Journal of General Microbiology (1989), **135**, 2173–2179. *Printed in Great Britain*

Rise in Intracellular pH is Concurrent with 'Start' Progression of *Saccharomyces cerevisiae*

By SHALINI ANAND AND RAJENDRA PRASAD*

School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

(*Received 22 February 1989; accepted 5 May 1989*)

Intracellular pH (pH_i) was determined during arrest and recovery of temperature sensitive-cell division cycle mutants of *Saccharomyces cerevisiae*. In all mutants, pH_i decreased during arrest; but when the mutants were released from arrest a rapid increase in pH_i ensued in only *cdc28*- and *cdc37*-arrested cells. Both of these mutations cause arrest at 'start', the sole regulatory point in the *S. cerevisiae* cell cycle. In cells with *cdc4* or *cdc7* mutations, which arrest past start, pH_i remained constant and exhibited a decrease, respectively, upon recovery of growth. The activity of plasma membrane ATPase decreased during the first 30 min of recovery of *cdc28*-arrested cells, concomitant with the rise in pH_i. During the same period, there was no significant change in activity in *cdc4*-bearing cells, whereas an increase was observed for *cdc7*-bearing cells. Increase in pH_i may be used as a specific signal by *S. cerevisiae* for start traversal and commitment to a new cycle.

INTRODUCTION

The cell cycle of the budding yeast *Saccharomyces cerevisiae* is pleiotropically regulated in G1 at a point termed 'start' (Hartwell, 1974; Yanishevsky & Stein, 1981). This occurs in late G1 and is immediately followed by two divergent subcycles, one involving nuclear events of DNA synthesis and nuclear division, the other consisting of cytoplasmic events governing bud emergence and growth (Hartwell *et al.*, 1974). Although much is known with regard to morphological and genetical aspects of start, (Hartwell *et al.*, 1973; Reed, 1980; Bedard *et al.*, 1981; Pringle *et al.*, 1981) the underlying biochemistry is not so well defined. The gene product of *CDC28* is a protein kinase (Reed *et al.*, 1985*b*; Medenhall *et al.*, 1987). The gene product of *CDC7*, a G1 gene whose sequential expression after *CDC28* is essential for the initiation of DNA synthesis, is also a kinase (Patterson *et al.*, 1986).

Recent reports indicate that a transient alkalinization of intracellular pH (pH_i) precedes DNA synthesis when quiescent animal cells are allowed to proliferate in response to mitogens and growth factors (Roos & Boron, 1981; Schuldiner & Rozengurt, 1982; Bravo & Bravo, 1986; Hagag *et al.*, 1987; Kakinuma *et al.*, 1987; Ober & Pardee, 1987; Rotstein *et al.*, 1987). In addition, increase in pH_i is associated with fertilization of sea urchin eggs (Steinhardt & Mazia, 1973; Lopo & Vacquier, 1977), development of *Dictyostelium* and dimorphic transition of *Candida albicans* (Aerts *et al.*, 1987; Stewart *et al.*, 1988). In most cases control of pH_i is accomplished, at least in part, by Na^+/H^+ antiport (Schuldiner & Rozengurt, 1982; Doppler *et al.*, 1986; Hagag *et al.*, 1987; Kakinuma *et al.*, 1987; Ober & Pardee, 1987; Rotstein *et al.*, 1987).

We have been studying the biochemical events associated with G1 to S transition in *S. cerevisiae* using temperature-sensitive *cdc* mutants (Dudani & Prasad, 1983, 1984*a*, *b*; Dudani *et al.*, 1983; Anand & Prasad, 1987). The present study was undertaken in view of the importance of pH_i in growth and differentiation of various cells.

Abbreviations: pH_e, external pH; pH_i, intracellular pH.

METHODS

Strains and growth conditions. Various *cdc* mutants, H.185.3.4 (*cdc28-1*), H.135.1.1 (*cdc4-3*), 4008 (*cdc7-4*), SR.661.2 (*cdc36-16*), SR665.1 (*cdc39-1*), SR672 (*cdc37-1*), and the wild-type strain (A364A) were obtained from Yeast Genetic Stock Center, Berkeley, California, USA. The growth conditions and maintenance of all these strains have been described (Hartwell *et al.*, 1973; Hereford & Hartwell, 1974; Reed, 1980). Arrest conditions were as described by Anand & Prasad (1987). All the strains were grown in YEPD medium at 23 °C. The mutants were synchronized by shifting them to their respective non-permissive temperatures: 36 °C for *cdc36* and *cdc4*; 37 °C for *cdc37* and *cdc39*; 38 °C for *cdc28* and *cdc7* (Hartwell *et al.*, 1973; Reed, 1980). Cell synchrony was established microscopically by counting at least 200 cells and looking for terminal phenotype of individual cells and by following DNA synthesis (Hartwell, 1970). Cells were collected at indicated times, harvested and washed twice with distilled water at 1500 *g*. For pH_i measurements, 10% (w/v) cell suspensions were prepared in 100 mM-Tris/citrate buffer (pH 5·4).

Measurement of pH_i. pH_i values were calculated by determining the steady state distribution of propionic acid across the plasma membrane (Cartwright *et al.*, 1986). Assay conditions were similar to those described by Prasad & Hofer (1987). A typical reaction mixture containing 100 mM-Tris/citrate buffer (pH 5·4) and cells (10–15 mg dry wt ml^{-1}) was incubated at 30 °C. The distribution of propionic acid was initiated by the addition of 10 μM-sodium [2-^{14}C]propionate (1·85 kBq ml^{-1}). In pilot experiments with wild-type cells (A364A) it was established that 3–4 min were required for propionic acid to equilibrate across the plasma membrane. Triplicate samples (0·3 ml) were removed after 4 min, rapidly filtered through Maxflow filter discs (0·45 μm), and washed four times with 1 ml ice-cold unlabelled 10 μM-propionic acid. Radioactivity retained was counted in a Beckman LS 1801 Beta liquid scintillation counter using a toluene based scintillation fluid. pH_i values were calculated as described by Prasad & Hofer (1987).

Preparation of crude plasma membrane fraction. The method was similar to that described by Tuduri *et al.* (1985). Washed cells (450 mg wet wt) were suspended in 1 ml grinding medium containing 250 mM-sucrose, 10 mM-Tris/HCl, pH 7·5, and 1 mM-phenylmethylsulphonyl fluoride (PMSF). To 900 μl of this suspension, 900 mg of glass beads (0·45 mm diameter) were added and the mixture was agitated nine times for 17 s with intervals of 3 s using a CO_2-refrigerated MSK Braun homogenizer with a micro adapter. The homogenate was centrifuged for 5 min at 1000 *g* and the pellet was rinsed by centrifugation at 1000 *g* for 5 min with 500 μl grinding medium. The combined supernatants were centrifuged again for 5 min at 1000 *g*. The resulting supernatant was centrifuged for 40 min at 15000 *g*. The pellet was suspended in 300 μl of 250 mM-sucrose, 10 mM-Tris/HCl, pH 7·5. This final suspension was called the 'crude membrane fraction'.

ATPase assay. 'Crude membrane fraction' (20 μl) was incubated for 10 min at 30 °C in 450 μl of 9 mM-$MgCl_2$, 50 mM-MES/KOH, pH 6·0, 10 mM-NaN_3. After preincubation the reaction was initiated by the addition of 5 mM-ATP (vanadate free). After 8 min the reaction was stopped by the addition of 2 ml 2% (v/v) H_2SO_4, 0·5% ammonium molybdate and 0·5% SDS. The phosphomolybdate was reduced with 20 μl 10% (w/v) ascorbic acid and absorbance at 750 nm was read after 10 min. Protein was estimated according to the method of Bradford (1976).

RESULTS AND DISCUSSION

Synchronization and recovery of cells

The *cdc* mutants were arrested by shifting exponentially growing cultures to their respective non-permissive temperature. G1 arrest of these mutants was established by counting the cells of the respective terminal phenotype (Hartwell *et al.*, 1973; Bedard *et al.*, 1981). In all cases 90–95% synchrony was obtained after 7–7·5 h; this was confirmed by the incorporation of [^{3}H]uracil (Hartwell, 1970). On return to the permissive temperature arrested cells are able to enter the S phase and subsequently complete a round of DNA synthesis without further protein synthesis (Hereford & Hartwell, 1974).

pH_i

The pH of the assay medium for all the cells was 5·4, for two reasons. Firstly, the external pH (pH_e) for maximum growth varied between 5·2 and 5·5. Secondly, the values from the steady state distribution of propionic acid demonstrated that pH_i was maintained fairly constant between pH_e 3·5 and 5·5 (Fig. 1). However, it increased sharply with further increase in pH_e. It was evident that the cells maintained a constant pH_i at or about pH_e 5·4, which was also the pH_e of maximum growth rate (mid-exponential phase). There was not much change in pH_e during arrest and recovery of the different cells (data not shown).

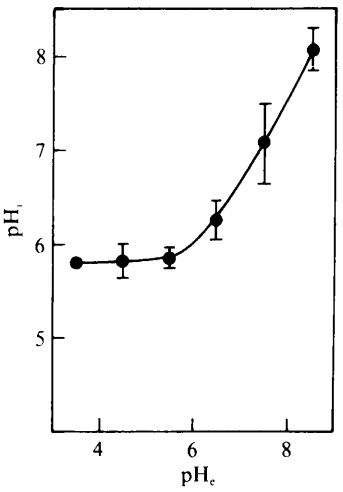

Fig. 1. pH_i of A364A cells suspended in 100 mM-Tris/citrate buffer of the indicated pH_e value. The conditions were as described in Methods. All data are means of three to four sets; bars represent SEM.

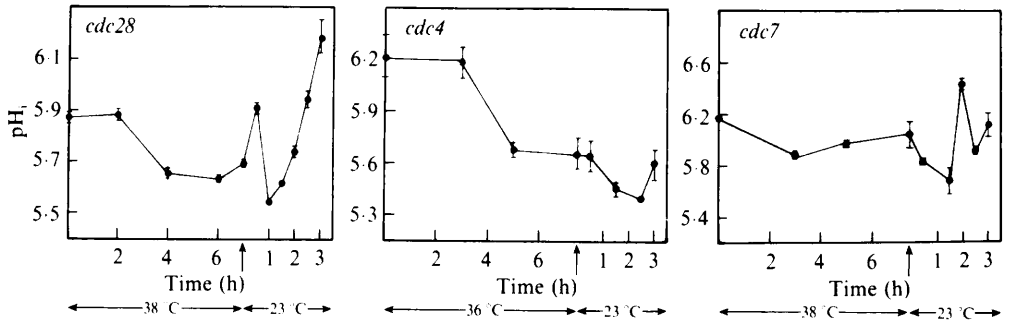

Fig. 2. pH_i during arrest and recovery of *cdc* mutants. The bars on each point represent SEM values.

Fig. 2 depicts the change in pH_i during the arrest and recovery of the three G1 mutants bearing *cdc28*, *cdc4* and *cdc7* mutations. During the 7–7·5 h taken for arrest, there was a gradual decrease in pH_i in all the cells. However, on recovery (shifting of cells to permissive temperature), the pH_i pattern among various mutants varied greatly. In the *cdc28*-bearing mutant, there was a rapid increase in pH_i within the first 30 min. This increase of 0·21 units was followed by a sharp decline up to 60 min, after which the pH_i continued to rise again. There was no significant change in pH_i in cells with the *cdc4* mutation during the initial period of recovery, whereas cells carrying a *cdc7* mutation exhibited an initial decrease in pH_i on return to permissive temperature, followed by an increase around 90 min.

The pH_i was also determined by exposure of unbuffered cell suspensions (2·5%, w/v) to nystatin (10 μg ml^{-1}) (Hauer *et al.*, 1981). Nystatin interacts with sterols of the plasma membrane to produce unspecific pores, which permit equilibration of protons across the membrane. Since it does not interact with the subcellular organelle membranes the change in proton concentration of the unbuffered suspension gives values close to true pH_i (Hauer *et al.*, 1981). By this method the pattern and magnitude of changes in pH_i observed were similar to those derived from propionate distribution, but the absolute values were slightly higher (data not shown). Although the pH_i values reported here are lower than those reported by Gillies *et al.* (1981) using ^{31}P NMR, they match with those of Cartwright *et al.* (1986), who also used the propionate distribution method. To rule out the possibility of a temperature effect on observed

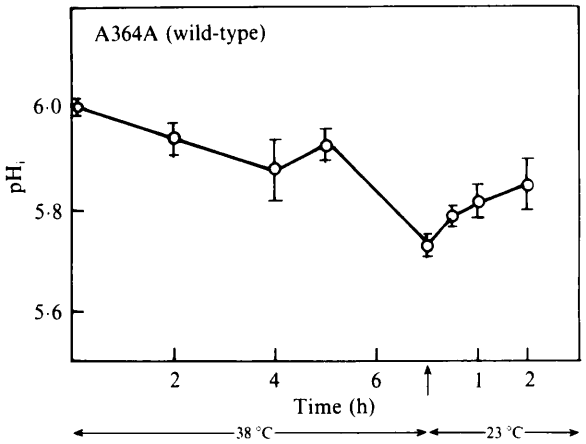

Fig. 3. Temperature effect on pH_i in wild-type cells (A364A). Mid-exponential cells were incubated at 38 °C for 7 h and subsequently shifted down to 23 °C. Similar results were obtained on incubation at other non-permissive temperatures (36 °C and 37 °C).

pH_i, it was measured in wild-type cells (A364A) subjected to a similar temperature shift-up and shift-down regimen (Fig. 3). Although a decrease in pH_i similar to that of mutants occurred during the 7 h incubation at non-permissive temperature (36, 37 or 38 °C), on shift down to 23 °C, pH_i was almost constant over a 120 min period. Therefore, the fluctuations in pH_i observed in the cell cycle mutants are specific to their arrest and recovery.

There is evidence that controls on initiation of a new cell cycle take place in steps that precede and include the *CDC28*-mediated step, and that passing start leads to commitment to the cell cycle (Hartwell, 1974; Yanishevsky & Stein, 1981). Moreover, *CDC4* and *CDC7* are expressed sequentially after *CDC28*, but prior to DNA synthesis (Hereford & Hartwell, 1974). Therefore, our observation that there is an elevation in pH_i specifically when start-arrested cells are allowed to re-enter cell cycling suggests that, like animal cells, *S. cerevisiae* cells can also use pH_i as a signal or regulatory factor for entering a new cell cycle. Gillies *et al.* (1981), using glucose-deprived synchronous cultures of *S. cerevisiae*, have also suggested that alkalinization of pH_i may accompany traversal of start. Although, because of low values of pH_i observed by us in comparison to ^{31}P NMR measurements, the rise in pH_i at start does not appear to be alkaline, nonetheless if one compares the change in pH_i with the progression of different mutants (observed by two independent methods), there is a definite pattern of increase in pH_i which is concurrent with start.

In order to confirm whether the observed increase in pH_i is start specific, three other strains, carrying *cdc36*, *cdc37* and *cdc39* mutations, were also studied. These mutants arrest with terminal phenotypes similar to the strain bearing *cdc28*, but they carry different mutations. Based on their conjugational efficiency and degree of viability at the restrictive temperature, Reed (1980) has suggested that the physiological state of *cdc28*- and *cdc37*-arrested cells is different from that of *cdc36*- and *cdc39*-arrested cells. Genetic evidence also suggests that functions of the *CDC28* and *CDC37* gene products may be related (Reed *et al.*, 1985*a*). The observed pH_i pattern strengthens these findings (Fig. 4). In SR672 (*cdc37*), as with H.185.3.4 (*cdc28*), there was a rapid increase in pH_i within 30 min of recovery from arrest. In the *cdc36*-bearing strain there was almost no change and strain SR665.1 (*cdc39*) exhibited a decrease in pH_i on release from arrest, followed by a gradual rise.

ATPase activity

In animal cells, an increase in pH_i is accompanied by the activation of Na^+/H^+ antiport (Roos & Boron, 1981; Schuldiner & Rozengurt, 1982; Doppler *et al.*, 1986; Hagag *et al.*, 1987; Kakinuma *et al.*, 1987; Ober & Pardee, 1987; Rotstein *et al.*, 1987). The movement of protons across the plasma membrane of yeast cells is controlled by a H^+-translocating ATPase which

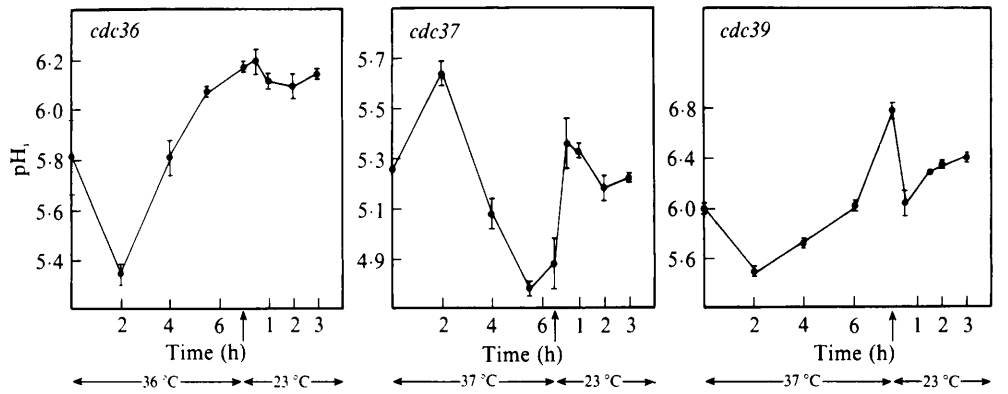

Fig. 4. pH_i during arrest and recovery in 'start' mutants. The bars on each point represent SEM values.

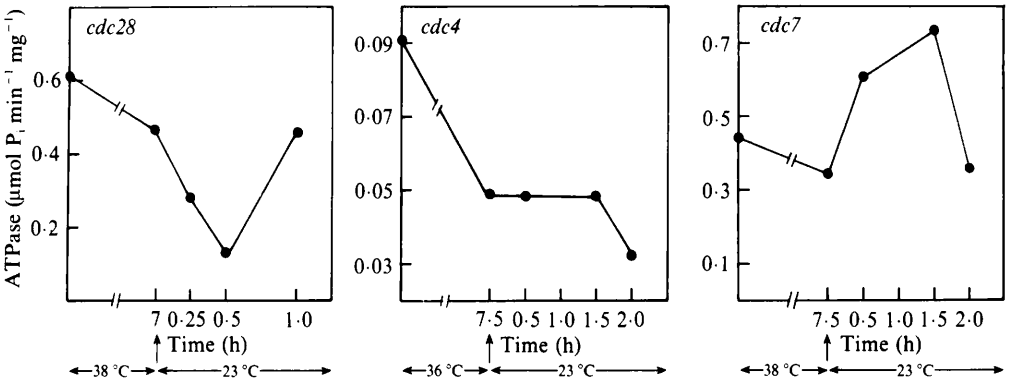

Fig. 5. Plasma membrane ATPase activity during arrest and recovery of *cdc* mutants. ATPase was assayed as described in Methods. The graph in each panel is representative of a typical experiment.

expels protons electrogenically (Goffeau & Slayman, 1981; Goffeau & Boutry, 1986; Serrano, 1988). In order to correlate the observed changes in pH_i, ATPase was assayed in crude membrane fractions isolated at the same time as for pH_i measurements (Fig. 5). The ATPase activity decreased as *cdc28*-carrying cells were allowed to recover. This coincided with the rapid increase in pH_i during that time. The activity increased during the next 30 min, with a simultaneous decline in pH_i. Similar to the pattern of pH_i values. ATPase activity in *cdc4*-bearing cells also exhibited no significant changes during recovery from arrest. However, in *cdc7*-bearing mutants ATPase activity increased at the time of shift to permissive temperature until 90 min, which was concomitant with a decrease in pH_i. The activity fell to a normal level during the next 30 min, with a simultaneous rise in pH_i (Fig. 5).

Since the changes in ATPase activity are specific to arrest at particular stages of the cell cycle, the temperature effect is ruled out. These changes correlate inversely with pH_i: whenever there is an increase in pH_i, ATPase activity goes down, and vice versa. This suggests that the plasma membrane ATPase activity, or in other words the rate of H^+ efflux, may be self-controlled by an internal H^+ regulatory site. A similar situation exists for the Na^+/K^+ ATPase in animal cells, which reportedly shuts off when the pH_i rises above a critical value and leads to the activation of Na^+/H^+ antiport (Pouyssegur, 1985). Such an antiport is not known in yeasts; however the possibility of a K^+/H^+ antiport exists, since flux of potassium has been associated as a counter-ion for ATPase-linked proton efflux (Eilam *et al.*, 1984; Blatt & Slayman, 1987; Glaser & Hofer, 1987). It is possible that *S. cerevisiae* has a functional K^+/H^+ antiport for rapid and fine-tuning of intracellular pH.

S.A. gratefully acknowledges an SRF award from the University Grants Commission, India. This work was supported in part by a grant from DST (no. 22(7P/22)84 STP II), ICMR (no. 5/11(1)/87-BMS-II (8700080)), New Delhi, India, and the special assistance of University Grants Commission under the COSIST Programme to the School of Life Sciences.

REFERENCES

AERTS, R. J., DURSTON, A. J. & KONIJN, T. M. (1987). Cytoplasmic pH at the onset of development in *Dictyostelium*. *Journal of Cell Science* **87**, 423–430.

ANAND, S. & PRASAD, R. (1987). Status of calcium influx in cell cycle of *S. cerevisiae*. *Biochemistry International* **14**, 963–970.

BEDARD, D. P., JOHNSTON, G. C. & SINGER, R. A. (1981). New mutations in the yeast *Saccharomyces cerevisiae* affecting completion of 'start'. *Current Genetics* **4**, 205–214.

BLATT, M. R. & SLAYMAN, C. L. (1987). Role of active potassium transport in the regulation of the cytoplasmic pH by non animal cells. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 2737–2741.

BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **72**, 248–254.

BRAVO, R. & BRAVO, H. M. (1986). Effect of pH on the induction of competence and progression to the S-phase in mouse fibroblasts. *FEBS Letters* **195**, 309–312.

CARTWRIGHT, C. P., JUROSZEK, J. R., BEAVEN, M., RUBY, M. S., DE MORAIS, S. M. F. & ROSE, A. H. (1986). Ethanol dissipates the proton motive force across the plasma membrane of *Saccharomyces cerevisiae*. *Journal of General Microbiology* **132**, 369–377.

DOPPLER, W., MALY, K. & GRUNICKE, H. (1986). Role of Na^+/H^+ antiport in the regulation of the internal pH of Ehrlich Ascites tumor cells in culture. *Journal of Membrane Biology* **91**, 147–155.

DUDANI, A. K. & PRASAD, R. (1983). Amino acid transport: its role in cell division and growth of *Saccharomyces cerevisiae* cells. *Biochemistry International* **7**, 15–22.

DUDANI, A. K. & PRASAD, R. (1984*a*). Coupling between phosphatidylinositol metabolism and *CDC28* gene product of *Saccharomyces cerevisiae*. *FEBS Letters* **167**, 151–154.

DUDANI, A. K. & PRASAD, R. (1984*b*). Glyoxalase-1 activity and cell cycle regulation in yeast. *Biochemical and Biophysical Research Communications* **119**, 962–967.

DUDANI, A. K., TRIVEDI, A. & PRASAD, R. (1983). The possible functional significance of phosphatidylinositol in G1 arrest of *Saccharomyces cerevisiae*. *FEBS Letters* **153**, 34–36.

EILAM, Y., LAVI, H. & GROSSOWICZ, N. (1984). Effect of inhibitors of plasma-membrane ATPase on potassium and calcium fluxes, membrane potential and proton motive force in the yeast *Saccharomyces cerevisiae*. *Microbios* **41**, 177–189.

GILLIES, R. J., UGURBIL, K., DEN HOLLANDER, J. A. & SHULMAN, R. G. (1981). ^{31}P NMR studies of intracellular pH and phosphate metabolism during cell division cycle of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 2125–2129.

GLASER, H. U. & HOFER, M. (1987). Ion dependent generation of the electrochemical gradient $\Delta\mu H^+$ in reconstituted plasma membrane vesicles from the yeast *Metschnikowia reukaufi*. *Biochimica et biophysica acta* **905**, 287–294.

GOFFEAU, A. & BOUTRY, M. (1986). Three proton pumping ATPases in yeast. *Microbiological Sciences* **3**, 164–168.

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

HAGAG, N., LACAL, J. C., GRABER, M., AARONSON, S. & VIOLA, M. V. (1987). Microinjection of ras p21 induces a rapid rise in intracellular pH. *Molecular and Cellular Biology* **7**, 1984–1988.

HARTWELL, L. H. (1970). Periodic density fluctuations during the yeast cell cycle and the selection of synchronous cultures. *Journal of Bacteriology* **104**, 1280–1285.

HARTWELL, L. H. (1974). *Saccharomyces cerevisiae* cell cycle. *Bacteriological Reviews* **38**, 164–198.

HARTWELL, L. H., MORTIMER, R. K., CULOTTI, J. & CULOTTI, M. (1973). Genetic control of the cell division cycle in yeast, V. Genetic analysis of *cdc* mutants. *Genetics* **74**, 267–286.

HARTWELL, L. H., CULOTTI, J., PRINGLE, J. R. & REID, B. J. (1974). Genetic control of the cell division cycle in yeast. *Science* **183**, 46–51.

HAUER, R., UHLEMANN, G., NEUMANN, J. & HOFER, M. (1981). Proton pumps of plasmalemma of *Rhodotorula gracilis*. *Biochimica et biophysica acta* **649**, 680–690.

HEREFORD, L. M. & HARTWELL, L. H. (1974). Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. *Journal of Molecular Biology* **84**, 445–461.

KAKINUMA, Y., SAKAMAKI, Y., ITO, K., CRAGOE, E. J. & IGARASHI, K. (1987). Relationship among activation of the Na^+/H^+ antiporter, ornithine decarboxylase induction, and DNA synthesis. *Archives of Biochemistry and Biophysics* **259**, 171–178.

LOPO, A. & VACQUIER, V. (1977). The rise and fall of intracellular pH of sea urchin eggs after fertilisation. *Nature, London* **269**, 590–592.

MEDENHALL, M. D., JONES, C. A. & REED, S. I. (1987). Dual regulation of the yeast *CDC28*-p40 protein kinase complex: cell cycle, pheromone and nutrient limitation effects. *Cell* **50**, 927–935.

OBER, S. S. & PARDEE, A. B. (1987). Intracellular pH is increased after transformation of chinese hamster embryo fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 2766–2770.

PATTERSON, M., SCLAFANI, R. A., FANGMAN, W. L. & ROSAMOND, J. (1986). Molecular characterisation of

cell cycle gene *CDC7* from *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **6**, 1590–1598.

POUYSSEGUR, J. (1985). The growth factor-activatable Na^+/H^+ exchange system, a genetic approach. *Trends in Biochemical Sciences* **10**, 453–455.

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.

PRINGLE, J. R. & HARTWELL, L. H. (1981). The *Saccharomyces cerevisiae* cell cycle. In *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, pp. 97–142. Edited by J. N. Strathern, E. W. Jones & J. R. Broach. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

REED, S. I. (1980). The selection of *S. cerevisiae* mutants defective in the 'start' event of cell division. *Genetics* **95**, 561–577.

REED, S. I., DE BARRES LOPES, M. A., FERGUSON, J., HADWIGER, J. A., HO, J. Y., HORWITZ, R., JONES, C. A., LORINCZ, A. T., MEDENHALL, M. D., PETERSON, T. A., RICHARDSON, S. & WITTENBURG, C. (1985*a*). Genetic and molecular analysis of division control in yeast. *Cold Spring Harbor Symposia on Quantitative Biology* **50**, 627–634.

REED, S. I., HADWIGER, J. A. & LORINCZ, A. T. (1985*b*). Protein kinase activity associated with the product of the yeast cell division cycle gene *CDC28*. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 4055–4059.

ROOS, A. & BORON, W. F. (1981). Intracellular pH. *Physiological Reviews* **61**, 296–434.

ROTSTEIN, O. D., HOUSTON, K. & GRINSTEIN, S. (1987). Control of cytoplasmic pH by Na^+/H^+ exchange in rat peritoneal macrophages activated with phorbol ester. *FEBS Letters* **215**, 223–227.

SCHULDINER, S. & ROZENGURT, E. (1982). Na^+/H^+ antiport in Swiss 3T3 cells: mitogenic stimulation leads to cytoplasmic alkalinization. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 7778–7782.

SERRANO, R. (1988). Structure and function of the proton translocating ATPase in plasma membranes of plants and fungi. *Biochimica et biophysica acta* **947**, 1–28.

STEINHARDT, R. & MAZIA, D. (1973). Development of K^+ conductance and membrane potentials in unfertilized sea urchin eggs after exposure to NH_4OH. *Nature, London* **241**, 400–401.

STEWART, E., GOW, N. A. R. & BOWEN, D. V. (1988). Cytoplasmic alkalinization during germ tube formation in *Candida albicans*. *Journal of General Microbiology* **134**, 1079–1087.

TUDURI, P., NSO, E., DUFUOR, J. & GOFFEAU, A. (1985). Decrease of plasma membrane H-ATPase activity during late exponential growth of *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications* **133**, 917–922.

YANISHEVSKY, R. M. & STEIN, G. H. (1981). Regulation of cell cycle in eukaryotic cells. *International Review of Cytology* **69**, 223–259.