

## Growth and Cell Division During Nitrogen Starvation of the Yeast *Saccharomyces cerevisiae*

GERALD C. JOHNSTON,\* RICHARD A. SINGER, AND E. SANDRA MCFARLANE

*Departments of Microbiology, Biochemistry, and Medicine, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7*

Received for publication 16 August 1977

During nitrogen starvation, cells of the yeast *Saccharomyces cerevisiae* increased threefold in number, and little ribonucleic acid (RNA) and protein were accumulated. Both RNA and protein were extensively degraded during starvation, suggesting that intracellular macromolecules could supply most of the growth requirements. The types and proportions of stable RNA synthesized during nitrogen deprivation were characteristic of exponentially growing cells; however, the complement of proteins synthesized was different. We conclude that, once events in the deoxyribonucleic acid division cycle are initiated, cells can complete division with little dependence on continued net cell growth.

Control of cell division in the budding yeast *Saccharomyces cerevisiae* occurs within the G1 portion of the cell cycle (10). Hartwell and his colleagues (10, 14) have defined "start" events within G1, the performance of which results in initiation of budding and deoxyribonucleic acid (DNA) synthesis. Before "start" can be completed, a number of physiological requirements, including growth to some critical size (14), must be met. Normally, cell division (the DNA division cycle) and growth (the growth cycle) are coordinated (19), but these two cycles are not necessarily interdependent (10, 14, 19, 29, 31). Recently we observed that during nitrogen starvation, which arrests yeast cells in the G1 portion of the cell cycle (10, 14), cell division was accompanied by less-than-normal amounts of growth. Under these conditions, a high proportion of abnormally small cells was produced (13, 14).

We report here the results of an investigation to define the relationship between growth and cell division in *S. cerevisiae* during nitrogen deprivation. Diverse aspects of growth, metabolism, and cell division during starvation have been investigated in a number of cell types other than yeast (2-6, 19, 21). However, no systematic study has yet been made with *S. cerevisiae*. If we are to employ this organism as a model, such data are necessary. To measure growth, we examined macromolecular metabolism during the initial stages of starvation and observed that, although cell number increased during starvation, there was little net accumulation of ribonucleic acid (RNA) or protein. Starvation was accompanied by extensive synthesis and breakdown of the macromole-

cules. We propose, as a consequence, that, once DNA division cycle events are initiated, cells can progress through the cell cycle with little need for net growth.

### MATERIALS AND METHODS

**Strains.** Two diploid strains of *S. cerevisiae*, AG-1 and AG1-7, were used throughout this study. Both were derived from two haploid strains, M56-20C (a *his6 ura1 aga2 gal1*) and S 185 ( $\alpha$  *ade6 leu1 gal1*), kindly provided by P. Whitney. Strain AG-1 is a prototrophic diploid resulting from the mating of strains M56-20C and S 185. The diploid auxotrophic strain AG1-7 resulted from mitotic recombination for *his6* and *ura1* in strain AG-1.

**Media and culture conditions.** Cells were grown in a liquid synthetic medium (YNB) described previously (14). For nitrogen-free medium (YNB-N),  $(\text{NH}_4)_2\text{SO}_4$  was omitted. All cultures were grown at room temperature (20 to 23°C) in Erlenmeyer flasks on a gyrotory shaker (New Brunswick Scientific, New Brunswick, N.J.). Cells were shifted from nitrogen-containing to nitrogen-free medium by collection on membrane filters (0.45- $\mu\text{m}$  pore size; Millipore Corp., Bedford, Mass.), followed by several washes with YNB-N. Filters were then placed in fresh medium and swirled to suspend the cells, and the filter was then removed. For growth of strain AG1-7, all media were supplemented with histidine (4  $\mu\text{g}/\text{ml}$ ) and uracil (20  $\mu\text{g}/\text{ml}$ ). Increased histidine and uracil concentrations did not allow increased growth during nitrogen starvation; thus, these compounds were not general nitrogen sources.

**Measurement of cellular parameters.** Cell numbers were routinely determined as described previously (8) after brief sonic oscillation. Viable-cell determinations were made after plating on YEPD medium (9, 25). The proportion of cells with buds was determined by direct microscopic examination (8). Volume distributions were determined with a

Coulter Channelyzer (Coulter Electronics, Hialeah, Fla.).

**Measurement of protein, RNA, and DNA.** Two methods were used to quantitate accumulated protein. In the first, samples of cells labeled with [ $^3\text{H}$ ]histidine (2  $\mu\text{Ci/ml}$ ; specific activity, 12 Ci/mmol; New England Nuclear Corp., Boston, Mass.) were added to equal volumes of 10% trichloroacetic acid ( $\text{CCl}_3\text{COOH}$ ) containing a 10-fold excess of unlabeled histidine. Samples were kept on ice for at least 30 min. Cells were then collected on glass-fiber filters and washed twice with 5%  $\text{CCl}_3\text{COOH}$  containing unlabeled histidine. Filters were dried and placed in liquid scintillation fluid {per liter of toluene: 2,5-diphenyloxazole, 4.0 g; *p*-bis[2-(5-phenyloxazolyl)]benzene, 0.05 g} and counted in a Nuclear-Chicago scintillation counter (Isocap 300).

The second method employed was a modification of the Lowry method, which has been described elsewhere (14).

Accumulation of RNA and DNA was determined by labeling cells with [ $^3\text{H}$ ]uracil (2  $\mu\text{Ci/ml}$ ; specific activity, 25 Ci/mmol; New England Nuclear Corp.) (9). For estimation of RNA, samples were added to equal volumes of 10%  $\text{CCl}_3\text{COOH}$  and kept at  $0^\circ\text{C}$  for 30 min before filtering. For DNA, samples were added to equal volumes of 2 N NaOH, left at room temperature for 16 h, and then chilled. Calf thymus DNA (50- $\mu\text{g/ml}$  final concentration) and 50%  $\text{CCl}_3\text{COOH}$  (16% final concentration) were added, and these mixtures were left for 30 min at  $0^\circ\text{C}$ . Both RNA and DNA were collected on glass-fiber filters and washed with 5%  $\text{CCl}_3\text{COOH}$  containing unlabeled uracil at a concentration 10 times that in the growth medium.

**Measurement of RNA and protein breakdown.** Breakdown of RNA and protein was measured as loss of labeled acid-precipitable material from cells. Cells were labeled for six generations with either [ $^3\text{H}$ ]uracil (2  $\mu\text{Ci/ml}$ ) or [ $^3\text{H}$ ]histidine (2  $\mu\text{Ci/ml}$ ), washed, and placed in YNB-N. At intervals, 1.0 ml was withdrawn from each culture and centrifuged at  $2,000 \times g$  for 5 min, and 10  $\mu\text{l}$  of the supernatant fluid was transferred onto a glass-fiber filter. The pellet and remaining supernatant fluid were mixed with an equal volume of 10%  $\text{CCl}_3\text{COOH}$  and treated as described above.

**Analysis of amino acid pools.** Cells were grown in YNB to  $2 \times 10^6/\text{ml}$ , collected by centrifugation, and suspended in YNB-N. For analysis of amino acid pools, cells were collected by centrifugation, washed once with distilled water (20), and suspended in 5%  $\text{CCl}_3\text{COOH}$  for 30 min at  $0^\circ\text{C}$ . This treatment extracts the acid-soluble pool of amino acids (30; P. Whitney, personal communication). Cells were removed from the  $\text{CCl}_3\text{COOH}$  by centrifugation, and  $\text{CCl}_3\text{COOH}$  in the supernatant fluid was extracted three times with ether. The aqueous fraction was lyophilized, and the dried samples were taken up in buffer and analyzed on a Beckman amino acid analyzer.

**RNA extraction and fractionation.** Cultures were incubated (see Results) with [ $^{14}\text{C}$ ]uracil (specific activity, 40 mCi/mmol) or [ $^3\text{H}$ ]uracil (specific activity, 25 Ci/mmol) and mixed with an equal volume

of cold 95% ethanol, and the cells were then collected by centrifugation (10 min at  $1,000 \times g$  at  $4^\circ\text{C}$ ).

The cell pellet was suspended in 0.5 ml of cold 1.0 mM sodium acetate buffer (pH 5.1) containing 5 mM sodium chloride and 0.1 mM magnesium acetate (32). Approximately 2 g of 0.50-mm glass beads (Matheson Scientific, Inc., Elk Grove Village, Ill.) was added, and cells were lysed by mixing in a Vortex mixer for 30 s (L. Olson, personal communication). Then 4.5 ml of cold acetate buffer and 5.0 ml of phenol were added, and the mixture was shaken at room temperature for 10 min. After centrifugation at  $1,000 \times g$  for 30 min, the aqueous phase was removed, mixed with 2 volumes of 95% ethanol containing 0.2 M sodium acetate, and left overnight at  $-20^\circ\text{C}$ . The RNA precipitate was collected by centrifugation and dissolved in 1.0 ml of sodium acetate extraction buffer.

The RNA preparation was layered on a 5 to 20% sucrose gradient in 0.1 M acetate buffer (pH 5.1) and centrifuged for 20 h at  $55,000 \times g$ . Fractions were collected from the bottom of the tube, and radioactivity in the portions was determined in Aquasol (New England Nuclear Corp.), using a Nuclear-Chicago Mark I liquid scintillation system.

**Cell lysis and electrophoresis of cellular proteins.** Cells were labeled with either [ $^3\text{H}$ ]histidine or [ $^{14}\text{C}$ ]histidine (New England Nuclear Corp.) and harvested by centrifugation for 5 min at room temperature and  $6,000 \times g$ . Cell pellets were frozen and then thawed with the addition, at  $4^\circ\text{C}$ , of 0.05 M sodium phosphate buffer (pH 7.45) containing the protease inhibitor phenylmethylsulfonyl fluoride at 0.3 mg/ml (24). The cell suspension was passed through a chilled Aminco French pressure cell at  $1.25 \times 10^7 \text{ kg/m}^2$ . After removal of cell debris by centrifugation for 5 min at  $300 \times g$ , proteins in the cell lysate were precipitated by the addition of an equal volume of 10%  $\text{CCl}_3\text{COOH}$ . The precipitated proteins were collected by centrifugation at  $16,000 \times g$  for 10 min, washed twice with cold absolute ethanol, and dried under vacuum. The proteins were taken up in sample buffer (33) containing 0.3 mg of phenylmethylsulfonyl fluoride per ml, heated in a boiling-water bath for 3 min, and subjected to electrophoresis (33) in cylindrical 7.5% polyacrylamide gels. After electrophoresis, gels were frozen on dry ice and sliced into 1-mm disks. Each slice was digested in 5 ml of toluene containing 3% Protosol and 0.8% Omnifluor (both New England Nuclear Corp.), and radioactivity, corrected for crossover and quenching, was determined with a Philips multichannel scintillation counter.

## RESULTS

**Metabolism of macromolecules during nitrogen starvation.** Cells of *S. cerevisiae* AG1-7 growing exponentially in synthetic medium (YNB) were transferred to a nitrogen-free medium (YNB-N). Half of this culture was supplemented with  $(\text{NH}_4)_2\text{SO}_4$  to the concentration present in YNB. The number of cells in the supplemented culture continued to increase exponentially, whereas the number in the nitro-

gen-deprived culture increased threefold (Fig. 1D). The threefold increase in number was paralleled by a threefold increase in viable count (data not shown). Upon cessation of growth, 98% of the nitrogen-deprived cells had no buds and, therefore, were arrested in the G1 phase of the cell cycle (10).

To examine net accumulation of macromolecules, cells of strain AG1-7 were labeled for several generations with [ $^3\text{H}$ ]uracil ( $0.2 \mu\text{Ci/ml}$ ) or [ $^3\text{H}$ ]histidine ( $2 \mu\text{Ci/ml}$ ), shifted to nitrogen-free medium supplemented with the same radioactive precursor at the same specific activity, and sampled at intervals for incorporated label. Under conditions of nitrogen deprivation, there was a 2.5-fold increase in labeled DNA in the culture (Fig. 1A), but there was little detectable increase in labeled protein (Fig. 1B) or RNA (Fig. 1C). The lack of [ $^3\text{H}$ ]histidine incorporation by these nitrogen-deprived cells was consistent with an absence of net accumulation of protein, as measured chemically by a modified Lowry procedure (14). At most, the protein content of the culture increased by 6% during nitrogen starvation. The negligible increase in label appearing in RNA during this period cannot be accounted for by precursor exclusion during starvation, since labeled DNA increased along with cell number, and both RNA and DNA were labeled with the same radioactive precursor (see Materials and Methods).

Although significant amounts of protein

were not accumulated by nitrogen-deprived cells, continued protein synthesis was necessary for completion of DNA division cycles under these conditions. Cells were shifted to nitrogen-free medium, and at various times samples were exposed to cycloheximide, an inhibitor of protein synthesis (28). At a concentration of  $20 \mu\text{g/ml}$ , cycloheximide prevented further increase in cell number regardless of its time of addition (data not shown).

Immediately after shift to nitrogen-free medium and again after 9 h of nitrogen starvation, amino acid pools were extracted and measured as described in Materials and Methods. Amino acid pools were measured in the otherwise isogenic prototrophic strain AG-1, because strain AG1-7 requires exogenous histidine, which might have interfered with pool size determinations. The growth response to nitrogen deprivation of this strain was identical to that of strain AG1-7. By 9 h of nitrogen deprivation, cell number increased from  $0.4 \times 10^6$  to  $1.2 \times 10^6/\text{ml}$ , whereas the free amino acid pool was reduced from 0.68 to less than  $0.03 \mu\text{g/ml}$ . Incorporation of this pool material alone could account for the small increase ( $0.15 \mu\text{g/ml}$ ) in protein content that occurred upon starvation.

The observations that protein synthesis continued during nitrogen starvation but that no significant net protein accumulated suggested that macromolecular degradation was initiated by cells under deprivation conditions. Results of an experiment to measure such degradation

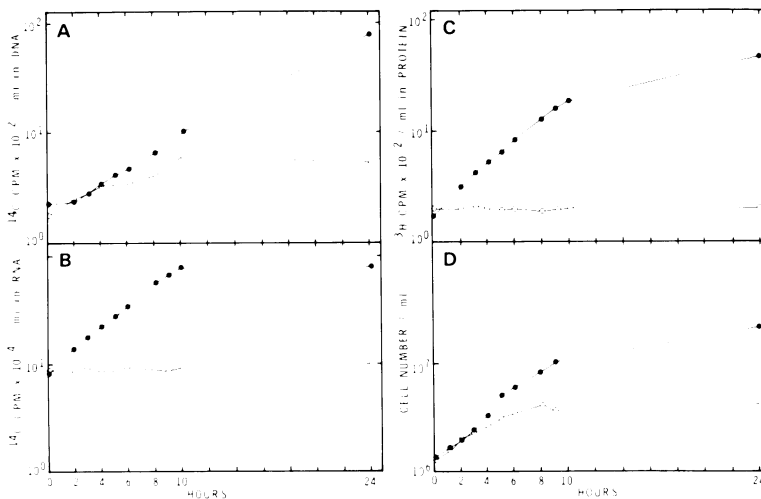


FIG. 1. Synthesis of DNA, RNA, and protein during nitrogen starvation. Cells of AG1-7 were transferred to either YNB (●) or YNB-N (○). (A) DNA content of cultures as determined by incorporation of [ $^3\text{H}$ ]uracil into alkali-resistant, acid-precipitable material; (B) RNA content of cultures as determined by incorporation of [ $^3\text{H}$ ]uracil into acid-precipitable material; (C) protein content of cultures as determined by incorporation of [ $^3\text{H}$ ]histidine into acid-precipitable material; (D) cell number per milliliter.

are shown in Fig. 2. Loss of acid-precipitable label in protein began immediately upon shift to nitrogen-free medium and continued for at least 9 h. Loss of acid-precipitable material from [ $^3\text{H}$ ]uracil-labeled cells was similarly noted 2 h after transfer of cells to nitrogen-free medium. There was no such release of label from cells in  $(\text{NH}_4)_2\text{SO}_4$ -supplemented medium. An increase in the radioactivity of the soluble acid extracts of cells accounted for all that lost from the acid-precipitable fractions. The degradation rates derived from these data, 3.7%/h for protein and 3%/h for RNA, are not corrected for reutilization of labeled degradation products and, thus, represent minimum estimates of degradation rates of these macromolecules.

**Patterns of protein synthesis during starvation.** Using analytical sodium dodecyl sulfate-gel electrophoresis, we compared the populations of proteins accumulated by nitrogen-deprived cells with those accumulated by cells growing in  $(\text{NH}_4)_2\text{SO}_4$ -supplemented medium (Fig. 3). Gels stained for proteins exhibited many discrete bands (Fig. 3A), although when they were sliced and processed for scintillation counting this resolution was lost (Fig. 3B). Nonetheless, it is evident that, upon starvation, cells of strain AG1-7 did not accumulate the same protein complement as those in nitrogen-replete medium (Fig. 3). Since the proteins from both  $^3\text{H}$ -labeled, nitrogen-deprived cells and  $^{14}\text{C}$ -labeled, nitrogen-replete cells were subjected to electrophoresis in the same gel,

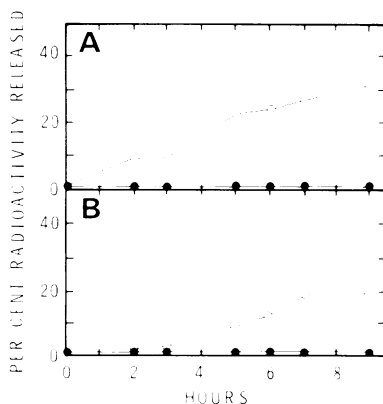


FIG. 2. Protein and RNA degradation. Cells of AG1-7 were grown in the presence of radioactive precursors for six generations and then transferred to either YNB ( $\bullet$ ) or YNB-N ( $\circ$ ). Each medium was supplemented with histidine and uracil as described in the text. Cultures were sampled and radioactivity was determined as described in the text. (A) Cells grown in [ $^3\text{H}$ ]histidine before medium shift; (B) cells grown in [ $^3\text{H}$ ]uracil before medium shift.

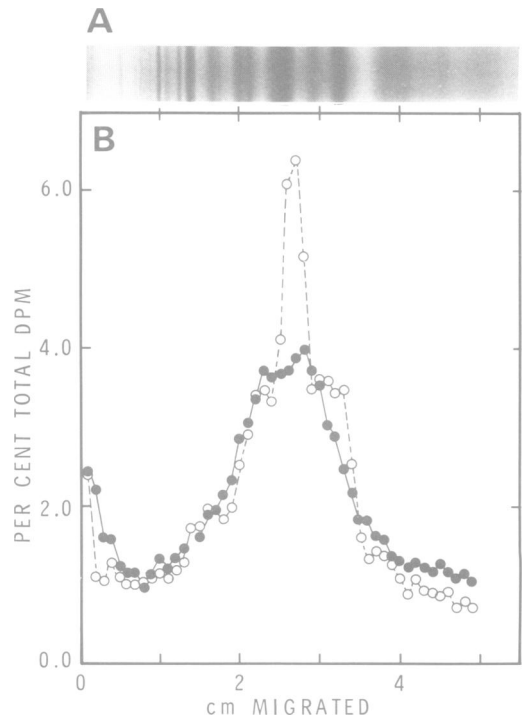


FIG. 3. Patterns of proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After 2 h of nitrogen deprivation in YNB-N, cells were incubated for 30 min with [ $^3\text{H}$ ]histidine to a  $2\text{-}\mu\text{Ci/ml}$  final concentration, harvested by centrifugation, and frozen. Before lysis, these cells were mixed with exponentially growing cells previously incubated for 30 min in YNB containing [ $^{14}\text{C}$ ]histidine, and then harvested and frozen in small portions. The pooled  $^3\text{H}$ - and  $^{14}\text{C}$ -labeled cells, mixed at a  $^3\text{H}/^{14}\text{C}$  ratio greater than 4, were lysed, and the soluble proteins were processed for electrophoresis as described in the text. (A)  $^{14}\text{C}$ -labeled proteins alone, stained for protein. (B) Proteins labeled with  $^3\text{H}$  after 2 h of nitrogen deprivation ( $\bullet$ ); proteins labeled with  $^{14}\text{C}$  ( $\circ$ ).

the relative labeling in each region of the gel may be compared directly. Plots of the  $^3\text{H}/^{14}\text{C}$  ratios along gels are a much more sensitive measure of the differences and similarities of the protein populations than are the gel profiles themselves. These ratios are shown in Fig. 4. At zero time, cells in nitrogen-free medium (and those in nitrogen-supplemented medium) displayed gross patterns of protein similar to those of exponentially growing cells. After 2 h of nitrogen starvation, the protein pattern was markedly different from that of growing cells (Fig. 3B and 4C). These differences persisted after 4 h (Fig. 4D) and 7 h (data not shown) of nitrogen starvation. Parallel differences were

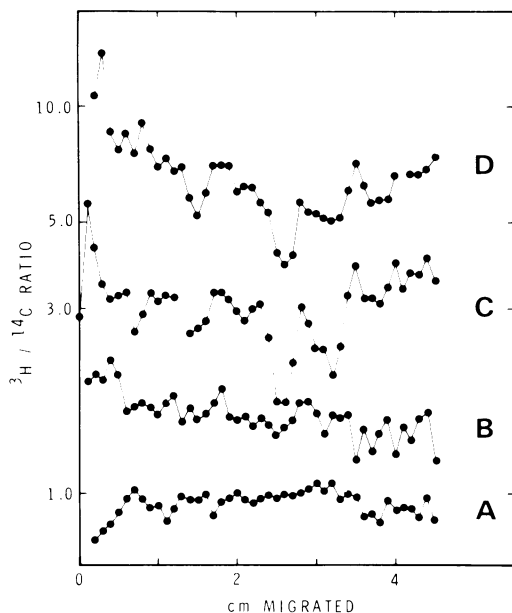


FIG. 4. Channels ratios from electrophoresis profiles of double-labeled protein samples. Cells of AG1-7 grown in YNB were collected, washed, and suspended in YNB-N. One portion of the culture was supplemented with  $(\text{NH}_4)_2\text{SO}_4$ . Immediately after the medium change (0 h) for the nitrogen-supplemented culture, and at 0, 2, and 4 h for the nitrogen-deprived culture, portions were labeled with  $[^3\text{H}]$ histidine and processed for electrophoresis as described in the legend to Fig. 3. The  $^3\text{H}/^{14}\text{C}$  (disintegrations per minute) ratio in each gel slice is shown. For clarity, plots were shifted along the ordinate, each by an arbitrary factor. (A) 0 h, YNB-N plus  $(\text{NH}_4)_2\text{SO}_4$ ; (B) 0 h, YNB-N; (C) 2 h, YNB-N; (D) 4 h, YNB-N.

also seen when cells were labeled with radioactive leucine (data not shown).

Changes in the patterns of protein labeling in nitrogen-deprived cells may reflect different synthetic capabilities of cells. Alternatively, different patterns may reflect new synthesis of enzymes, such as those for arginine catabolism induced by nitrogen deprivation (18, 34) or selective (preferential) protein degradation.

To test the first alternative, cells of strain AG1-7 were grown for six generations in medium containing arginine (200  $\mu\text{g}/\text{ml}$ ) as a nitrogen source—conditions sufficient to induce the arginase enzyme system (17, 35). The cells were then placed for 30 min in medium containing both arginine and  $[^{14}\text{C}]$ histidine, collected by centrifugation, and frozen. For electrophoresis, the  $^{14}\text{C}$ -labeled cell pellets were mixed with cells from a  $[^3\text{H}]$ histidine-labeled exponentially growing culture or from a  $[^3\text{H}]$ histidine-

labeled culture that had been starved for nitrogen for 4 h. The channel ratio plots of cells growing exponentially with either arginine or  $(\text{NH}_4)_2\text{SO}_4$  as the sole nitrogen source were similar. However, the ratios comparing proteins of arginine-grown cells with those of nitrogen-starved cells showed the characteristic differences seen in Fig. 4C or D. Thus, the pattern of soluble protein synthesis in nitrogen-starved cells was not merely that of a cell induced for arginine catabolism.

To test the second alternative, the patterns of prelabeled proteins in starving cells and the pattern of proteins in growing cells were compared. No difference was observed (Fig. 5). The extensive protein degradation observed in nitrogen-deprived cells was not generally directed toward one class of soluble proteins and did not produce detectable polypeptide fragments. Therefore, degradation alone does not account for the differences in patterns of protein labeling between growing and starving cells.

#### Stable RNA metabolism during starvation.

During starvation for nitrogen, there was no net accumulation of RNA (Fig. 1); however, there was significant RNA degradation (Fig. 2). These findings, plus the observation (1, 22) that multiple RNA polymerases were involved in stable RNA synthesis, led us to examine the synthesis of stable RNA during starvation.

Cells were transferred to nitrogen-free medium and at intervals were exposed to  $[^3\text{H}]$ uracil for 30 min. These cells were then combined with cells incubated in YNB with  $[^{14}\text{C}]$ uracil for 30 min, and the RNA from these

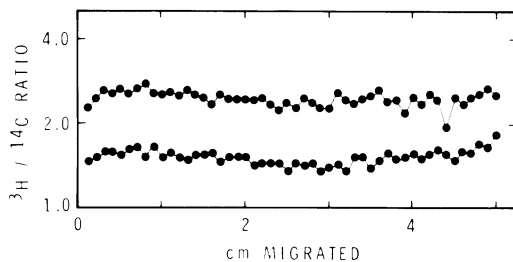


FIG. 5. Protein degradation during nitrogen starvation. Cells of AG1-7 were grown for several generations in YNB containing 2  $\mu\text{Ci}$  of  $[^3\text{H}]$ histidine per ml, washed, and transferred to nonradioactive YNB-N. Samples were collected for protein extraction immediately after transfer (lower plot) and again 4 h later (upper plot). The  $^3\text{H}$ -labeled proteins from these cells were extracted along with  $^{14}\text{C}$ -labeled proteins from cells growing exponentially in YNB and were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Channels ratios were calculated as described in the legend to Fig. 4.

pooled samples was isolated. The overall proportions and types of stable RNA labeled during nitrogen starvation were similar to those of exponentially growing cells (Fig. 6).

To determine whether preferential breakdown of one RNA species occurred during starvation, cells of strain AG1-7 were labeled for at least six generations with [ $^3\text{H}$ ]uracil and then transferred to YNB-N. The radioactivity patterns observed in samples extracted at intervals were characteristic of the types and proportions of stable RNA from exponentially growing cells. Loss of prelabeled stable RNA was noted after 1 to 2 h of nitrogen starvation, in agreement with data presented in Fig. 2.

### DISCUSSION

The results reported here suggest that after the "start" event little net growth is needed to allow cells to complete events in the DNA division cycle. The increase in DNA and cell number during nitrogen deprivation occurred without protein accumulation. During this period, however, active protein synthesis was required for completion of DNA division cycle events, as indicated by the inhibitory effect of cycloheximide.

Analysis by sodium dodecyl sulfate-poly-

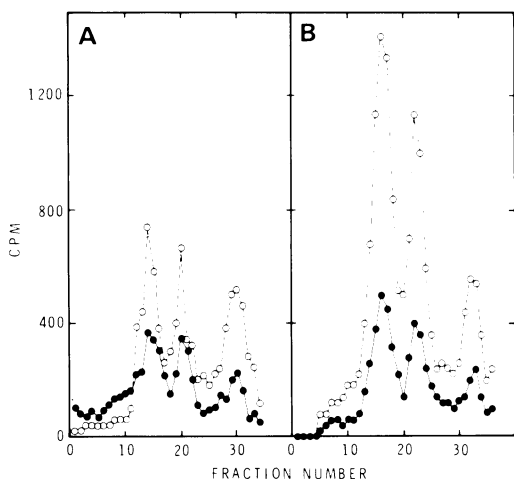


FIG. 6. Stable RNA synthesis during nitrogen starvation. Cells of AG1-7 growing in YNB were transferred by filtration (see text) to YNB-N. At 0 h (A) and 3 h (B), 12.5 ml of cells was transferred to a flask containing 25  $\mu\text{Ci}$  of [ $^3\text{H}$ ]uracil. After 30 min of incubation, cells were collected by centrifugation and combined with 12.5 ml of exponentially growing cells incubated for 30 min with 25  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]uracil. Cells were lysed and RNA was extracted and resolved on sucrose gradients as described in the text. Symbols: ●,  $^3\text{H}$ -labeled RNA; ○,  $^{14}\text{C}$ -labeled RNA.

acrylamide gel electrophoresis showed that the spectrum of labeled proteins in nitrogen-deprived cells differed from that in growing cells. Differences were not simply the result of induced enzymes for arginine catabolism or of preferential protein breakdown. Although these processes may have contributed to altered protein patterns in starving cells, we conclude that the altered protein complements are a result of different patterns of protein synthesis. We cannot distinguish between synthesis of different proteins or synthesis of different proportions of the same proteins found in exponentially growing cells.

The synthesis of an altered spectrum of proteins by cells under poor growth conditions has also been noted in mammalian cells (3,4; inter alia, reference 2). Hopper et al. (11) also observed differences in patterns of proteins synthesized when *S. cerevisiae* was placed under sporulation conditions.

Protein synthesis during nitrogen deprivation was also accompanied by extensive protein degradation. There was no evidence of selective or preferential loss of protein subpopulations.

Long-term labeling experiments suggested that little or no RNA accumulated during nitrogen starvation. The rate of stable RNA synthesis in nitrogen-deprived cultures was balanced by the rate of RNA degradation.

Degradation of protein and RNA is characteristic of starvation in both prokaryotes (5, 6, 19, 21) and some eucaryotes (5, 6, 9, 10, 27, 34), including *S. cerevisiae* (7). Hopper et al. (11) examined macromolecular metabolism accompanying sporulation in *S. cerevisiae* and found that cells placed in sporulation medium also degraded both protein and RNA. Diploid strains of genotype a/a, although incapable of completing sporulation, showed patterns of degradation similar to those of sporulating a/ $\alpha$  strains (11). The experiments measuring degradation as reported here involved nitrogen deprivation in the presence of glucose. These conditions, however, inhibit sporulation. Therefore, protein and RNA degradation may merely be an initial response to unfavorable conditions, unlinked to subsequent events that determine the fate of the cell.

The ability of cells to complete events in the DNA division cycle with less-than-normal amounts of growth is not unique to *S. cerevisiae*. Stationary-phase cells of the fission yeast *Schizosaccharomyces pombe* (12) and of some protozoa (23) are small compared to growing cells. Several types of mammalian cells become characteristically smaller as they arrest DNA division cycles under conditions of nutrient

limitation (15, 16, 26). Kimball et al. (16) noted that Chinese hamster cells entering stationary phase could progress through the cell cycle "with very little net increase in protein content." Salzman (26) observed a decrease in protein and RNA content of HeLa cells as they approached stationary phase. His data indicated that the final doubling of cell number was accompanied by less than a doubling of cellular protein and RNA. Similarly, 3T3 cells in stationary phase have markedly less RNA than cells in a growing population (15).

We conclude that, once a cell of *S. cerevisiae* has completed the "start" event and initiated the DNA division cycle, it then has the capacity to complete the DNA division cycle uninfluenced by physiological changes affecting growth. Completion of the cell cycle only requires continued cellular metabolism. In the absence of essential nutrients, generalized protein and RNA degradation may be stimulated nonspecifically to provide precursors for new synthesis. The altered pattern of protein synthesis thus may reflect the response in the growth cycle to conditions leading to cell division arrest.

#### ACKNOWLEDGMENTS

We thank C. Ehrhardt and D. MacKeigan for expert technical assistance. We also thank K. B. Easterbrook for critical reading of the manuscript.

The work was supported by grant MA-5776 from the Medical Research Council of Canada and by the National Cancer Institute of Canada.

#### LITERATURE CITED

- Adman, R., L. D. Schultz, and B. D. Hall. 1972. Transcription in yeast; separation and properties of multiple RNA polymerases. *Proc. Natl. Acad. Sci. U.S.A.* 69:1702-1706.
- Baserga, R. 1976. Multiplication and division in mammalian cells. Marcel Dekker Inc., New York.
- Becker, H., and C. P. Stanner. 1972. Control of macromolecular synthesis in proliferating and resting Syrian hamster cells in monolayer culture. III. Electrophoretic patterns of newly synthesized proteins in synchronized proliferating cells and resting cells. *J. Cell. Physiol.* 80:51-62.
- Fox, T. O., and A. B. Pardee. 1971. Proteins made in the mammalian cell cycle. *J. Biol. Chem.* 246:6159-6165.
- Goldberg, A. L., and A. C. St. John. 1974. Intracellular protein degradation in mammalian and bacterial cells. *Annu. Rev. Biochem.* 43:835-869.
- Goldberg, A. L., and A. C. St. John. 1976. Intracellular protein degradation in mammalian and bacterial cells: part 2. *Annu. Rev. Biochem.* 45:747-803.
- Halvorson, H. 1958. Intracellular protein and nucleic acid turnover in resting yeast cells. *Biochim. Biophys. Acta* 27:255-266.
- Hartwell, L. H. 1970. Periodic density fluctuation during the yeast cell cycle and the selection of synchronous cultures. *J. Bacteriol.* 104:1280-1285.
- Hartwell, L. H. 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. *J. Bacteriol.* 93:1662-1670.
- Hartwell, L. H. 1974. *Saccharomyces cerevisiae* cell cycle. *Bacteriol. Rev.* 38:164-198.
- Hopper, A. K., P. T. Magee, S. K. Welch, M. Friedman, and B. D. Hall. 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. *J. Bacteriol.* 119:619-628.
- Johnson, B. F. 1968. Morphometric analysis of yeast cells. II. Cell size of *Schizosaccharomyces pombe* during the growth cycle. *Exp. Cell Res.* 49:59-68.
- Johnston, G. C. 1977. Cell size and budding during starvation of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 132:738-739.
- Johnston, G. C., J. R. Pringle, and L. H. Hartwell. 1977. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Exp. Cell Res.* 105:79-98.
- Johnson, L. F., H. T. Abelson, H. Green, and S. Penman. 1974. Changes in RNA in relation to growth of the fibroblast. I. Amount of mRNA, rRNA and tRNA in resting and growing cells. *Cell* 1:95-100.
- Kimball, R. F., S. W. Perdue, E. H. Y. Chu, and J. R. Ortiz. 1971. Microphotometric and autoradiographic studies on the cell cycle and cell size during the growth and decline of Chinese hamster cell cultures. *Exp. Cell Res.* 66:17-32.
- Middelhoven, W. J. 1964. The pathway of arginine breakdown in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 93:650-652.
- Middelhoven, W. J. 1968. The derepression of arginase and ornithine transaminase in nitrogen-starved baker's yeast. *Biochim. Biophys. Acta* 156:440-443.
- Mitchison, J. M. 1971. The biology of the cell cycle. Cambridge University Press, Cambridge.
- Nurse, P., and A. Wiemken. 1974. Amino acid pools and metabolism during the cell division cycle of arginine-grown *Candida utilis*. *J. Bacteriol.* 117:1108-1116.
- Pine, M. J. 1972. Turnover of intracellular protein. *Annu. Rev. Microbiol.* 26:103-126.
- Ponta, H., U. Ponta, and E. Wintersberger. 1972. Purification and properties of DNA-dependent RNA polymerase from yeast. *Eur. J. Biochem.* 29:110-118.
- Prescott, D. M. 1956. Relation between cell growth and cell division. II. The effect of cell size on cell growth rate and generation time in *Amoeba proteus*. *Exp. Cell Res.* 11:86-98.
- Pringle, J. R. 1975. Avoiding proteolytic artifacts, p. 179-184. *In* D. M. Prescott (ed.), *Methods in cell biology*, vol. 12. Academic Press Inc., New York.
- Pringle, J. R., and J. R. Mor. 1975. Methods for monitoring the growth of yeast cultures and for dealing with the clumping problem, p. 131-138. *In* D. M. Prescott (ed.), *Methods in cell biology*, vol. 11. Academic Press Inc., New York.
- Salzman, N. P. 1959. Systematic fluctuations in the cellular protein, RNA and DNA during growth of mammalian cell cultures. *Biochim. Biophys. Acta* 31:158-163.
- Schimke, R. T., and D. Doyle. 1970. Control of enzyme levels in animal tissues. *Annu. Rev. Biochem.* 39:929-976.
- Siegel, M. R., and H. D. Sislet. 1964. Site of action of cycloheximide in cells of *Saccharomyces pastorianus*. II. The nature of inhibition of protein synthesis in a cell-free system. *Biochim. Biophys. Acta* 87:83-89.
- Slater, M., and M. Schaechter. 1974. Control of cell division in bacteria. *Bacteriol. Rev.* 38:199-221.
- Stewart, P. R. 1975. Analytical methods for yeasts, p. 111-147. *In* D. M. Prescott (ed.), *Methods in cell biology*, vol. 12. Academic Press Inc., New York.
- Unger, M. W., and L. H. Hartwell. 1976. Control of cell division in *Saccharomyces cerevisiae* by methio-

- nyl-tRNA. Proc. Natl. Acad. Sci. U.S.A. 73:1664-1668.
32. **Waldron, C. , and F. Lacroute.** 1975. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acid in yeast. *J. Bacteriol.* 122:855-865.
  33. **Weber, K., J. R. Pringle, and M. Osborn.** 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. *Methods Enzymol.* 26:3-27.
  34. **Whitney, P. A., and B. Magasanik.** 1975. The induction of arginase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 248:6197-6202.
  35. **Wiame, J. M.** 1971. The regulation of arginine metabolism in *Saccharomyces cerevisiae*; exclusion mechanisms. *Curr. Top. Cell. Regul.* 4:1-38.