Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4384–4388, September 1978
Cell Biology

Rate of macromolecular synthesis through the cell cycle of the yeast *Saccharomyces cerevisiae*

(two-dimensional gel electrophoresis/protein synthesis/RNA synthesis/DNA synthesis)

S. G. ELLIOTT AND C. S. MCLAUGHLIN*

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

Communicated by Howard A. Schneiderman, June 26, 1978

ABSTRACT **Centrifugal elutriation was used to separate cells of *Saccharomyces cerevisiae* in balanced exponential growth according to position in the cell cycle. Macromolecular synthesis was examined. DNA synthesis was found to be periodic, but RNA and protein synthesis showed an exponential increase in rate. Two-dimensional electrophoresis was used to determine the rate of synthesis of individual proteins, with 111 of the more abundant cellular proteins selected for analysis from among the more than 1000 proteins that migrate in the system. All the examined proteins showed an exponentially increasing rate of synthesis.**

The total amount of protein increases continuously throughout the cell cycle in bacteria and yeast (1). However, both periodic and continuous increases in the activities of a variety of enzymes from many species of prokaryotic and eukaryotic organisms have been reported (1–4). Four general patterns of activity increase through the cell cycle have been considered. The patterns of continuous increase can be subdivided into two types: (*i*) exponential increase and (*ii*) linear increase with a doubling in the rate at some point during the cell cycle. Additionally, patterns of periodic increase can be subdivided into: (*iii*) step patterns like that of DNA accumulation and (*iv*) patterns in which activity reaches a peak followed by inactivation or degradation of the enzyme.

Despite the extensive literature on periodic changes in enzymatic activity, speculations on the periodic nature of the synthesis of individual enzymatic proteins have been viewed with skepticism for the following reasons:

(*i*) The various researchers reporting on enzyme accumulation measured enzyme activity and not enzyme synthesis. Mitchison and Creanor (5) reported that there is a delay of 20% of the cell cycle in the yeast *Schizosaccharomyces pombe* between synthesis of the enzyme precursor and its activation. This indicates that increased enzyme activity is not necessarily synonymous with enzyme synthesis. Furthermore, because the activities of many enzymes are regulated by feedback mechanisms, the periodicity that many researchers reported may be due to periodic changes in the synthesis of regulatory molecules affecting enzyme activity and not enzyme synthesis.

(*ii*) Many studies of periodic synthesis utilized synchronous cultures prepared by induction techniques that involve media changes. These methods might be expected to drastically affect the nature of periodic synthesis of enzymes during at least the first synchronous division cycle. Furthermore, a study in *Schizosaccharomyces pombe* (6) showed that when total protein was subdivided into small groups on one-dimensional sodium dodecyl sulfate gels, their synthesis was exponential. In addition, in *Saccharomyces cerevisiae*, the synthesis of a well-defined class of proteins, the ribosomal proteins, is nonperiodic (ref. 7; unpublished data).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

In light of these problems, we decided to reexamine enzyme synthesis in *S. cerevisiae*. Our reexamination is based on two recent technical innovations, the application of centrifugal elutriation to yeast (8) and the development of a high-resolution, two-dimensional gel electrophoretic technique capable of resolving over 1000 individual cellular proteins (9).

Centrifugal elutriation is a selective technique that circumvents the problem of metabolic disruption by removing cells at various stages of their cell division cycle from a culture in balanced growth. The system separates cells on the basis of size and thus by their position in the cell cycle. In this procedure, a centrifugal force tends to sediment cells while liquid flowing in the opposite direction tends to flush them out of the rotor chamber. By centrifuging at a constant rotor speed and incrementally increasing the flow rate, discrete size classes of cells are obtained. This technique has an advantage over other selection techniques in that virtually any liquid can be used to generate the separation. Furthermore, the potential resolution of events during the cell cycle is enormous because the number of fractions obtained can be increased by decreasing the magnitude of the incremental changes in flow rate (8).

The development by O'Farrell (9) of a high resolution two-dimensional gel electrophoretic technique allows a direct examination of the rate of synthesis of a large number of individual proteins during the cell cycle. To a first approximation, the rate of synthesis of a specific protein is proportional to the amount of radioactive precursor incorporated into the protein during a short pulse. This measure of the rate of synthesis can be determined qualititatively by autoradiography and quantitatively by a dual-label procedure involving long-term and pulse labels. The long-term label uniformly labels all proteins. The pulse labels only proteins made during a short period of the cell cycle. The ratio of the two is then a measure of the rate of synthesis during a short period of the cell cycle. The method is simple, has internal controls for recovery, and minimizes sample manipulation because any amount of protein can be used to determine the rate of synthesis. Each of the four patterns of protein accumulation through the cell cycle can be distinguished by measuring the ratio of the pulse label to the long term label through the cell cycle. This ratio is essentially a measure of $(dA/dt)/A$, in which A = amount and t = time. For a component that is increasing at an exponential rate, this ratio should be constant through the cell cycle. All the other models predict a 2-fold or greater variation in the ratio through the cell cycle.

MATERIALS AND METHODS

Strains. A diploid of *Saccharomyces cerevisiae*, SKQ2n (*a*/α, *ade1*/+, +/*ade2*, +/*his1*), was used in all the experiments. It was obtained from Brian Cox (University of Oxford).

Chemicals. Glusulase was from Endo Laboratories. Electrophoresis grade acrylamide, *N,N'*-methylene bisacrylamide

* To whom reprint requests should be addressed.

and *N,N,N',N'*-tetramethylenediamine (TEMED) were from Eastman Kodak. Ampholines were from LKB. Urea was ultrapure and obtained from Schwarz/Mann. [^{35}S]Methionine was prepared from carrier-free sulfate (Amersham) by the procedure of Graham and Stanley (10). All other radioactive components were from Schwarz/Mann.

Growth and Labeling of Cells. To label protein, cells were grown to midlogarithmic phase at 23°C on a rotary shaker in medium containing, per liter, 6.7 g of yeast nitrogen base minus amino acids (Difco), 20 g of glucose, and 10 mg each of adenine and uracil. Long-term labeling of DNA was done in medium that was the same as above except that 22 mg of each amino acid, with the exception of methionine and cysteine, was added back. However, to pulse label DNA, adenine and uracil were left out of the medium. To label RNA, yeast nitrogen base was substituted for yeast nitrogen base minus amino acids, and the amounts of adenine and uracil were reduced to 1/10th.

The type of proteins made during the cell cycle was determined by pulse labeling with [^{35}S]methionine at 0.2 μCi/ml for 10 min, during which time 60% of the label was taken up. The rate of protein synthesis was determined by a dual-label method. ^{3}H-Labeled yeast protein hydrolysate or [*methyl*-^{3}H]methionine (1 μCi/ml and 2 μCi/ml, respectively) was added to a logarithmic phase culture for 3 hr (1.5 generations) to uniformly label proteins. [^{35}S]Methionine (0.6 μCi/ml) was then added for 10 min. Two cultures were used to measure the rate of DNA synthesis. [2-^{14}C]Uracil (0.10 μCi/ml) was used in one culture for the long-term label and [5-^{3}H]uracil (4.0 μCi/ml) for the pulse in the other culture. The pulse time was extended from 10 to 15 min to allow for the additional time necessary to process uracil into DNA. Following the labeling, equal volumes of the cultures were mixed. The rate of RNA synthesis was determined by using 0.5 μCi of [^{3}H]uracil per ml for the long-term label and 0.25 μCi of [^{14}C]uracil per ml for a 10-min pulse. In all cases, incorporation was stopped by the addition of ice to the medium. The cells were immediately pelleted, washed once, and resuspended in ice-cold distilled water. Radioactive labels in DNA (alkali-stable material) and RNA (trichloroacetic acid-insoluble material) were determined as described (11). After correcting for quench, the ratios of pulse to long-term radioactivities were calculated to determine the rates of synthesis of protein, DNA, or RNA.

Cell Cycle Fractionation. The procedure used has been described (8). Cells grown and labeled as described above were sonified and loaded at 4°C into a Beckman JE-6 rotor spinning at 3000 rpm in a J-21 centrifuge at a flow rate of 9 ml/min. One-hundred and fifty ml was then washed through the chamber. The first fraction was collected at 11 ml/min, and successive fractions were obtained by increasing the flow rate in 2 ml/min increments. The last fraction was collected at 27 ml/min. Sodium chloride was added to a final concentration of 0.10 M and the cells were collected by centrifugation. Aliquots of cells from each fraction were stained with Giemsa (12) and examined microscopically under oil immersion.

Two-Dimensional Gel Electrophoresis. Pellets of cells, chilled to 0°–4°C and containing approximately 5 × 10^{7} cells, were resuspended in 50 μl of 1.2 M sorbitol and 5 μl of Glusulase was added. After 30 min on ice, the spheroplasts were collected by centrifugation and lysed in 10 μl of sodium dodecyl sulfate lysis buffer (1% sodium dodecyl sulfate/1.6% pH 5–7 Ampholines/1.6% pH 5–8 Ampholines/0.8% pH 3–10 Ampholines). Ten microliters of DNase/RNase solution was then added (20 mM $CaCl_2$/50 mM $MgCl_2$/0.5 M Tris·HCl, pH 7.0/0.5 mg of RNase A per ml/1 mg of DNase I per ml). Solid urea was then added to a final concentration of 9.5 M. Finally, 20 μl of sample buffer was added [9.5 M urea/8% (vol/vol) Nonidet P-40/10% (vol/vol) 2-mercaptoethanol/2% Ampholines (0.8% pH 5–7/0.8% pH 5–8/0.4% pH 3–10)]. The final sample was 0.25% sodium dodecyl sulfate, 2% Ampholines, 9.5 M urea, 4% Nonidet P-40 and 5% 2-mercaptoethanol.

Electrophoresis was basically that of O'Farrell (9), with the following modifications. Isoelectric focusing gels were 3% acrylamide. *N,N,N',N'*-Tetramethylethylenediamine was not used during polymerization and ammonium persulfate was increased 3-fold. The sample overlay contained 9 M urea, 4% Nonidet P-40, 5% 2-mercaptoethanol, 0.8% pH 5–7 Ampholines, 0.8% pH 5–8 Ampholines, and 0.4% pH 3–10 Ampholines. The sample overlay was used to overlay both the gel during polymerization and the sample during electrophoresis. The gels were dried according to the procedure of Irie *et al.* (13), exposed to No Screen medical x-ray film, and processed according to standard procedures.

Determination of the Rate of Synthesis of Individual Proteins. Spots on gels from cell fractions representing distinct positions in the cell cycle were selected at random, cut out, and digested in toluene-based scintillation fluid containing 3.5% Protosol at 23°C in tightly capped vials. After 2–4 days, the radioactivities in the vials were determined in a scintillation counter and the data were corrected for quench. The ratio of ^{35}S/^{3}H cpm in individual spots represents the rate of synthesis of each polypeptide.

RESULTS

Cell Separation. To demonstrate that centrifugal elutriation can effect a separation of cells in different parts of the cell cycle, a logarithmic phase culture was fractionated. In Fig. 1, the percentage of four Giemsa-stained cell types indicated in the *inset* is plotted vs. flow rate. According to Fig. 1, unbudded cells peak in the fraction eluted at 13 ml/min, budded cells with 1 nucleus peak in the fraction collected at 19 ml/min, cells with migrating nuclei peak in the fraction collected at 21 ml/min, and binucleate cells peak in the fraction collected at 25 ml/min. Successive fractions eluted from the rotor contain cells that have progressed further through the cell cycle. Furthermore, we find the separation to be extremely reproducible. Thus, each fraction

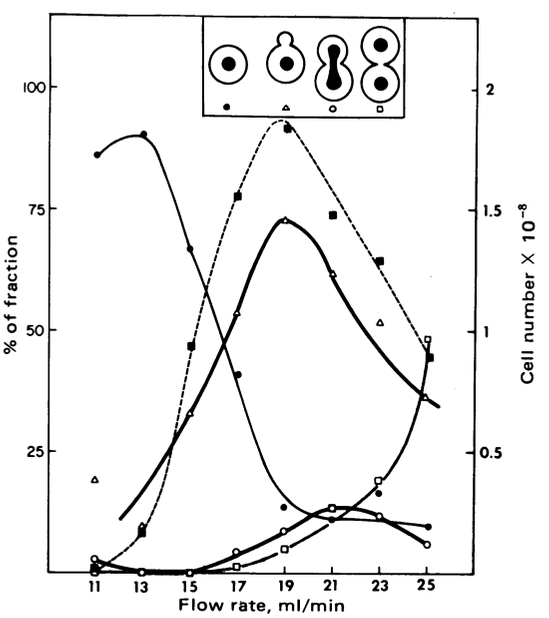

FIG. 1. Separation of cell markers by centrifugal elutriation. *Inset* shows four different cell types examined in fractions obtained at the various flow rates. ●, Unbudded cells; △, budded cells with a single nucleus; ○, cells with migrating nuclei; and □, binucleate cells. Cells were stained with Giemsa and the proportion of each cell type in each fraction was determined microscopically. Total cell number (broken line) was determined for each fraction by a model T Coulter Counter.

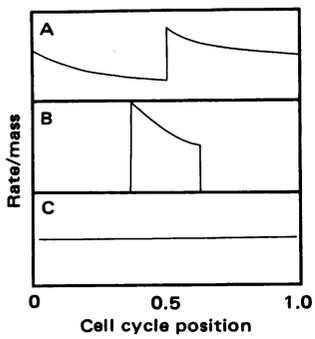

FIG. 2. Theoretical rate-to-mass ratios for three models of accumulation. (*A*) Linear doubling accumulation (step synthesis); (*B*) step accumulation (periodic synthesis); (*C*) exponential accumulation (exponential synthesis).

contains cells from specific segments of the cell cycle and the procedure offers a convenient method for analyzing macromolecular synthesis during the cell cycle.

Patterns of Synthesis during the Cell Cycle. The idealized rate-to-mass ratios for the three models discussed previously are shown in Fig. 2. Fig. 2*A* shows the linear doubling model. The ratio is the same at the beginning and end of the cell cycle. Initially the ratio decreases, then at a defined point in the cell cycle it exactly doubles, and then it decreases again. The peak synthesis model is shown in Fig. 2*B*. There is an abrupt increase in ratio followed by a slower decrease, and finally a cessation entirely. Exponential synthesis (Fig. 2*C*) is characterized by a constant ratio.

Rate of Protein, RNA, and DNA Synthesis during the Cell Cycle. A logarithmic-phase culture was treated with ^{3}H-labeled protein hydrolysate and [^{35}S]methionine to label protein by the dual-label method and fractionated by centrifugal elutriation. The medium contained all amino acids except methionine and cysteine at 22 mg/liter to allow continuous incorporation of the protein hydrolysate. The ratio of $^{35}S/^{3}H$ is plotted vs. flow rate in Fig. 3*A*. The ratio remains constant at a value of 2.1, indicating that the rate of synthesis of protein increases exponentially throughout the cell cycle. The same result was obtained when [^{3}H]methionine was substituted for ^{3}H-labeled yeast protein hydrolysate.

The rates of synthesis of RNA and DNA were determined in a similar manner, using uracil labels in both cases. The ratio of pulse to long-term incorporation is plotted vs. flow rate in Fig. 3*B* for DNA and in 3*C* for RNA. The results indicate that DNA synthesis is periodic, with a peak of synthesis occurring between 0.1 and 0.4 of the cell cycle. The ratio for RNA is constant; therefore, the rate of RNA synthesis increases exponentially through the cell cycle.

Qualitative Analysis of the Synthesis of Individual Proteins through the Cell Cycle. To test for gross changes in the rate of synthesis of individual proteins during the cell cycle, cells were pulse labeled and fractionated by elutriation, and gel electrophoresis was performed on aliquots from eight fractions. Fig. 4 shows a typical autoradiogram of the gels. Seventy percent of the ^{35}S pulse-labeled material entered the gel; however, 7% of the material is present in the streak on the left-hand margin of the gel. Thus, over 60% of the proteins made by the yeast cell do run as discrete spots on the O'Farrell gels. While the absolute position of a spot varies from gel to gel, the relative position is more constant. It is possible to unambiguously assign each one of about 550 spots a number (Fig. 5). Visual examination of the autoradiogram of the pulse label from different cell cycle fractions indicated that each of the 550 spots was present in each of the cell cycle fractions. Thus, we could find no evidence that the synthesis of any examined protein was confined to a small portion of the cell cycle or absent from any portion of the cell cycle.

Quantitative Analysis of the Synthesis of Individual Proteins through the Cell Cycle. The intensity of the spot in the autoradiogram is proportional to its rate of synthesis. However, variations in the shape and resolution of the spots from one autoradiogram to another make difficult a quantitative visual comparison of the rate of synthesis of a protein. To study the rate of synthesis of individual proteins, a logarithmic-phase culture was labeled continuously for 3 hr with ^{3}H-labeled yeast protein hydrolysate in buffered medium containing all the amino acids except methionine and cysteine at 22 mg/liter. At the end of 3 hr, the culture was pulse labeled for 10 min with [^{35}S]methionine. The culture was divided by centrifugal elutriation into eight fractions representing different positions in the cell cycle and two-dimensional electrophoresis was performed. Individual spots were cut out and the ratios of the pulse

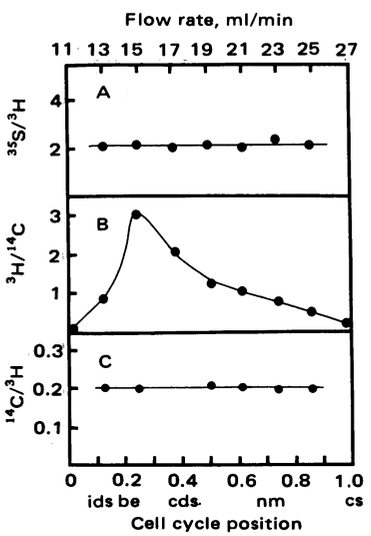

FIG. 3. The synthesis of protein, DNA, and RNA during the cell cycle. Cells were labeled by the dual-label method and separated by elutriation, and the ratio of pulse to long-term radioactivities was determined. (*A*) Ratio of radioactivities in protein; (*B*) alkali-stable trichloroacetic acid-insoluble radioactive material representing DNA: (*C*) acid-insoluble radioactive material in RNA. Timing of the various cell cycle markers was determined from the peaks in cell type (Fig. 1) and the peak in DNA synthesis (above). By using previously published timings (14, 15, 19) for DNA synthesis (0.1–0.4), bud emergence (0.2), and nuclear division (0.75), the cell cycle position vs. flow rate was obtained. Abbreviations: ids, initiation of DNA synthesis; be, bud emergence; cds, completion of DNA synthesis; nm, nuclear migration; cs, cell separation.

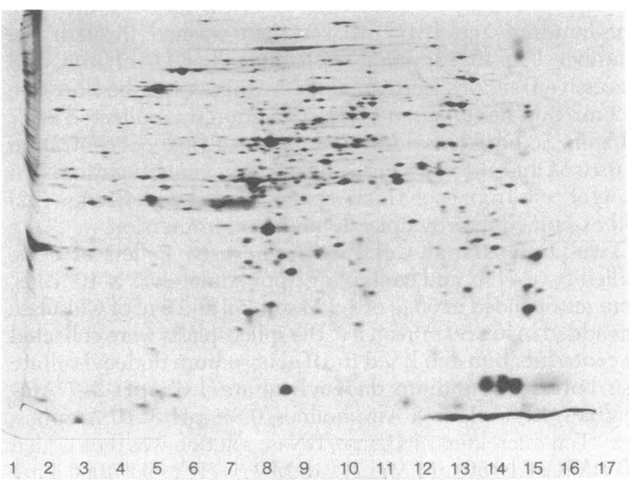

FIG. 4. Two-dimensional electrophoresis of yeast protein. ^{35}S-Labeled protein containing 1×10^{6} cpm was electrophoresed as described in *Materials and Methods*. After a 5-day exposure, the autoradiogram was developed according to standard procedures. Numbers (cm) were placed along the edge to establish relative position.

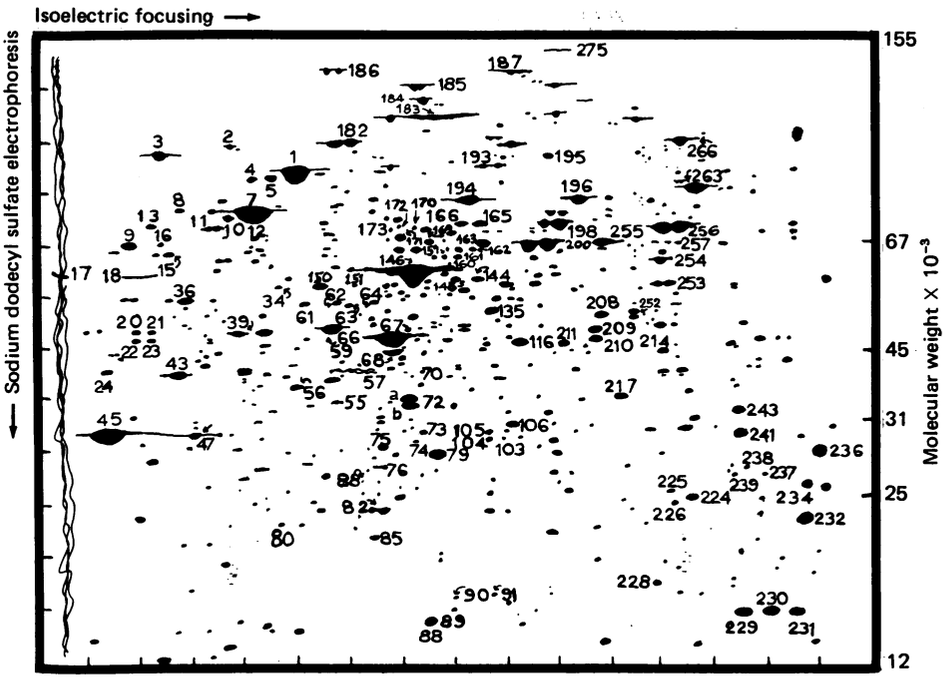

FIG. 5. Two-dimensional map of yeast protein. The map was prepared from Fig. 4. One-centimeter marks are placed along the edges to establish relative position. Numbers are assigned to proteins that were analyzed. Molecular weight markers are bovine serum albumin (67,000), ovalbumin (45,000), DNase 1 (31,000), and chymotrypsinogen (25,100).

and long-term incorporations were determined. The $^{35}S/^{3}H$ ratio for spot 67 remains constant through the cell cycle at a value of 0.15 (Fig. 6). Thus, the rate of synthesis of this polypeptide increases exponentially through the cell cycle.

One-hundred and eleven different polypeptide spots were cut out and analyzed as described above (Fig. 5). The results from ten typical polypeptide spots are listed in Table 1. The unvarying pulse-to-long-term label ratio indicates that the rate of synthesis of each of these proteins increases exponentially during the cell cycle. The rest of the 111 proteins examined also had invariant pulse-to-long-term label ratios. The percent standard deviation [(standard deviation/average) × 100] through the cell cycle was calculated for each of the 111 polypeptides examined. The average percent standard deviation was 11%. In contrast, DNA synthesis (Fig. 3*B*), which is clearly an example of periodic synthesis, showed a 75% standard deviation. The linear doubling model (Fig. 2*A*) predicts a 22% standard deviation (Fig. 2*C*). Additionally, the pattern of the rate-to-mass ratio between the two models is quite distinctive. Only 8 out of the 111 polypeptides examined had percent standard deviations through the cell cycle greater than 22%. No recognizable pattern of change in the rate-to-mass ratio through the cell cycle could be determined for these polypeptides. The points appeared to be randomly distributed around the exponential accumulation line. Thus, all 111 polypeptides show an exponentially increasing pattern of synthesis through the cell cycle.

DISCUSSION

Previous workers have established the importance of balanced growth during cell cycle studies (17, 18). The analysis of the cell cycle by centrifugal elutriation presented here is nearly ideal from that standpoint because the cells are labeled during exponential growth and subsequently separated into various cell cycle fractions under conditions that preclude macromolecular synthesis. Gordon and Elliott (8) have shown that separations of yeast cells according to their position in the cell cycle by centrifugal elutriation compares favorably with other separation methods that have been developed. Distinctive cell types are well separated and the relative number of cells of each cell type, summed from all the fractions, is the same as in the starting balanced growth culture.

Our evidence indicates that the rates of RNA and protein synthesis increase in an exponential manner through the cell cycle, while the rate of DNA synthesis varies periodically. The increased rate of DNA synthesis that marks the S phase of the cycle begins near the time of bud emergence and takes about one-fourth of the cell cycle for completion, in agreement with previous studies by Williamson (19) and Hartwell *et al.* (16).

The rate of RNA synthesis divided by the total RNA in the

Table 1. $^{35}S/^{3}H$ ratios of individual proteins through the cell cycle

Protein	Flow rate,* ml/min							
	13	15	17	19	21	23	25	27
1	0.12	0.13	0.13	0.14	0.13	0.14	0.13	0.13
7	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10
10	0.11	0.11	0.11	0.10	0.10	0.10	0.09	0.10
79	0.10	0.10	0.10	0.10	0.09	0.10	0.09	0.10
146	0.11	0.13	0.15	0.15	0.15	0.14	0.14	0.15
209	0.12	0.12	0.13	0.13	0.13	0.13	0.13	0.13
229	0.13	0.14	0.14	0.15	0.13	0.14	0.15	0.14
252	0.11	0.12	0.12	0.13	0.13	0.12	0.12	0.12
254	0.12	0.14	0.12	0.13	0.14	0.14	0.12	0.13
266	0.19	0.22	0.21	0.19	0.22	0.22	0.21	0.23

* The relationship between flow rate and cell cycle position can be deduced from Fig. 5.

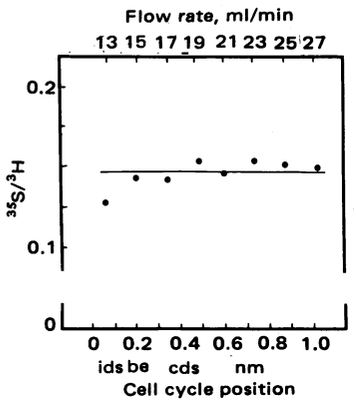

FIG. 6. Synthesis of a protein through the cell cycle. Protein was dual-labeled and fractionated by elutriation, and electrophoresis was performed on each fraction. For each fraction, spot 67 (Fig. 5) was cut out and the ratio of radioactivities in it was determined. For explanation of the assignment of cell cycle position, see Fig. 3.

cell is constant during the cell cycle (Fig. 3C), indicating that the apparent rate of RNA synthesis shows an exponential increase through the cell cycle. Our results then appear to support the conclusion of Sogin *et al.* (20), who noted that the rate of RNA synthesis is unaffected by the DNA doubling during S phase. Recently, however, Fraser and Carter (21) reported a doubling in the rate of synthesis of rRNA and poly(A)-containing RNA in *S. cerevisiae* during S phase. The same results were reported in *Schizosaccharomyces pombe*, by Fraser and Moreno (22). These discrepancies may be explained by medium or strain differences. Further studies will be required to determine the effect of DNA replication on synthesis of different RNA species during the cell cycle.

We find that the overall rate of protein synthesis increases exponentially through the cell cycle. Fig. 3A shows that the apparent rate of protein synthesis divided by the total amount of protein is a constant through the cell cycle. Because the apparent rate is based on a 10-min pulse label, corrections are required for precursor pool saturation to determine absolute rates. However, the corrections are negligible because the methionine pool is half saturated less than 30 sec after the introduction of labeled methionine (23). Thus, the apparent rate of protein synthesis (Fig. 3A) accurately reflects the absolute rate of synthesis. Previous results appeared to suggest that protein showed a linear rather than an exponential rate of accumulation during the cell cycle (24–26). However, the previous data do not allow a clear distinction between linear and exponential rates of increase and are not inconsistent with our observation that the overall rate of increase of protein is exponential. Our observations in *S. cerevisiae* appear to be inconsistent with simulated linear patterns of protein synthesis deduced from the apparent rate of polyadenylated mRNA synthesis during synchronous culture in *Schizosaccharomyces pombe* (22). Using the pulse–long-term dual-label method, it was found that all of the 111 different polypeptides examined quantitatively showed an exponential increase in their rate of synthesis through the cell cycle. In addition, visual examination of the autoradiograms from the O'Farrell gels demonstrated that each of the 550 major polypeptides was synthesized in every fraction of the cell cycle. These results suggest that periodic variations in the rate of synthesis of individual proteins must occur in a small fraction of proteins if variation occurs at all. However, it should be noted that three potentially interesting classes of proteins fail to focus in the O'Farrell system. These are the very acidic proteins, the very basic proteins, and the proteins with special solubility problems.

Because a number of enzymes do focus in our electrophoretic system, our results suggest that the periodic variations in enzymatic activity that have been reported may be mainly due to the periodic enzyme activation rather than periodic enzyme synthesis. This makes unlikely a number of general theories on periodic synthesis—e.g., linear reading, oscillatory repression, or a relationship to the periodic doubling in DNA content. Furthermore, it suggests that control of cell division may be mediated via a measurement of the amount of regulatory proteins present; or, alternately, it may be regulated by periodic changes in activity or proteins without a corresponding change in synthesis. In any case, the subject is now open to direct experimental test. Purified enzyme preparations can be electrophoresed in the two-dimensional system and the spots corresponding to the enzymes can be identified. It is then straightforward to determine if changes in enzymatic activity correlate with changes in the rate of synthesis of the enzyme.

We wish to thank Steve Oliver and John Stephens for helpful discussions in the preparation of this manuscript. This investigation was supported by grant number CA10628 awarded by the National Cancer Institute, Department of Health, Education, and Welfare.

1. Mitchison, J. M. (1971) *The Biology of the Cell Cycle* (Cambridge University Press, Cambridge, England).
2. Mitchison, J. M. (1969) *Science* **165**, 657–663.
3. Donachie, W. D. & Masters, M. (1969) in *The Cell Cycle-Gene Enzyme Interactions*, eds. Padilla, G. M., Whitson, G. L. & Cameron, J. L. (Academic, New York), p. 37.
4. Halvorson, H. O., Carter, B. L. A. & Tauro, P. (1971) *Adv. Microbiol. Physiol.* **6**, 47–105.
5. Mitchison, J. M. & Creanor, J. (1969) *J. Cell Sci.* **5**, 373–391.
6. Wain, W. H. (1971) *Exp. Cell Res.* **69**, 49–56.
7. Shulman, R. W., Hartwell, L. H. & Warner, J. R. (1973) *J. Mol. Biol.* **73**, 513–525.
8. Gordon, C. N. & Elliott, S. G. (1977) *J. Bacteriol.* **129**, 97–100.
9. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
10. Graham, R. & Stanley, W. M. (1972) *Anal. Biochem.* **47**, 505–513.
11. Simchin, G., Piñon, R. & Salts, Y. (1972) *Exp. Cell Res.* **75**, 207–218.
12. Hartwell, L. H. (1970) *J. Bacteriol.* **104**, 1280–1285.
13. Irie, R., Yano, N., Morichi, T. & Kembo, H. (1965) *Biochem. Biophys. Res. Commun.* **20**, 389–442.
14. Culotti, J. & Hartwell, L. H. (1971) *Exp. Cell Res.* **67**, 389–401.
15. Hartwell, L. H. (1971) *Exp. Cell Res.* **69**, 265–276.
16. Hartwell, L. H., Culotti, J., Pringle, J. R. & Reid, B. J. (1974) *Science* **183**, 46–51.
17. Campbell, A. (1957) *Bacteriol. Rev.* **21**, 263–272.
18. Sebastian, J., Carter, B. L. A. & Halvorson, H. O. (1971) *J. Bacteriol.* **108**, 1045–1050.
19. Williamson, D. H. (1965) *Biochem. J.* **90**, 25–26.
20. Sogin, S. J., Carter, B. L. A. & Halvorson, H. O. (1974) *Exp. Cell Res.* **89**, 127–138.
21. Fraser, R. S. S. & Carter, B. L. A. (1976) *J. Mol. Biol.* **104**, 223–242.
22. Fraser, R. S. S. & Moreno, F. (1976) *J. Cell Sci.* **21**, 497–521.
23. Warner, J. R., Morgan, S. A. & Shulman, R. W. (1976) *J. Bacteriol.* **125**, 887–891.
24. Williamson, D. H. & Scopes, A. W. (1961) *J. Inst. Brew. London* **67**, 39–42.
25. Gorman, J., Tauro, P., LaBerge, M. & Halvorson, H. (1974) *Biochem. Biophys. Res. Commun.* **15**, 43–49.
26. Hilz, H. & Eckstein, H. (1964) *Biochem. Z.* **340**, 351–382.