). Studies on the mechanism of the antifungal action of benzoate. *Biochemical Journal* **214**, 657–663.

LEE, K. L., BUCKLEY, H. R. & CAMPBELL, C. C. (1975). An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. *Sabouraudia* **13**, 148–153.

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., CARRUBA, G., ANGIOLELLA, L. & CASSONE, A. (1982). Induction of germ tube formation by *N*-acetyl-D-glucosamine in *Candida albicans*: uptake of inducer and germinative response. *Journal of Bacteriology* **152**, 555–562.

MUTHUKUMAR, G. & NICKERSON, K. W. (1984). Ca(II)–calmodulin regulation of fungal dimorphism in *Ceratocystis ulmi*. *Journal of Bacteriology* **159**, 390–392.

MUTHUKUMAR, G., NICKERSON, A. W. & NICKERSON, K. W. (1987). Calmodulin levels in yeasts and filamentous fungi. *FEMS Microbiology Letters* **41**, 253–255.

NIIMI, M., NIIMI, K., TOKUNAGA, J. & NAKAYAMA, H. (1980). Changes in cyclic nucleotide levels and dimorphic transition in *Candida albicans*. *Journal of Bacteriology* **142**, 1010–1014.

ODDS, F. C. (1979). *Candida and Candidosis*. Leicester: Leicester University Press.

ODDS, F. C. (1985). Morphogenesis in *Candida albicans*. *Critical Reviews in Microbiology* **12**, 45–93.

PRASAD, R. & HOFER, M. (1987). The electrochemical gradient of H^+ in *Candida albicans* and its relevance to the uptake of nutrients. *Biochemistry International* **14**, 617–626.

SAN-BLAS, G. & SAN-BLAS, F. (1984). Molecular aspects of fungal dimorphism. *Critical Reviews in Microbiology* **11**, 101–127.

SANDERS, D. & SLAYMAN, C. L. (1982). Control of intracellular pH. Predominant role of oxidative metabolism, not proton transport, in the eukaryotic microorganism *Neurospora*. *Journal of General Physiology* **80**, 377–402.

SHEPHERD, M. G., POULTER, R. T. M. & SULLIVAN, P. A. (1985). *Candida albicans*: biology, genetics and pathogenicity. *Annual Review of Microbiology* **39**, 579–614.

SOLL, D. R. (1985). The role of zinc in dimorphism. *Current Topics in Medical Mycology* **1**, 258–285.

SOLL, D. R. (1986). The regulation of cellular differentiation in the dimorphic yeast *Candida albicans*. *Bioessays* **5**, 5–11.

TKACHUK, V. A. & MEN'SHIKOV, M. Y. (1981). Effect of pH on calcium binding properties of calmodulin and its interaction with the Ca-dependent form of cyclic nucleotide phosphodiesterase. *Biokhimiya* **138**, 779–788.

WINKLER, M. M. & GRAINGER, J. L. (1978). Mechanism of action of NH_4Cl and other weak bases in the activation of sea urchin eggs. *Nature, London* **273**, 536–538.