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# $^{31}$P NMR studies of intracellular pH and phosphate metabolism during cell division cycle of *Saccharomyces cerevisiae*

(ATP/polyphosphate)

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**ABSTRACT** **We have analyzed changes in intracellular pH and phosphate metabolism during the cell cycle of *Saccharomyces cerevisiae* (NCYC 239) by using high-resolution $^{31}$P NMR spectroscopy. High-density yeast cultures ($2 \times 10^8$ cells per ml) were arrested prior to "start" by sequential glucose deprivation, after which they synchronously replicated DNA and divided after a final glucose feeding. Oxygenation of arrested cultures in the absence of glucose led to increased levels of sugar phosphates and ATP and an increase in intracellular pH. However, these conditions did not initiate cell cycle progression, indicating that energization is not used as an intracellular signal for initiation of the cell division cycle and that the cells need exogenous carbon sources for growth. Glucose refeeding initiated an alkaline intracellular pH transient only in the synchronous cultures, showing that increased intracellular pH accompanies the traversal of start. Changes in phosphate flow and utilization also were observed in the synchronous cultures. In particular, there was increased consumption of external phosphate during DNA synthesis. When external phosphate levels were low, the cells consumed their internal polyphosphate stores. This shows that, under these conditions, polyphosphate acts as a phosphate supply.**

$^{31}$P NMR spectroscopy is rapidly gaining in importance as a technique for the noninvasive analysis of phosphorus-containing metabolites and intracellular pH *in vivo*. Since the first $^{31}$P NMR spectra of yeast were published in 1975 (1), sensitivity and resolution have been improved to allow for the investigation of glycolytic mutants (2), the control of glycolysis (3), and compartmentation of pH in ascospores (4). In this communication, we present the results of a $^{31}$P NMR analysis of synchronous suspensions of yeast cells in which we have investigated intracellular pH and phosphorus metabolism during the cell division cycle.

Yeast are an ideal system for investigating the cell division cycle. We have found that they can be maintained for long periods at the high densities ($2 \times 10^8$ cells per ml) necessary to obtain good signal-to-noise in the NMR experiments. In addition, many temperature-sensitive cell division cycle mutants of *Saccharomyces cerevisiae* are available, allowing for more detailed analyses in the future.

Concepts of the cell division cycle in yeast have changed in recent years. Using the cell division cycle mutant system, Hartwell and his colleagues (5–10) have identified the presence of two loosely coupled subcycles, each comprising a series of discrete, interdependent steps. One subcycle involves the nuclear events of DNA replication and nuclear division; the other consists of the cytoplasmic events governing bud emergence and growth. The subcycles converge prior to mitosis and diverge again after a point called "start"; this point bears some similarity to the R point of mammalian cells (11). Although much is known with regard to the morphologic and genetic aspects of "start," its biochemistry is relatively unknown. Most reports to date have indicated that traversal of "start" is dependent on protein synthesis (12–14).

Recent reports have indicated the presence of alkaline shifts in intracellular pH prior to DNA synthesis in synchronous cultures of *Tetrahymena* (15) and *Physarum* (16). Increased intracellular pH has been shown to hasten the initiation of protein synthesis (17) and increase polyadenylylation of mRNA (18) in some systems. Alkaline intracellular pH has also been shown to increase the rate of glycolysis in diverse cell types (see ref. 19 for review). In addition, increased pH in the medium has been demonstrated to increase the rate of cell cycle initiation in yeast (20). A major question in the present investigation is whether or not intracellular pH changes during the cell cycle in yeast.

Phosphate metabolism in yeast is relatively well studied, yet it is incompletely understood. The most abundant phosphate-containing compound in *Saccharomyces* is polyphosphate, a polymer of phosphate residues primarily contained in vacuoles (21). A recent report indicates that 85% of the cellular polyphosphate is a single large ($M_r \approx 240{,}000$) polymer and the remaining 15% is comprised of oligophosphate with an average chain length of 10 phosphate residues (22).

The fact that the phosphate bonds are high energy has prompted many researchers to suggest that polyphosphate acts as a phosphogen or energy store (23). Other groups have suggested that polyphosphate is the preferred substrate for the hexokinase reaction (24); some indicate that polyphosphate is most importantly a phosphate store to be used during phosphate deprivation (25). These various hypotheses are not mutually exclusive and, in fact, the proper question to ask is: To what extent, and under what circumstances, does polyphosphate function as a phosphogen, substrate, or phosphate store? In the present study we followed the concentrations of polyphosphate during discrete periods of the cell division cycle.

## METHODS

**Chemicals.** Growth medium (YEPD) contained 2% Bacto-Peptone (Difco), 1% yeast extract (Difco), and 2% D-glucose. Starvation buffer (SB) contained 2.5 mM $MgCl_2$, 10 mM KCl, and 2.4 mM $CaCl_2$. Infusion medium used in the NMR experiments was 10% Bacto-Peptone/5% yeast extract/20% D-glucose. Prior to each experiment, 100 $\mu$l of Antifoam B (Sigma) was added per 10 ml of infusion medium. The infusion medium contained approximately 8 mM endogenous orthophosphate. High-phosphate infusion medium was prepared by addition of potassium phosphate to a final concentration of 15 mM. Glycerol phosphorylcholine was obtained from P-L Biochemicals as a 50 mg/ml aqueous solution and was used without dilution.

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**Cells.** *S. cerevisiae* strain NCYC 239 was grown on YEPD into late stationary phase and synchronized according to the method of Williamson and Scopes (26). This procedure first selects for large cells by velocity gradient centrifugation and then successively subjects the culture to aerobic feeding, aerobic starvation, and anaerobic starvation. At the end of the treatments, the culture is resuspended in SB to a concentration of $2 \times 10^8$ cells per ml and stored in 2°C for up to 1 week. For storage periods greater than 1 week, synchronous cultures were treated to two additional refeedings. This procedure maintained the stock cultures in a synchronous state. Asynchronous stocks were obtained from late stationary cultures from which the small cells had been removed by velocity gradient centrifugation. These were also stored at 2°C until used.

**Experimental.** At the time of the experiments, 17 ml of the cell suspension was placed in a 20-mm NMR tube with 25 μl of Antifoam B, capped with a manifold containing aeration and feeding tubes, and placed in the probe. A 95% $N_2$/5% $CO_2$ mixture was passed for 15 min through the upper and lower bubblers at 300 and 20 ml/min, respectively, to allow for convective equilibration. Experiments were begun by substituting 95% $O_2$/5% $CO_2$ for the nitrogen mixture; 20–30 min later, 4.0 ml of infusion medium was injected into the suspension through the manifold. Thereafter, the infusion medium was introduced into the suspension at a rate of 3.3 ml/hr. The conditions as described have allowed for uninterrupted synchronous growth for up to 5 hr.

Synchrony was quantified with respect to both DNA synthesis and degree of budding. DNA synthesis was determined by a modification of the procedure introduced by Simchen *et al.* (27). Synchronous cultures were incubated with 100 μCi (1 Ci = $3.7 \times 10^{10}$ becquerels) of [$^3$H]uracil (New England Nuclear). At different times after feeding, 0.2-ml aliquots were added to 1 ml of 2.0 M NaOH and incubated at room temperature overnight. The samples were then made 5% in trichloroacetic acid and kept ice cold for 30 min. The remaining DNA was pelleted, resuspended in 95% EtOH, and collected by filtration on Whatman GF/C filters. Radioactivity was determined in a Beckman LS200 scintillation counter. Budding index was determined microscopically on glutaraldehyde-fixed aliquots from the samples.

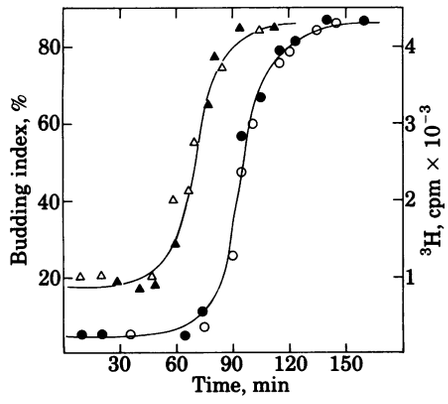


FIG. 1. DNA synthesis (▲, △) and budding index (●, ○) as a function of time after addition of food to synchronous *S. cerevisiae* NCYC 239. Cultures were synchronized by sequential glucose deprivation and resuspended in starvation buffer to a density of $2 \times 10^8$ cells per ml. Then, 17 ml of this stock suspension was placed in a 20-mm NMR tube at 25°C and either $N_2$ (●, ▲) or $O_2$ (○, △) was passed through the suspension for 30 min prior to feeding. At time 0, $O_2$ was bubbled through suspension at 300 ml/min, and 4 ml of infusion medium was added to the cultures; thereafter, medium was injected at 3.3 ml/hr. Also at time 0, 75 μCi of [$^3$H]uracil was added to the suspension. At times indicated, 100-μl aliquots were removed for determination of either budding index or $^3$H incorporation into DNA. Budding index is expressed as % cells with buds; DNA synthesis is expressed as cpm as determined by liquid scintillation counting of base- and acid-extracted cellular precipitate.

**Data Acquisition and Analysis.** The spectrometer used was a Brucker WH 360 WB operating at 145.78 MHz in pulsed Fourier transform mode; 70° pulses were generated every 0.5 sec and data were stored every 600 scans. At the end of each experiment, 100 μl of glycerol phosphorylcholine and 100 μl of 1.0 M phosphate buffer (pH 5.5) were added for calibration purposes. The glycerol phosphorylcholine is known to resonate at −0.49 ppm and was used to calibrate the chemical shifts of orthophosphates for the estimation of intra- and extracellular pH. The phosphate was used to calibrate concentrations in each experiment. The partial saturation of resonances stemming from rapid pulsing were corrected by comparison with fully relaxed spectra. Under our conditions, external phosphate and polyphosphate were saturated by 22% and 33%, respectively. The concentration of phosphate under each peak was estimated by using the correction for peak saturation, the calibration for concentration, and the number of cells present in the suspension. Intensities reported in Figs. 4 and 5 for polyphosphate are ap-

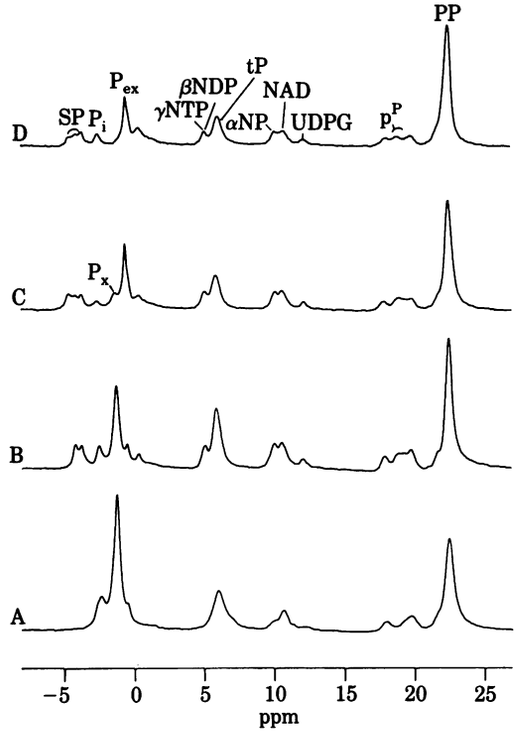


FIG. 2. Representative $^{31}$P NMR spectra of synchronous *S. cerevisiae* NCYC 239. A 17-ml culture (containing $2 \times 10^8$ cells per ml) in a 20-mm NMR tube was placed in a Brucker WH-360-WB spectrometer operating at 145.78 MHz; 45-μsec (75°) pulses were generated every 500 msec, and each spectrum represents the time average of 600 free induction decays. Spectra: A, from culture aerated with 95% $N_2$/5% $CO_2$ in starvation buffer; B, 10 min after switch to 95% $O_2$/5% $CO_2$; C and D, cultures 10 min and 60 min after addition of 4 ml of infusion medium, respectively. Peaks were assigned according to the findings of Navon *et al.* (2). Chemical shifts are reported as ppm relative to phosphoric acid. SP, phosphomonoesters (sugar phosphates); $P_i$, intracellular orthophosphate; $P_{ex}$, extracellular orthophosphate; γNTP, terminal phosphate of nucleoside triphosphates; βNDP, terminal phosphate of nucleoside diphosphates; αNP, primary phosphate of nucleoside phosphates; NAD, nicotinimideadenine dinucleotide (oxidized); UDPG, uridine diphosphorylglucose; $p^P$, penultimate phosphates of tri- and polyphosphates; PP, middle peaks of polyphosphate chains; $P_x$, orthophosphate possibly in polyphosphate vacuoles; tP, terminal phosphates of pyro-, oligo-, and polyphosphates.

proximately μmol of $P_i$ per $10^8$ cells, and external $P_i$ intensity units are approximately mM. Under our conditions, 1 g (wet weight) equals $3 \times 10^9$ cells.

## RESULTS

Yeast cultures prepared as described in *Methods* synchronously replicate DNA and bud after a final glucose feeding. These results are shown in Fig. 1 for cultures containing $2 \times 10^8$ cells per ml. The degree of synchrony shown here is essentially identical to that reported elsewhere for more dilute cellular suspensions (26).

The oxygenation of the synchronized cell suspension prior to the addition of infusion medium was designed to mimic the aerobic starvation encountered during the synchronization procedure. Oxygenation increased cellular stores of ATP and sugar phosphates and increased intracellular pH (Fig. 2, spectra A and B). The increase in intracellular pH is manifested by the downfield shift of the internal orthophosphate peak. These changes show that oxygen induces catabolism of an endogenous carbon source (see *Discussion*). We have seen that this response to oxygen is independent of synchrony (2). Initiation of the cell division cycle occurred in response to glucose addition. Glucose feeding induced rapid changes in the spectra (Fig. 2, spectrum C). Most notably, the sugar phosphate pool became more heterogenous, the concentration of intracellular orthophosphate decreased, and intracellular pH rose higher. After some time, the spectrum changed as illustrated by spectrum D in Fig. 2. The complete time courses for intracellular pH, polyphosphate, and external phosphate levels are given in Figs. 3–5.

Fig. 3 illustrates the intracellular pH changes of cells in response to oxygenation and feeding. Data are presented for asynchronous and synchronous cultures. Some of the synchronous cultures were preoxygenated prior to feeding and some were not. Clearly, synchronous cultures undergo a transient intracellular pH alkalinization upon feeding, whereas asynchronous cultures do not.

The external $P_i$ level in the sample is determined by the rate of infusion of the phosphate-containing medium and the rate of consumption by the cells. In the initial 20–30 min after refeeding, external $P_i$ intensity increases (Fig. 4). Subsequently, the intensity started to decrease, indicating that, after a lag time of 20–30 min, cells begin consuming phosphate more rapidly than it is being infused. During the initial 20–30 min, there is a complex "early" net consumption of polyphosphate. In cultures fed with low-phosphate (0.8 mM) infusion medium, external orthophosphate was virtually depleted within 60 min, after which polyphosphate was consumed. In contrast, cells fed with high-phosphate (5 mM) infusion medium began rapid utilization of exogeneous $P_i$ 20–30 min after refeeding but a nonzero steady-state level of external $P_i$ was achieved approximately 60 min after refeeding and was maintained for several hours. This shows that, in the presence of an adequate supply of exogeneous $P_i$, polyphosphate is not consumed. Hence, although the rapid consumption of phosphate seems to be characteristic of the period of the cell cycle during DNA synthesis, the use of external orthophosphate or internal polyphosphate as a phosphate source depends on whether or not the external $P_i$ supply is adequate. In other words, the "late" consumption of polyphosphate seen in the case of low-phosphate infusion seems to be solely in response to the depletion of phosphate from the medium. However, neither the lag time for external phosphate consumption nor the early consumption of polyphosphate is affected by the amount of orthophosphate in the medium.

The behaviors of stored polyphosphate and phosphate consumption in asynchronous cultures are illustrated in Fig. 5. As shown by these data, asynchronous cells also exhibit a 30-min lag prior to the commencement of phosphate consumption. However, the early consumption of polyphosphate seen in the synchronous cultures was absent. Under the first condition (solid symbols), cells were grown in phosphate-rich (5 mM) YEPD prior to harvest. These cultures contain a moderately high level of polyphosphate. Under these conditions, there was a slight, rapid consumption of polyphosphate in response to feeding. These stores were replenished upon commencement of external phosphate consumption 30 min later. To test whether asynchronous cultures exhibit a fixed response under our conditions, cells were grown in low-phosphate (0.8 mM) YEPD, harvested, and stored at 2°C for 3 weeks prior to use. As shown by the open figures, these cells had no appreciable polyphosphate stores. The polyphosphate levels in this system increased somewhat upon refeeding and increased dramatically 30 min later upon consumption of external phosphate. This phenomenon has been described earlier as *Überkompensation* (25).

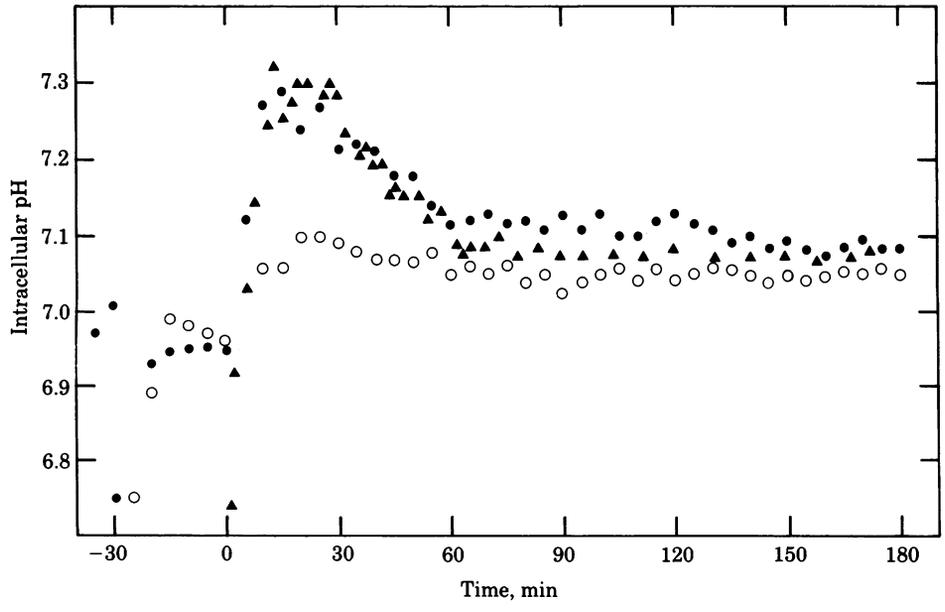


FIG. 3. Intracellular pH as a function of time after refeeding in synchronous (●, ▲) and asynchronous (○) cultures. The asynchronous sample and one synchronous sample (●) were preoxygenated for 30 min prior to addition of food at time 0. The other synchronous suspension (▲) was aerated with 95% $N_2$/5% $CO_2$ for 30 min before feeding. At time 0, 4 ml of infusion medium was added to 17 ml of cell suspension containing $2 \times 10^8$ cells per ml in starvation buffer. Intracellular pH was estimated from the chemical shift of the intracellular orthophosphate peak by comparison with a standard curve of chemical shift vs. pH. The standard curve was determined at 20°C in M9 medium (2).

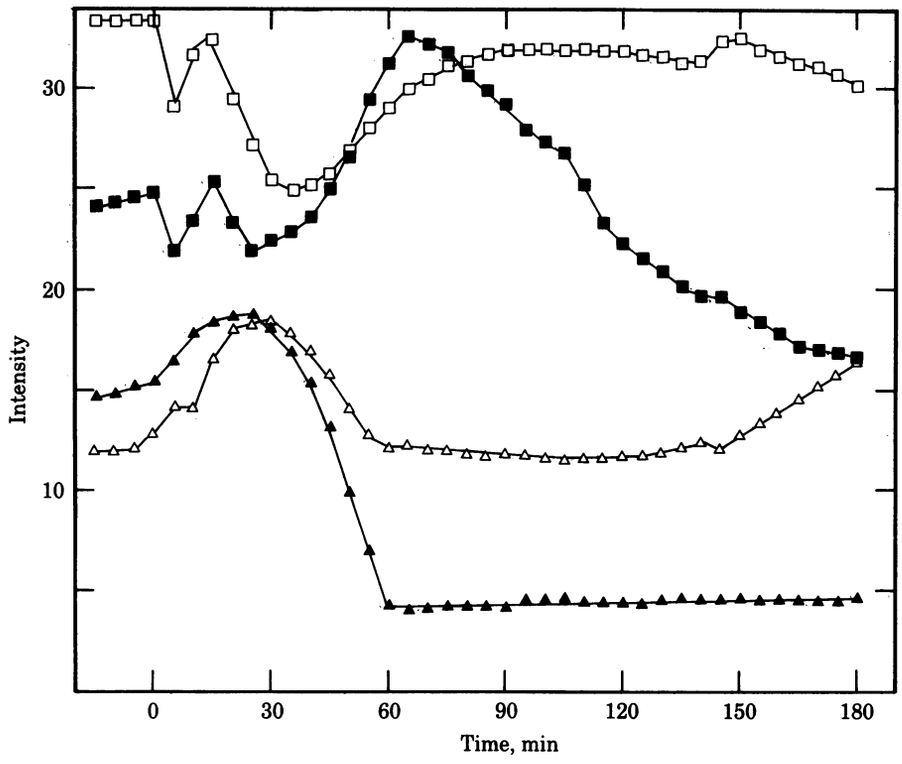


FIG. 4. Polyphosphate (□, ■) and extracellular orthophosphate (△, ▲) as a function of time after addition of infusion medium to synchronous cultures of *S. cerevisiae* NCYC 239. Culture conditions were as in Fig. 2. Intensities are approximately μmol of P per $10^8$ cells for polyphosphate and mmol per liter for external orthophosphate. Solid symbols represent cultures fed with low-phosphate (0.8 mM) infusion medium. Open symbols represent cultures fed with high-phosphate (5 mM) infusion medium. Phosphate flows fall into three discrete periods: 0–30 min = strict polyphosphate consumption; 30–60 min = rapid external orthophosphate consumption; 60–180 min = preferred external orthophosphate consumption.

## DISCUSSION

Under the experimental conditions described in this communication, yeast cells synchronously replicated their DNA and budded at densities 2 orders of magnitude greater than previously reported (26), enabling us to follow the cell cycle under NMR conditions. Conditions appropriate for high-density synchronous growth were achieved primarily through adequate oxygenation and delivery of nutrients.

The synchronous suspensions were arrested at a point prior to "start" by cycles of starvation and refeeding. At the beginning of the synchronization procedure, cells are in stationary phase and are, by definition, randomly distributed throughout $G_0$. The 30-min refeeding schedule energizes the cells for a time and, in this way, brings them towards the $G_0/G_1$ interface. After refeeding, cultures are starved aerobically for 6 hr. This treatment does not deenergize the cells (Fig. 2), but the absence of an exogenous carbon source prevents cell cycle initiation. Cells thus poised will synchronously exit $G_0$ if presented with a glucose stimulus for longer than 30 min. It is interesting to note

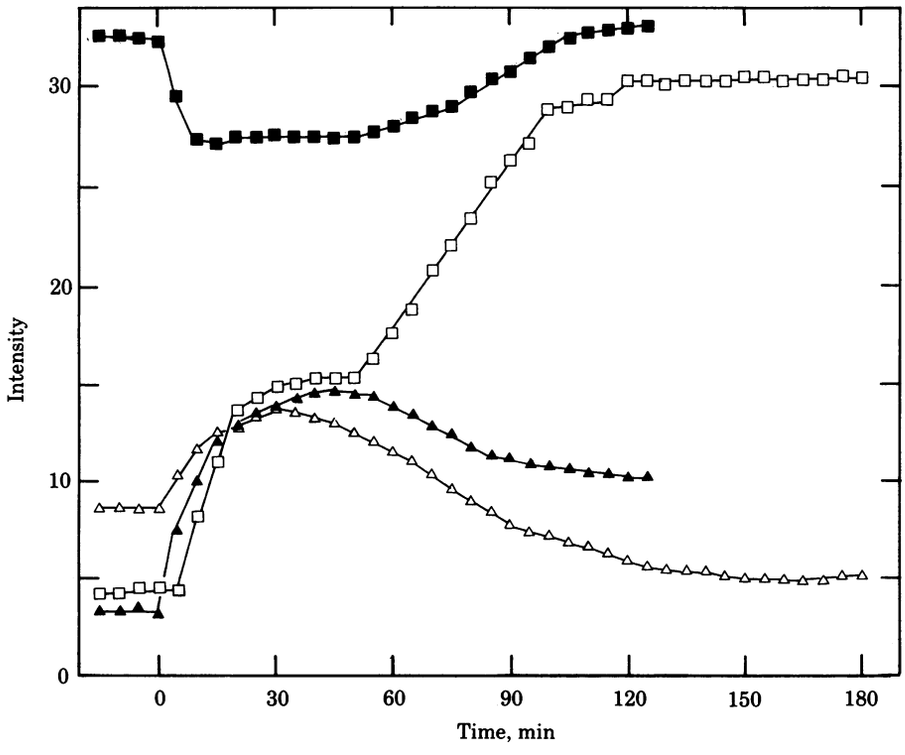


FIG. 5. Polyphosphate (□, ■) and external orthophosphate (△, ▲) as a function of time after addition of infusion media to asynchronous cultures of *S. cerevisiae* NCYC 239. Conditions were as in Fig. 4, except that asynchronous cultures were used. Solid symbols represent data from stationary cultures grown on phosphate-rich (5 mM) YEPD and refed with low-phosphate infusion medium. Open symbols represent cultures grown on low-phosphate (0.8 mM) YEPD and refed with low-phosphate infusion medium.

that changes in intracellular pH and phosphate metabolism occur 30 min after refeeding (Figs. 3–5). It is not known whether these parameters are essential to the synchronization process.

Intracellular pH is gaining recognition as being a reproducible variable during stages of cell growth. It was first postulated to change reproducibly throughout the cell cycle by Frydenberg and Zeuthen (28). To date, such changes have been demonstrated in at least two systems (15, 16). In *Tetrahymena*, these shifts have been proposed to correlate with the nuclear subcycle and not the cytoplasmic. The alkaline transient demonstrated here for *S. cerevisiae* is similar to previous reports in that it is virtually complete prior to the initiation of DNA synthesis. It is possible that increased pH acts as a signal for the initiation of DNA synthesis. Increased intracellular pH has been demonstrated to increase the rates of glycolysis and protein synthesis, two events necessary for the initiation of DNA synthesis (reviewed in ref. 19). The questions remaining in this system include understanding the cause of the pH shift and its direct metabolic consequences. We note that polyphosphate hydrolysis occurs at the same time as the pH jump. However, phosphate ester hydrolysis by itself should lead to a lower pH, indicating that an increase in pH cannot be caused by polyphosphate consumption.

An important observation from this study is that oxygenated cells in starvation buffer contain a visible amount of ATP (≈3 mM) yet do not initiate cell cycle progression. In parallel studies, we have seen that starved cells are able to synthesize ATP upon oxygenation and that this response is independent of glycolysis (2). It seems likely that the cells are oxidizing readily available substrates such as amino acids or fatty acids for ATP production. These results clearly show that the signal for cell cycle initiation is created during the transport and catabolism of glucose and not by the energy supplied in the process.

Glucose phosphorylation might act as a signal through rapid depletion of internal orthophosphate stores, as seen in Fig. 2 (cf. ref. 3). However, a similar decrease in intracellular $P_i$ and an equivalent increase in the sugar phosphate and ATP peaks has been observed within the first few minutes of feeding glucose to asynchronous cells. Hence, this flow is not correlated with the traversal of "start" in the cell division cycle. The absence of a correlation does not mean that this flow does not contain the "start" signal for those cells that are ready. It does mean, though, that the decrease in intracellular $P_i$ upon glucose feeding is not caused by traversal of start.

The synchronous suspension allows us to follow the phosphate flow during different periods of the cells division cycle. Immediately after glucose feeding the polyphosphate level decreases (Fig. 4). The increase occurring 10–20 min after feeding possibly can be explained by the disappearance of an inorganic phosphate peak attributed to the polyphosphate vacuoles (2) (Fig. 2). If that is the case, then the total intensity of that peak plus the polyphosphate decreases slowly for the first 30 min. In the asynchronous cells (Fig. 5), there is a similar decrease in the high concentrations of polyphosphate that occur in the first 10 min. Because of kinetic differences, it is not definite that these two initial decreases are identical, but they are qualitatively similar. In this event, this initial decrease of polyphosphate would not be cell cycle dependent. However, shortly afterward, there is a rapid decrease of external phosphate and a simultaneous increase in polyphosphate, continuing for about 60 min. DNA synthesis is initiated during this period. There are no corresponding changes in the asynchronous suspensions, indicating that these flows are related to the cell division cycle.

Finally, the third period starts at about 60 min and continues until about 180 min. This is the period starting with DNA synthesis and shows phosphate flows that are observed only in the synchronous suspensions. In this period (Fig. 4), if there is a high level of external orthophosphate, the polyphosphate remains constant. However, if there is a low level of external phosphate, polyphosphate is consumed. Hence, in this period it is clear that polyphosphate can act as a substitute phosphate store, in place of external phosphate. It is not known whether the external phosphate, on its way to being consumed during some biosynthesis pathway, goes through the polyphosphate pool or not. However, this can be easily measured by radioactive tracer techniques, which will also determine the end products of phosphate consumption. We do wish to emphasize that, in the third period, polyphosphate can serve as a substitute source of phosphate, in place of external orthophosphate. It remains to be seen whether the polyphosphate used in this way contributes energy as well as phosphate to the cells.


1. Salhany, J. M., Yamane, T., Shulman, R. G. & Ogawa, S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4966–4970.
2. Navon, G., Shulman, R. G., Yamane, T., Eccleshall, J. R., Lam, K. B., Baronofsky, J. J. & Marmur, J. (1979) *Biochemistry* **18**, 4487–4499.
3. den Hollander, J. A., Brown, T. R., Ugurbil, K. & Shulman, R. G. (1981) *Biochemistry*, in press.
4. Barton, J. K., den Hollander, J. A., Lee, T. M., Maclaughlin, A. & Shulman, R. G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2470–2473.
5. Hartwell, L. H., Culotti, J. & Reid, B. (1970) *Proc. Natl. Acad. Sci. USA* **66**, 352–359.
6. Hartwell, L. H. (1971) *J. Mol. Biol.* **59**, 183–194.
7. Hartwell, L. H. (1971) *Exp. Cell Res.* **69**, 265–276.
8. Hartwell, L. H., Mortimer, R. K. & Culotti, M. (1973) *Genetics* **74**, 267–286.
9. Hartwell, L. H., Culotti, J., Pringle, J. R. & Reid, B. J. (1974) *Science* **183**, 46–51.
10. Hartwell, L. H. (1976) *J. Mol. Biol.* **104**, 803–817.
11. Pardee, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1286–1290.
12. Wolfner, M., Yep, D., Messenguy, F. & Fink, G. R. (1975) *J. Mol. Biol.* **96**, 273–290.
13. Unger, M. W. & Hartwell, L. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1664–1668.
14. Shilo, B., Simchen, G. & Pardee, A. B. (1978) *J. Cell. Physiol.* **97**, 177–188.
15. Gillies, R. J. & Deamer, D. W. (1979) *J. Cell. Physiol.* **100**, 23–32.
16. Gerson, D. F. & Burton, A. C. (1976) *J. Cell Physiol.* **91**, 297–304.
17. Brandis, J. & Raff, R. (1979) *Nature (London)* **278**, 467–469.
18. Wilt, F. H. & Mazia, D. (1974) *Dev. Biol.* **37**, 422–424.
19. Gillies, R. J. (1981) in *The Transformed Cell*, eds. Cameron, I. & Pool, T. B. (Academic, New York), pp. 347–395.
20. Thomas, K. C. (1981) *J. Bacteriol*, in press.
21. Urech, K., Duerr, M., Boller, Th. & Wiemken, A. (1978) *Arch. Microbiol.* **116**, 275–278.
22. Jacobson, L., Halmann, M. & Yariv, J. (1981) *Eur. J. Biochem.*, in press.
23. Felter, S. & Stahl, A. J. C. (1973) *Biochimie* **55**, 245–251.
24. Van Steveninck, J. (1969) *Arch. Biochem. Biophys.* **130**, 244–252.
25. Weinberg, R. (1975) *J. Bacteriol.* **121**, 1122–1130.
26. Williamson, D. H. & Scopes, A. W. (1960) *Exp. Cell. Res.* **20**, 338–349.
27. Simchen, G., Pinyon, R. & Salts, Y. (1972) *Exp. Cell. Res.* **75**, 207–218.
28. Frydenberg, O. & Zeuthen, E. (1960) *C. R. Lab. Trav. Carlsberg* **31**, 423–455.