RATES OF SYNTHESIS OF POLYADENYLATED MESSENGER RNA AND RIBOSOMAL RNA DURING THE CELL CYCLE OF SCHIZOSACCHAROMYCES POMBE

WITH AN APPENDIX

CALCULATION OF THE PATTERN OF PROTEIN ACCUMULATION FROM OBSERVED CHANGES IN THE RATE OF MESSENGER RNA SYNTHESIS

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SUMMARY

The rates of polyadenylated messenger RNA and ribosomal RNA synthesis were measured in synchronously dividing cultures of fission yeast (Schizosaccharomyces pombe). Control asynchronous cultures, which had been exposed to the conditions used for preparing synchronous cultures, were investigated to check for effects of the synchronization procedure itself on RNA synthesis. After each period of DNA synthesis in synchronous culture, the rates of messenger and ribosomal RNA synthesis doubled, suggesting that gene number controls the rate of messenger and ribosomal RNA synthesis. This was confirmed by experiments with asynchronous, exponential-phase cultures in which DNA synthesis was inhibited by hydroxyurea. Both synchronous culture and hydroxyurea experiments suggested that there is a delay of 15 min (0.1 of the cell generation time) between replication of the DNA and transcription of both gene copies.

A pattern of protein accumulation was calculated from changes in the rate of polyadenylated messenger RNA synthesis during synchronous culture. This simulated pattern indicates that protein is accumulated linearly, with a doubling in the rate of accumulation once per cell cycle. The simulated pattern of protein accumulation is very similar to measurements previously reported by other workers of changes in activities of 3 enzymes in synchronous cultures.

It is suggested that the doubling of the rate of messenger RNA synthesis, as a consequence of the replication of the DNA once per cycle, provides the basis of a mechanism for control of the doubling of other cellular constituents during the cell cycle.

INTRODUCTION

Exponential growth of eukaryotic cells involves the doubling, during one cell cycle, of the DNA and all other cellular components, to produce 2 daughter cells identical to the parent. The mechanisms which control the balanced duplication of cellular components other than DNA are not understood. However, it is possible

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that the doubling of gene number during DNA replication may serve as a framework for the control of doubling of at least some other cellular components. It is therefore of interest to examine changes in the pattern of gene activity during the cell cycle by following rates of messenger RNA synthesis. Since the discovery that many eukaryotic messenger RNAs contain a polyadenylic acid sequence (Darnell, Wall & Tushinski, 1971; Lee, Mendecki & Braverman, 1971; Edmonds, Vaughan & Nakazato, 1971; McLaughlin et al. 1973) and the development of methods for the isolation of this polyadenylated messenger RNA (Edmonds & Caramela, 1969; Aviv & Leder, 1972), it has become possible to study directly the rate of synthesis of a defined fraction of cellular messenger RNA.

We have measured changes in the rates of synthesis of messenger and ribosomal RNA in synchronously dividing cultures of the fission yeast *Schizosaccharomyces pombe*. This organism was chosen for study because the patterns of increase of several enzymes through the cell cycle have been studied in detail (Mitchison & Creanor, 1969). Knowledge of changes in the rate of messenger RNA synthesis during the cell cycle enables us to relate the changes in activity of these enzymes to gene number.

Materials and Methods

Yeast and culture conditions

*Schizosaccharomyces pombe* Lindner, Strain N.C.Y.C. 132; A.T.C.C. 24751 was grown in a minimal medium EMM 2 (Mitchison, 1970) at 32 °C. Cultures of 200—2000 ml were grown in conical flasks on a rotary shaker operating at 220 rev. min⁻¹; 20-l. cultures were stirred by a motor-driven propellor. Cell growth was followed by measuring absorbance of cultures at 595 nm.

Uptake and incorporation of [2-³H]adenine

One-millilitre samples of culture were added to 3·3 µCi [2-³H]adenine (Radiochemical Centre, Amersham, U.K.; sp. act. 22 Ci. mmol⁻¹) and 1 µg non-radioactive adenine, and incubated for 5 min at 32 °C.

For adenine uptake measurements, 5 ml ice-cold water containing 100 µg ml⁻¹ non-radioactive adenine were added to the incubation. The cells were collected by filtration on Whatman GF/A glass-fibre paper, washed 3 times with 5 ml cold adenine solution, then ³H-radioactivity on the dried filters was measured by counting in 5 ml 0·5 % (w/v) butyl-PBD-toluene scintillator.

For measurement of adenine incorporation, 5 ml ice-cold 10% (w/v) CCl₄COOH containing 100 µg ml⁻¹ non-radioactive adenine were added to the incubation. The cells were collected by filtration, washed 3 times with 5 ml CCl₄COOH solution and radioactivity incorporated into acid-insoluble material determined by scintillation counting as above.

Synchronous cultures

Cells were harvested by filtration on Whatman No. 50 paper from 10—20 l. of exponential-phase culture grown to a cell concentration of 4 × 10⁸ ml⁻¹. Small cells near the beginning of the cell cycle were selected by centrifugation on sucrose gradients (Mitchison & Vincent, 1962) and were re-inoculated into fresh EMM 2 to give a cell concentration of 1—1·5 × 10⁴ ml⁻¹. Progress of division in synchronous cultures was followed by monitoring the percentage of cells with a cell plate, which appears shortly before cell division.

Asynchronous control cultures were prepared to check for artifacts introduced by the
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synchronization procedure. Cells were fractionated on sucrose gradients as above, then the gradients were mixed thoroughly before taking a sample containing cells at all stages of the cell cycle as inoculum for the asynchronous culture. Alternatively, cells from an asynchronous, exponential-phase culture were collected by filtration and resuspended in culture medium containing 27% sucrose for 15 min before reinoculation.

Radioactive incubations

At 20-min intervals after inoculation of the synchronous or asynchronous control cultures, samples of 60-100 ml, containing 1-2 x 10^8 cells, were mixed with 3.3 µCi ml^-1 [2-3H]adenine for a 10-min pulse incubation. The adenine pool in fission yeast is expandable, and adenine uptake is extremely rapid (Cummins & Mitchison, 1967). In most experiments, the total adenine concentration of the pulse-incubation medium was raised to 1 µg ml^-1 by addition of non-radioactive adenine. Under these conditions, the concentration of adenine in the medium did not change significantly during the pulse incubation: less than 10% of the total adenine supplied was taken up by the densest cultures labelled.

As one approach to testing whether the rate of adenine uptake controlled the rate of incorporation, we also pulse-labelled one culture with 3.3 µCi ml^-1 [2-3H]adenine at a total adenine concentration of 0.03 µg ml^-1. Under these conditions, almost all of the adenine supplied was taken up by the cells during the pulse incubation, and uptake per ml culture remained constant during culture growth. All adenine incorporation results obtained under these labelling conditions were corrected to allow for this exhaustion of the adenine supply by dividing each incorporation value by the level of adenine uptake per cell for that pulse incubation. The effect of this procedure is to produce a curve for changes in adenine incorporation during culture growth which would result if adenine uptake per cell remained constant.

After the 10-min pulse incubation, the sample was chilled by addition of crushed ice. The cells were collected by filtration on Oxoid 6-cm diameter 0.45-µm pore-size filters, and resuspended in 5 ml ice-cold extraction medium (2% (w/v) sodium tri-isopropylnaphthalene sulfonate in 15 mM NaCl; 50 mM Tris-HCl, pH 7.8) and stored frozen at -20 °C. Control experiments showed that incorporation of adenine into nucleic acids ceased immediately on chilling the culture.

The cell suspension was thawed at 0 °C. Ten-microlitre samples were dried on Whatman GF/A paper and counted in butyl-PBD-toluene scintillator as above to measure adenine uptake. Ten-microlitre samples were mixed with 5 ml ice-cold 10% CCl_4COOH containing 100 µg ml^-1 adenine. The cells were collected by filtration, washed 3 times with 5 ml CCl_4COOH solution and total adenine incorporation determined by scintillation counting as above. One-hundred-microlitre samples of cell suspension were diluted with 5 ml 0.9% (w/v) NaCl. The cells were separated by sonication for 75 s in the M.S.E. sonicator, further diluted with saline, and cell number was determined using a Coulter Counter. Suspension of cells in extraction medium and freezing did not influence the cell number determination.

Extraction of nucleic acids

Total nucleic acid was extracted from the remainder of the cell suspension. The cells were broken by agitation with 2 g of glass beads (40 mesh) for 5 min in a Vibromixer (Shandon Scientific) and nucleic acids were extracted and deproteinized by successive phenol/chloroform treatments as explained in detail elsewhere (Fraser, Creanor & Mitchison, 1973; Fraser, 1975). The extracted nucleic acids were ethanol-precipitated and further purified by 3 cycles of reprecipitation from 0.5% (w/v) sodium dodecyl sulphate in 150 mM sodium acetate pH 6.0 by 2.5 volumes of ethanol (Loening, 1969). The nucleic acids were stored as a precipitate in 80% ethanol; 0.1% sodium dodecyl sulphate; 30 mM sodium acetate, pH 6.0, at 4 °C.

A portion of the nucleic acid precipitate was collected by centrifugation at 2000 g for 5 min, then hydrolysed in 2.5 ml 0.5 N HClO_4 at 70 °C for 20 min. The ultraviolet absorption spectrum of the hydrolysate was measured in the Unicam SP800 recording spectrophotometer. Nucleic acid concentration was calculated from the absorbance at 260 nm, using a molar absorption coefficient of 12400 M^-1 cm^-1, calculated for a solution of equal molar quantities of the 4 ribonucleotides at acid pH. In fission yeast, about 99% of the total nucleic acid is RNA;
the absorption spectra therefore provided values for RNA per ml culture. The total yield of nucleic acid from each incubation was within the range 150 to 300 μg.

**Measurement of DNA synthesis**

Two equal aliquots of nucleic acid suspension were sedimented at 2000 g for 5 min. One was incubated with 0.5 ml 1 M KOH at 45 °C for 12 h to hydrolyse RNA. The other was washed with 1 ml 70 % ethanol; 0.1 M NaCl, to remove sodium dodecyl sulphate, then dissolved in 0.1 ml 50 mM 2-(N-morpholino)ethanesulphonic acid, pH 7.0; 2 mM magnesium acetate. DNase (Sigma, electrophoretically purified) was added to 10 μg ml⁻¹ and DNA was digested for 30 min at 20 °C. The sample was then digested with KOH as above.

Sixty per cent HClO₄ was added to each sample to reduce the pH to 1 and to precipitate KClO₄ as carrier, then 0.5 ml 10 % CCl₄ COOH containing 100 μg ml⁻¹ adenine was added. Acid-insoluble material was collected by filtration on Whatman GF/A glass-fibre paper, washed 3 times with ice-cold CCl₄ COOH and radioactivity on the dried filters was determined as above. Radioactivity incorporated into DNA was calculated by subtracting the radioactivity of the DNase + KOH-digested sample from the radioactivity of the parallel, KOH-digested sample. At the mid-point of the period of DNA synthesis in synchronous cultures, about 90 % of the KOH-resistant radioactivity was rendered acid-soluble by the DNase treatment.

The 'specific activity' of the DNA was expressed as DNA radioactivity per unit total nucleic acid in the sample. The specific activity could not be expressed as radioactivity per unit weight of DNA, as the amount of DNA in the samples was too small to measure accurately.

**Synthesis of polyadenylated messenger RNA**

Messenger RNA containing a polyadenylic acid sequence (poly(A)⁺mRNA) was isolated from total nucleic acid using the specific binding of the poly(A) sequences, at high salt concentration, to the complementary homopolymer oligodeoxythymidylic acid (oligo(dT)), immobilized on cellulose (Edmonds & Caramela, 1969; Aviv & Leder, 1972). Between 5 and 10 μg total nucleic acid were sedimented and dissolved in 0.5 ml binding buffer (400 mM NaCl; 10 mM Tris-HCl, pH 7.8; 1 mM EDTA; 0.2 % sodium dodecyl sulphate) and mixed with 20 mg oligo (dT) cellulose (Collaborative Research Inc., Waltham, Mass., U.S.A.; binding capacity 100 A₄₅₀ units poly(A) g⁻¹) for 30 min at 20 °C. The oligo (dT) cellulose was sedimented at 10000 g for 1 min, then washed 5 times with 0.5 ml of binding buffer to remove non-specifically bound nucleic acids. The oligo (dT) cellulose was then washed 4 times with 0.25 ml of elution buffer (Binding buffer minus NaCl) to release the poly(A)⁺mRNA.

To determine the total radioactivity of the poly(A)⁺mRNA, the combined elution buffer washes were dried at 70 °C in scintillation vials, then hydrolysed in 0.3 ml 0.5 M HClO₄ at 70 °C for 20 min. Seven millilitres of water-miscible scintillator (0.5 % (w/v) butyl PBD; 40 % (v/v) 2-methoxyethanol; 60 % (v/v) toluene) were added and poly(A)⁺mRNA radioactivity determined by scintillation counting. Correction of counts was by external standard counting, calibrated by addition of [³H]toluene to a range of samples with different degrees of quenching. The 'specific activity' of the poly(A)⁺mRNA was expressed as radioactivity per unit weight total nucleic acid fractionated on oligo(dT) cellulose, since the actual weight of poly(A)⁺mRNA prepared by binding to oligo(dT) cellulose was too low to measure.

Examples of fractionations on sucrose gradients of fission yeast poly(A)⁺mRNA samples are published elsewhere (Fraser, 1975). The poly(A)⁺mRNA sediments on gradients to give a polydisperse distribution, ranging from 6 to over 30 s, with a broad peak around 12 s. There is no sign of contamination of the poly(A)⁺mRNA by ribosomal or transfer RNAs.

**Specific activity of ribosomal RNA**

Ten microgrammes of total nucleic acid were fractionated on a 2.5 % polyacrylamide gel for 2-5 h at 5 mA per gel, 8 V cm⁻¹ gel length (Loening, 1967). The gels were scanned for absorbance at 265 nm in a Joyce Loebel Gel Scanner. The weight of ribosomal RNA present on the gels was found, in relative units, from the areas of the peaks for 25 s and 18 s rRNAs on
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the absorbance scan. Peak area is linearly proportional to weight of RNA present (Fraser, 1971). The gel was frozen in solid CO$_2$ and sliced transversely at 1.0-mm intervals. Each slice was incubated with 0.3 ml 0.5 M HClO$_4$ at 70 °C for 20 min to hydrolyse the RNA; 7 ml of water-miscible scintillator were added and the radioactivity in each slice determined by scintillation counting. The total radioactivity of ribosomal RNA was calculated by addition of the radioactivities of the individual gel slices in the rRNA radioactivity peaks, after subtracting the background, polydisperse radioactivity. Radioactivity in the rRNA precursor was included in the total rRNA radioactivity. The specific activity of the rRNA was calculated as total rRNA radioactivity divided by the combined areas of the 25 and 18 s rRNA absorbance peaks. Ultraviolet absorption and radioactivity scans of gels which illustrate the measurement of rRNA amount and radioactivity by this method are published elsewhere (Fraser & Loening, 1974).

RESULTS

Artifacts caused by the synchronization procedure

It is essential when preparing synchronous cultures by a selection technique to check that the conditions of selection do not themselves induce changes which could be mistaken for cell-cycle related events. The disturbing factors to which yeast cells

![Fig. 1. Changes in optical density at 595 nm (△—△) and in rates of adenine uptake (□—□) and incorporation (●—●) (10$^{-4}$ x cpm ml$^{-1}$) in an asynchronous culture of fission yeast. Cells from an exponential-phase culture were fractionated on a 10-40% sucrose gradient (Mitchison & Vincent, 1965). The gradient was shaken after centrifugation and cells from all stages of the cell cycle re-inoculated into fresh medium. Samples were pulse-labelled for 5 min with 3.3 µCi ml$^{-1}$ [2-3H]adenine at a total adenine concentration of 1 µg ml$^{-1}$.](image-url)
were exposed during selection synchronization included concentration by filtration, centrifugation, high sucrose concentration and re-inoculation into fresh culture medium. The two methods used to prepare asynchronous control cultures together exposed cells to all of these disturbing factors, but did not select cells by size before re-inoculation.

Fig. 2. Changes in specific activity of rRNA (△—△) (relative units) and poly(A)+ mRNA (●—●) (10^4 x cpm µg^-1 total nucleic acid) in an asynchronous culture of fission yeast. Cells from an exponential-phase culture were harvested and resuspended in medium containing 27% sucrose for 15 min, then re-inoculated into conditioned culture medium. Samples of the culture were pulse-labelled for 10 min with 3-3 µCi ml^-1 [2-3H]adenine; 1 µg ml^-1 total adenine concentration, every 20 min during culture growth.

Fig. 1 shows changes in optical density, adenine uptake and incorporation rates in an asynchronous control culture inoculated with cells from a shaken sucrose density gradient. The rate of increase of optical density of the culture was low for the first 45 min, then optical density rose at a rate corresponding to a cell doubling time of 145 min, which is the time in normal exponential culture. The rates of adenine uptake and incorporation were low for the first 30 min, then rose exponentially until at least 4.5 h after inoculation. Thus the process of synchronization appeared to reduce growth of the culture for about the first 30 min after inoculation. There was no sign of any periodic changes in the rates of adenine incorporation or uptake during the period from 30 min to the end of the experiment. However, during this period the rates of increase of adenine uptake and incorporation were greater than the rate of increase of the optical density of the culture. In an asynchronous culture undergoing balanced exponential growth, the rates of increase should be identical. The explanation of this discrepancy is that adenine uptake rises with decreasing pH of the culture medium (R. S. S. Fraser, unpublished results). Fresh EMM 2, into which the cells were inoculated, has a pH of 5.3; during growth of the culture the pH falls to about 4.6.

The effects of the synchronization procedure on synthesis of specific types of RNA were also examined. Cells from an exponential-phase culture were concentrated, suspended at high concentration in 27% sucrose for 15 min, then re-inoculated into conditioned culture medium; 27% sucrose was used as it is the concentration of sucrose in the region of sucrose gradients from which cells are taken for inoculation.
of synchronous cultures. The length of exposure to this sucrose concentration was the time required to load, centrifuge and fractionate a gradient. At intervals after re-inoculation of the asynchronous control culture, samples were pulse-labelled for 10 min with [2-3H]adenine, and the specific activities of poly(A)+mRNA and rRNA were determined. In asynchronous, exponentially growing cultures, the specific activities of pulse-labelled RNA fractions should remain constant with time. Fig. 2 shows that the specific activity of the rRNA rose for 30 min, then remained constant. This result is consistent with the changes in rate of adenine incorporation in the asynchronous control culture inoculated with cells from a shaken sucrose gradient (Fig. 1). The specific activity of the poly(A)+mRNA did not become constant until 1.5 h after re-inoculation. The synchronization procedure therefore causes a depression of both rRNA and poly(A)+mRNA syntheses; poly(A)+mRNA synthesis takes much longer to recover. In some experiments we found that the initial depression of rRNA synthesis was much greater than of poly(A)+mRNA, but recovery to a normal rate of rRNA synthesis still took only 30–60 min.

Changes in RNA synthesis during growth of synchronous cultures

Fig. 3 shows changes in cell number and cell plate index in a typical synchronous culture. The first division occurred between 2 and 3 h; the second between 4 and 5 h. Peaks of cell plate index at 2 h and 4 h 20 min were slightly in advance of the increases in cell numbers. The curve for DNA specific activity shows that DNA synthesis occurred in 2 periods, between 2 and 3 h, and between 4 and 5 h. This is consistent with previous results obtained by measuring total DNA by the diphenylamine method, which showed that the period of DNA synthesis (S-period) coincides with cell division (Bostock, Donachie, Masters & Mitchison, 1966; Mitchison & Creanor, 1971 a). The 2 horizontal lines labelled SI and SII in Fig. 3c show the times of the first and second periods of DNA synthesis in the culture. These lines are reproduced in later figures showing changes in rates of RNA synthesis in the culture. Because of the imperfect synchrony of the culture, the duration of S-period in the entire culture will be longer than the duration of S in the individual cell.

Fig. 4A shows changes in total nucleic acid per ml culture during growth of the synchronous culture. There is considerable scatter of points, as the yield of nucleic acids by the phenol-detergent extraction method is variable. This is entirely a consequence of variable degrees of breakage of cells during the extraction. Total nucleic acid per ml culture increased approximately exponentially, with a doubling time equal to the cell generation time.

Fig. 4B shows that rates of uptake and incorporation of [2-3H]adenine changed in a periodic manner during growth of the synchronous culture. The portion of the curves drawn in broken lines at the start of culture indicates those points which were probably depressed by the effects of the synchronization conditions (Fig. 1). While the pattern of periodic change is the same for uptake and incorporation, we consistently found that the timings of the changes were different, with incorporation changing about 15 min before uptake.

Changes in the specific activities of pulse-labelled rRNA and poly(A)+mRNA in
2 synchronous cultures are shown in Fig. 5. The results are expressed as specific activities to eliminate variation arising from differences in efficiency of extraction of nucleic acids. Poly(A)\(^+\)mRNA specific activity was fairly constant until the middle of the first S-period, rose during the second half of the first S-period, then declined between the first and second S-periods. A second rise in poly(A)\(^+\)mRNA specific activity occurred late in the second S-period. rRNA specific activity was initially very low in both cultures, but rose rapidly, then declined during most of the first S-period. Late in the first S-period, rRNA specific activity rose, then declined again between the first and second S-periods, and during most of the second S-period. Late in the second S-period the specific activity of rRNA again rose. Each rise in rRNA
specific activity during S was later than the corresponding rise in the specific activity of poly(A)+mRNA.

The culture shown in Fig. 5A was labelled at a total adenine concentration of 1 μg ml⁻¹; that shown in Fig. 5B with 0.03 μg ml⁻¹, and the results were corrected for exhaustion of adenine during the pulse as explained in Materials and methods. It is clear that the patterns of changes in specific activities of RNA in the 2 cultures were similar, despite the different labelling conditions.

The rises in specific activities of pulse-labelled poly(A)+mRNA and rRNA after commencement of DNA synthesis suggest a connexion between DNA content and rate of RNA synthesis. However, the expression of the data as specific activities,

Fig. 4. Changes in A, total phenol-detergent extracted nucleic acid per ml culture; and B, incorporation (■—■) and uptake (○—○) (10⁻⁴ x cpm ml⁻¹) of [2-³H]adenine during growth of a synchronous culture. Samples of culture were pulse-labelled for 10 min with 3.3 μCi ml⁻¹ [2-³H]adenine and 1 μg ml⁻¹ total adenine concentration. SI and SII indicate the times of the first and second periods of DNA synthesis; CPI and CII show times of first and second peaks of cell plate index (from Fig. 3).
Fig. 5. Changes in specific activities of rRNA (△—△) (relative units) and poly(A)+ mRNA (○—○) ($10^4$ x cpm $\mu$g$^{-1}$ total nucleic acid) during synchronous cultures of fission yeast. The vertical lines and cross-bars on the poly(A)+mRNA points show the standard errors of the means. Each point is the mean of 6 determinations by oligo(dT)-cellulose fractionation. S1 and SII show the times of the first and second periods of DNA synthesis in each culture, determined as shown in Fig. 3c. CPI and CPII indicate the times of the first and second peaks of cell plate index. The results shown in A are from a culture pulse-labelled with 3.3 $\mu$Ci ml$^{-1}$ [3-$^{3}$H]adenine and a total adenine concentration of 1 $\mu$g ml$^{-1}$. In B, samples of culture were pulse-labelled with 3.3 $\mu$Ci ml$^{-1}$ [3-$^{3}$H]adenine and a total adenine concentration of 0.03 $\mu$g ml$^{-1}$. The results shown in B have been corrected for exhaustion of adenine in the pulse medium by dividing each incorporation value by the level of adenine uptake per cell at the time of the pulse.
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while avoiding variation arising from different efficiencies of extraction of nucleic acids, does not make immediately apparent the full relationship of the rate of RNA synthesis to the occurrence and timing of DNA synthesis. To investigate this relationship, the primary data can be transformed in 2 ways. Firstly, the rate of poly(A)+ mRNA synthesis per ml culture can be calculated by multiplying the specific activity (cpm µg⁻¹ total nucleic acid) by the value for total nucleic acid per ml culture at the

Fig. 6. Changes in the rate of rRNA synthesis (Δ—Δ) (relative units) and poly(A)+-mRNA synthesis (■—■)(10⁻⁸ x cpm) per ml culture during synchronous culture, calculated from changes in specific activities of rRNA and poly(A)+mRNA shown in Fig. 5 A and changes in total nucleic acid extracted per ml culture shown in Fig. 4 A. S1 and SII show the times of the first and second periods of DNA synthesis; CPI and CP II, the times of the first and second peaks of cell plate index (from Fig. 3 c, b, respectively).

time of the pulse incubation. Specific activity data for rRNA can be similarly treated to give rate of rRNA synthesis per ml culture. We have taken values for total nucleic acid per ml culture from the line calculated to give the best fit to the experimental points in Fig. 4 A, rather than using the actual determined values. This avoids variation from differences in the efficiency of extraction of nucleic acids.

Fig. 6 shows changes in the rates of poly(A)+mRNA and rRNA synthesis per ml culture, calculated from the changes in specific activities in the synchronous culture shown in Fig. 5 A. The initial rate of rRNA synthesis was very low, a consequence of the transitory depression of rRNA synthesis by the conditions of synchronization (Fig. 2). Poly(A)+mRNA synthesis was also depressed in the early stages of culture
Following the commencement of the first S-period, the rates of synthesis of both rRNA and poly(A)+mRNA doubled. Between the first and second S-periods, the rates of synthesis of both types of RNA remained constant. After the beginning of the second S-period, the rate of poly(A)+mRNA synthesis per ml culture again doubled; rRNA synthesis also increased. The doubling of rates of RNA synthesis during S-periods suggests that gene number controls the rate of RNA synthesis.

A more sensitive test of the relationship of poly(A)+mRNA synthesis to the occurrence and timing of DNA synthesis is obtained by calculating a curve for changes in mRNA specific activity which would be produced if gene number alone were controlling the rate of RNA synthesis. From the changes in DNA specific activity during synchronous culture (Fig. 3c), we obtain a curve for DNA synthesis per ml culture by multiplying the specific activity (cpm μg⁻¹ total nucleic acid) by total nucleic acid ml⁻¹ at the time of the pulse incubation (from Fig. 4A). Integration of the curve for DNA synthesis per ml culture over successive time increments gives a curve for DNA content per ml culture. The amount of DNA at time o is taken to equal the increment in DNA during the first S-period. This curve is shown in Fig. 7. If the rate of mRNA
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RNA synthesis is controlled only by gene number, the amount of mRNA synthesized in any time period is proportional to the average DNA content during that period. Dividing this figure for synthesized mRNA by the total nucleic acid per ml culture at this time gives a curve of changes in mRNA specific activity where mRNA synthesis is limited only by gene number. This theoretical specific activity curve (Fig. 7) rises during periods of DNA synthesis, and falls between S-periods, as total nucleic acid accumulates. The amplitude of the periodic fluctuation gradually decreases, reflecting the gradual loss of synchrony of division and DNA synthesis in the culture.

It is clear that the calculated changes in mRNA specific activity are broadly similar to the experimentally measured changes in poly(A)+mRNA specific activity (Fig. 7). However, there are 2 important differences between the 2 curves. In the first 1.5 h of culture, the pattern of changes in the experimental data is very different from that of the calculated data; the experimental values are lower than expected. This is a consequence of the depression of early poly(A)+mRNA synthesis by the synchronization procedure (Fig. 2). Secondly, while thereafter the periodic fluctuations of experimental and calculated specific activities were similar, attempts to superimpose the calculated data on the experimental data consistently showed that the changes in the experimental curve were about 15 min later than the corresponding changes in the calculated curve. This suggests that while the rate of poly(A)+mRNA synthesis may be basically regulated by gene number, there is a short delay between the replication of the DNA and the transcription of both copies of the gene. An alternative explanation is that DNA which is actively transcribed may be replicated late in the S-period, but this is made unlikely by the evidence to be discussed below.

RNA synthesis after inhibition of DNA synthesis

The results described above suggest a dependence of RNA synthesis rate on gene number, but do not establish a causal relationship. Further evidence for the control of the rate of poly(A)+mRNA synthesis by gene number, and for the 15-min delay between DNA replication and transcription of both gene copies, comes from experiments with asynchronous, exponential-phase cultures in which DNA synthesis was inhibited. The rate of poly(A)+mRNA synthesis in control cultures rose exponentially, at the same rate as cell growth (Fig. 8). Twelve millimolar hydroxyurea was added to one half of the culture to inhibit DNA synthesis (Mitchison & Creanor, 1971b). Within 5 min of adding the inhibitor, the specific activity of DNA after a pulse-label with [2-3H]adenine was one-quarter that in the control, and no significant pulse-labelling of DNA was detectable by 10 min after addition of hydroxyurea. The rate of poly(A)+mRNA synthesis in hydroxyurea-treated cells rose at the same rate as in control cultures for 15-20 min, then became stable for at least 1 h. By 100 min after addition of hydroxyurea, there was again a small rise in the rate of poly(A)+mRNA synthesis. The rate of uptake of adenine by hydroxyurea-treated cells rose at the same rate as in control cells for about 50 min, then became constant. This suggests that the effects of hydroxyurea on labelling of poly(A)+mRNA were not merely a consequence of an effect of the inhibitor on isotope uptake, but were an effect on poly(A)+mRNA synthesis. Overall cell growth, measured by increase in the optical
Fig. 8. A, changes in the rates of adenine uptake (Δ, ▲) (10^{-8} \times \text{cpm ml}^{-1}) and poly-(A)^{+}RNA synthesis (○, ●) (10^{-8} \times \text{cpm ml}^{-1}) in asynchronous, exponential cultures of fission yeast; 12 mM hydroxyurea was added to one half of the culture at 40 min. Results from the control culture are shown (Δ, ○); and from the hydroxyurea-treated culture (▲, ●). Horizontal bars and vertical lines on the points for poly(A)^{+}RNA synthesis indicate the standard errors of the means. Each point is the mean of 6 determinations by oligo(dT) cellulose fractionation. B, changes in optical density at 595 nm, of the control culture (○) and the hydroxyurea-treated culture (▲). C, changes in total adenine uptake (10^{-8} \times \text{cpm ml}^{-1}), and D, adenine incorporation (10^{-8} \times \text{cpm ml}^{-1}), in a separate experiment from A and B: (Δ, ○), control cultures; (▲, ■), cultures to which 12 mM hydroxyurea was added at 40 min.
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density of the culture, was not affected by hydroxyurea within the time of the experiment.

The change from an exponentially increasing rate of poly(A)$^+$mRNA synthesis to a constant rate after addition of hydroxyurea to inhibit DNA synthesis confirms that synthesis of poly(A)$^+$mRNA is controlled by gene number. The 15-20-min delay between addition of the inhibitor and the onset of a constant rate of poly(A)$^+$mRNA synthesis again suggests that there is a short delay between gene replication and a doubled rate of transcription. The late recovery in rate of poly(A)$^+$mRNA synthesis in hydroxyurea-treated cells probably occurred because the inhibition of DNA synthesis by hydroxyurea in fission yeast is not permanent (Mitchison & Creanor, 1971).

In a separate experiment, the rate of total RNA synthesis became constant 10–15 min after addition of hydroxyurea (Fig. 8D). In this case the adenine uptake rate increased at the same rate as in control cells for 30 min after addition of hydroxyurea, then became stable (Fig. 8c).

DISCUSSION

Cell cycle analysis

The measurement of changes in the rate of RNA synthesis during the cell cycle using selection-synchronized cultures has one disadvantage. The synchronization procedure itself seriously affects RNA synthesis, especially poly(A)$^+$mRNA synthesis, and these artifacts must be investigated before interpreting changes in relation to the cell cycle. We have shown that the first cell cycle in synchronous culture, that is the period from inoculation to the first division, is subject to these artifacts. Thereafter, the changes observed can be taken to be cell cycle-related events.

A better approach to investigation of changes in RNA synthesis during the cell cycle would be to pulse-label an asynchronous, exponentially growing culture, then separate cells by cell volume, hence stage in the cell cycle, by zonal centrifugation (Carter, Sebastian & Halvorson, 1971). We have used this method in a parallel study of RNA synthesis during the cell cycle in the budding yeast Saccharomyces cerevisiae (Fraser & Carter, 1976). However, for our study of fission yeast, synchronous cultures were chosen, because previous investigations of changes in enzyme activity were carried out with synchronous cultures; it was our aim to correlate gene number, messenger RNA synthesis and patterns of enzyme activity.

Throughout these experiments, we have assumed that the rate of incorporation of $[2^{-3}H]$adenine into RNA is a measure of the rate of RNA synthesis, and that changes in the rate of incorporation do not merely reflect changes in the rate of adenine uptake or precursor pool specific activity. We have several reasons for concluding that incorporation rate is a true measure of synthesis. Firstly, where incorporation and uptake rates were followed in synchronous cultures or after addition of hydroxyurea, both uptake and incorporation displayed the same overall pattern of changes. However, changes in uptake rate were always later than corresponding changes in rate of incorporation. This suggests that uptake rate is determined by the rate of incorporation,
and not vice versa. Secondly, in synchronous cultures, the rise in specific activity of poly(A)$^+$mRNA during S-period was consistently earlier than the rise in rRNA specific activity (Fig. 5). It is difficult to reconcile this difference with a situation where incorporation is controlled by uptake rate or precursor pool specific activity, unless there are separate precursor pools for mRNA and rRNA. Thirdly, the size of the adenine-labelled acid-soluble pool, as a proportion of cell dry mass, remains essentially constant during the cell cycle (Mitchison, Cummins, Gross & Creanor, 1969). Finally, the same basic patterns of changes in specific activities of poly(A)$^+$mRNA and rRNA were found when synchronous cultures were labelled with high or low adenine concentrations. At the high adenine concentration, the amount of uptake depended on the activity of the adenine uptake mechanism of the cells. Results from the culture labelled at the low adenine concentration were expressed so as to show the incorporation pattern in an artificial situation where uptake per cell remained constant during culture. The effect of this is to nullify any effects of changes in uptake rate on the rate of incorporation.

**rRNA synthesis during the cell cycle**

In synchronous cultures, the specific activity of pulse-labelled rRNA rose after commencement of the period of DNA synthesis. The rate of rRNA synthesis per ml culture doubled at the end of the first S-period, and began to rise again late in the second S-period. Experiments with hydroxyurea, showing that the rate of total RNA synthesis became stable after inhibition of DNA synthesis, suggested that the doubling during synchronous culture of the rate of rRNA synthesis was a result of the increase in DNA content. These results confirm earlier experiments with *S. pombe* by Wain & Staatz (1973) which showed a rise in the rate of labelling of total, long-pulse-labelled RNA at about the time of DNA synthesis.

The rRNA in *S. pombe* in exponential growth is essentially stable (Fraser, 1975). Therefore a doubling of the rate of rRNA synthesis once per cycle will lead to a doubling of rRNA content over one cell cycle. This provides a basis for control of the doubling of the size of the ribosomal population in each cell cycle.

The rise in specific activity of rRNA during $S$ consistently occurred later than the rise in specific activity of poly(A)$^+$mRNA. This may mean that the DNA coding for rRNA is replicated very late during $S$. The alternative explanation, that there is a long lag between replication of rDNA and transcription of both copies, is made unlikely by the rapid onset of a steady rate of total RNA synthesis after addition of hydroxyurea. In contrast to these results, we found in *Saccharomyces cerevisiae* that the rate of rRNA synthesis doubled very early in $S$, suggesting that in budding yeast the rDNA is replicated early in $S$ and is immediately available for transcription (Fraser & Carter, 1976).

**Poly(A)$^+$mRNA synthesis during the cell cycle**

Our results show that shortly after the commencement of the period of DNA synthesis, the rate of poly(A)$^+$mRNA synthesis per ml culture doubles. A causal relationship between DNA content and the rate of poly(A)$^+$mRNA synthesis was
confirmed by the experiment in which DNA synthesis was inhibited by hydroxyurea. In Saccharomyces cerevisiae, we have also found that the rate of poly(A)\(^{+}\)mRNA synthesis doubles during the S-period (Fraser & Carter, 1976).

We conclude that the rate of poly(A)\(^{+}\)mRNA synthesis is controlled by gene number. This offers the basis of a mechanism for control of the doubling of cellular components once per cell cycle. A doubling of the rate of messenger synthesis once per cycle should lead, in the simplest case, to a doubling in the rate of enzyme accumulation once per cycle, and hence to a doubling in the metabolic capacity of the cell. The simplest interpretation of our data for poly(A)\(^{+}\)mRNA synthesis per ml culture is that an 'active fraction' of the genome is transcribed at a constant rate throughout the cell cycle; doubling of the numbers of these genes during S doubles the rate of messenger RNA synthesis. This simple pattern would account for the bulk of total messenger synthesized in the cell, but could obscure a more complex situation where a small proportion of total mRNA synthesis was from other genes which were transcribed periodically or at varying rates through the cycle. We are also unable to estimate what proportion of the total genome is represented by the 'constantly transcribed fraction' of our simple model.

Some further qualifications must be made to the model relating gene number and rate of mRNA synthesis to doubling of cell components. The model is based on measurements of the rate of synthesis of polyadenylated mRNA, which probably does not represent the entire mRNA metabolism of yeast (McLaughlin et al. 1973). The poly(A)\(^{+}\)mRNA we studied was prepared from whole cell nucleic acid, and thus contained not only cytoplasmic mRNA, but also nuclear precursors and any special stored or transit forms. Any cell cycle-specific variation in processing or storage could complicate the situation. Furthermore, we have examined cells growing at close to their maximum growth rate. It is possible that in cells growing at a much slower growth rate, factors other than gene dosage might be limiting, in which case a different mechanism for the co-ordination of balanced duplication of cellular components during the cell cycle might exist. Differences in the pattern of rRNA synthesis during the cell cycle have been reported for mammalian tissue culture cells growing at different growth rates (Pfeiffer & Tolmach, 1968; Scharff & Robbins, 1965; Klevecz & Stubblefield, 1967; Enger & Tobey, 1969).

Studies on the dependence of poly(A)\(^{+}\)mRNA synthesis on gene number using synchronous cultures or hydroxyurea both indicated that there is a lag of 15–20 min between DNA replication and an increase in the rate of transcription. This lag might be caused by physical unavailability of the new DNA for transcription, or by temporary limitation, by some factor other than gene number, of the rate of transcription. It is interesting that when cells are grown at a slightly faster growth rate, in malt extract broth, there is no lag between DNA replication and a doubled rate of poly(A)\(^{+}\)mRNA synthesis (R. S. S. Fraser, unpublished results).

Gene number, mRNA synthesis and patterns of enzyme activity in synchronous cultures

The best test of whether the pattern of changes in rate of poly(A)\(^{+}\)mRNA synthesis we have observed during the cell cycle is meaningful in the control of cell growth is
to examine whether it can account for observed changes in enzyme activities during the cell cycle. We have calculated the pattern of protein accumulation which would be produced from the changes in rate of poly(A)\textsuperscript{+}mRNA synthesis during synchronous culture shown in Fig. 6. The Appendix describes how the protein accumulation data were calculated, and how the goodness of fit of different curves to the calculated points was tested. Fig. 9 shows the calculated protein-accumulation data. The curve which gave the best fit to the points was for linear accumulation of protein, with a doubling in the rate of accumulation at 2 h 50 min and 5 h 15 min after inoculation.

Fig. 9. The pattern of protein accumulation (relative units) during growth of a synchronous culture, calculated from changes in the rate of poly(A)\textsuperscript{+}mRNA synthesis during culture as explained in the appendix. The data are plotted on linear (●—●) and logarithmic (□—□) scales. The cell cycle scale (1.0 unit equals one cell generation time) is drawn from the cell number data of Fig. 3A; 1.0 and 2.0 represent the mid-points of the first and second divisions respectively. The 2 arrows indicate the times of the mid-points of the first and second periods of DNA synthesis (from Fig. 3C).
Taking the cell cycle from one division to the following division as a unit, these rate-change points occur at 0.2 of the second and third cell cycles after inoculation. We have not considered a possible rate change at 0.2 in the first cycle after inoculation, as there would be too little data available before the rate change to establish the initial rate.

The pattern of protein accumulation calculated from the rates of poly(A)+mRNA synthesis is very similar to the patterns of increase in enzyme activities measured in similar synchronous cultures by Mitchison & Creanor (1969). They found that sucrase, acid phosphatase and alkaline phosphatase all increased in activity linearly; the rate of synthesis of each enzyme doubled at close to 0.2 in each cell cycle.

The similarity of the calculated protein accumulation pattern (Fig. 9) to observed changes in enzyme activities (Mitchison & Creanor, 1969) suggests that the measured changes in the rate of poly(A)+mRNA synthesis are meaningful in the control of cell growth. These results lend support to our suggestion that the balanced duplication of cell components once per cycle may be largely controlled by constant transcription of a rate-limiting number of genes coding for mRNA. The explanation of observed patterns of enzyme increase purely by consideration of changes in mRNA synthesis further suggests that the content or availability of ribosomes does not play a part in the regulation of protein synthesis in this case.

Our data and calculations show that the doubling in rate of protein accumulation at 0.2 of the cell cycle is a consequence of the doubling of gene number during S. The data explain the paradox noted by Mitchison & Creanor (1969): that the DNA synthesis in S. pombe occurs at about 0.9 to 1.0 of the cell cycle, but the rate-doubling point for their enzymes was at 0.2 in the following cycle. This delay is in contrast to bacterial systems, where replication of the structural gene appears to be followed immediately by a doubled rate of enzyme accumulation (Kuempe & Masters & Pardee, 1965; Donachie, 1965; Helmstetter, 1968; Donachie & Masters, 1969). The delay of 0.2-0.3 of a cell cycle in S. pombe can be ascribed to 2 factors: a delay of 15-20 min (about 0.1 of a cycle) which remains to be explained, between replication of the DNA and a doubled rate of transcription, and a delay of up to 30 min (0.2 of a cycle) between the doubling of the rate of mRNA synthesis and the establishment in the cell of a sufficiently increased messenger content to raise the rate of protein synthesis by a detectable amount. It is clear from the calculated curves for messenger RNA accumulation (Appendix; Figs. 10, 11, pp. 519-520) that the approach to a doubled messenger content after doubling of the rate of transcription is slow.

We have compared a simulation of protein accumulation, derived from measurements of synthesis of a major fraction of mRNA, with experimental data for individual enzymes. It will be interesting to compare the simulated protein accumulation pattern with experimental results on total protein accumulation or synthesis during synchronous culture when such data become available. Total protein accumulation will represent the aggregate effects of all protein-synthesis control mechanisms, operating at transcriptional and translational levels (Creanor, May & Mitchison, 1975; Fraser, 1975) as well as turnover effects. It is possible that patterns of total protein accumulation may diverge from the pattern simulated from data of poly(A)+mRNA synthesis.
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RNA synthesis during the cell cycle


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**APPENDIX: CALCULATION OF THE PATTERN OF PROTEIN ACCUMULATION FROM OBSERVED CHANGES IN THE RATE OF MESSENGER RNA SYNTHESIS**

We will calculate the pattern of protein accumulation which would arise from the changes in rate of messenger RNA synthesis measured during growth of a synchronous culture (Fig. 6). The calculation has 2 steps: calculation of changes in messenger content per ml culture during growth, and calculation of a curve for protein accumulation from these changes in messenger content.

**Calculation of changes in messenger content**

To calculate changes in messenger RNA content from changes in the rate of mRNA synthesis, we require to know the average half-life time (t_{1/2}) of yeast poly(A)^+mRNA, and we have to be able to express the measured rate of mRNA synthesis in the same units as the rate of degradation. The mean t_{1/2} of fission yeast poly(A)^+mRNA has been measured by 2 independent methods, which both give a value of 40–45 min (Fraser, 1975). Assuming that at the start of a synchronous culture, the messenger
RNA content is 100 units ml⁻¹, we will calculate changes in content resulting from degradation and from changes in the relative rate of synthesis during culture.

Considering first only degradation, the amount of the original 100 units which degrades in a given time interval \( t \) is obtained from Equation (1):

\[
n_t = n_0 e^{-rt},
\]

where \( n_0 \) is the starting population, \( n_t \) is the population remaining after time \( t \), and \( r \) is the rate constant. The equation holds for decay which follows first-order kinetics; the decay of fission yeast poly(A)+mRNA does substantially show first order kinetics (Fraser, 1975). For \( t = t_{98} = 40 \) min, \( n_o = 2n_t \) and \( r = -0.0173 \) min⁻¹. We will consider mRNA content at 15-min intervals during culture; the fraction of \( n_0 \) degraded after 15 min is 0.229.

In a situation where synthesis also occurs, to find the net change in mRNA content over a 15-min time interval we must in addition consider:

(a) The mRNA synthesized during the 15 min. Let the unit of mRNA synthesis be \( s \), and let \( s = -r \). Let the relative rate of mRNA synthesis (shown in Fig. 6) at time 0 in the synchronous culture be \( m \), and at the mid-point of the 15-min interval of synchronous culture growth being considered be \( m' \). \( k \) is a constant, the significance of which will be explained later. Then the average rate of mRNA synthesis during the 15-min interval is \( ks.m'/m \) min⁻¹, and this rate is in the same units as the rate of degradation \( r \). Thus the amount of mRNA synthesized in the 15-min time interval is \( 15 ks.m'/m \).

(b) The degradation of messenger which was synthesized during the 15-min time interval must be subtracted. Taking the average content of newly synthesized mRNA during the interval as \( \frac{1}{2}.ks.m'/m \), the amount of the newly synthesized mRNA degraded is calculated as \( \frac{1}{2}.ks.m'/m \times 0.229 \).

Thus starting with \( n_o \) units mRNA ml⁻¹ at \( t = 0 \), the amount present at \( t = 15 \) min is:

\[
n_{15} = n_0 - (n_0 + \frac{1}{2}.ks.m'/m) \times 0.229 + 15 ks.m'/m.
\]

The function of the constant \( k \) is to modify the absolute rate of mRNA synthesis so that messenger content per ml culture exactly doubles in each cell cycle, which must happen in cells undergoing balanced, exponential growth. If the messenger RNA metabolism of the culture were such that it achieved a steady state condition in every cell cycle, i.e. if the rates of mRNA synthesis and degradation became equal, the value of \( k \) would be 1. As we cannot assume that steady state is in fact reached in the \( S. pombe \) cell cycle, we must derive a value for \( k \). This is done by considering an 'ideal' synchronous culture; that is, one in which mRNA synthesis is not influenced by artifacts from the synchronization procedure or loss of synchrony with time. As a basis for this ideal culture, we take the experimentally determined changes in the rate of poly(A)+mRNA synthesis per ml between 2 h and 4 h 25 min (equal to 1 cell generation time) from Fig. 6, and reproduce the same curve to represent the preceding and subsequent divisions. This is shown in Fig. 10. Starting with 100 units mRNA ml⁻¹, we calculate changes in mRNA content over successive 15-min intervals using
Eq. (2). This was initially performed with $k = 10$, and was repeated with different values of $k$ until the $k$ value was found which gave an exact doubling of mRNA content in every cell cycle. Fig. 10 shows the calculated curve for mRNA content which satisfied this condition; the $k$ value was 1.10. This value of $k$ implies that steady state is never reached, as at any time the rate of synthesis is always faster than the rate of degradation.

![Graph showing changes in mRNA content and poly(A)+mRNA synthesis](image)

**Fig. 10.** Changes in relative rate of synthesis of poly(A)+mRNA in an 'ideal' synchronous culture (-----), and in content of mRNA per ml (○—○) calculated by Eq. (2).

We can now calculate, using Eq. (2), with $k = 1.10$, changes in mRNA content in the real synchronous culture shown in Fig. 6. The value of $m$ we used is the relative rate of mRNA synthesis before the depression of poly(A)+mRNA synthesis by the synchronization procedure. This value was found by extrapolating the curve for changes in the rate of poly(A)+mRNA synthesis during the second division (2 h to 4 h 25 min) back to $t = 0$ (Fig. 11). The value of $m$ obtained is of course higher than the measured values of poly(A)+mRNA synthesis rate early in culture; the difference is similar to the measured depression of poly(A)+mRNA synthesis rate by the synchronization procedure shown in Fig. 2.

Fig. 11 shows the calculated curve of mRNA content in the real synchronous culture. It is clear that mRNA accumulation in the real culture is much less periodic than in the ideal culture (Fig. 10), and that the pattern of accumulation in the first cell cycle is markedly affected by the synchronization procedure. It is not until the second cycle that a periodic pattern of mRNA accumulation is established.
Calculation of a curve for protein accumulation

From the calculated curve of changes in mRNA content during synchronous culture (Fig. 11), we calculate a curve for protein accumulation. We assume that in any 15-min time interval, the amount of protein synthesized is proportional to the average mRNA content during the time interval. The protein is assumed to be stable. The amount of protein present at $t = 0$ is taken to equal the increment during the first cell-generation time in culture. Fig. 9 shows the calculated protein-accumulation data, plotted as linear and semilogarithmic plots.

![Graph showing changes in messenger RNA content per ml culture in a synchronous culture](image)

Fig. 11. Changes in messenger RNA content per ml culture in a synchronous culture (---), calculated using Eq. (2), p. 518, from changes in the rate of poly(A)$^+$mRNA synthesis per ml (---). The poly(A)$^+$mRNA synthesis data are re-drawn from Fig. 6. The broken line shows the extrapolation of the poly(A)$^+$mRNA synthesis rate used to find the value of $m$ unperturbed by the depressive effects of the synchronization procedure on poly(A)$^+$mRNA synthesis.

We then tried to fit to these data curves which seemed likely patterns of protein accumulation in cultures undergoing synchronous, exponential growth. The simpler model considered was an exponential increase in protein. A single straight line was fitted to the plot of log protein content against time by the method of least squares. The second model was derived from results with bacterial systems, where some stable enzymes are known to increase in activity linearly through the cell cycle, with a doubling in the rate of accumulation once per cycle (Kuempel et al., 1965; Donachie & Masters, 1969). We fitted a series of straight lines by inspection to the linear plot of
the protein accumulation data, with the restriction that there could be only one rate-change point per cell cycle. The goodness of fit in each case was tested by calculating the residual sum of squares between the calculated points and the fitted line. Fig. 9 shows the best fit obtained. Three straight lines were used, with rate changes at 2h 50 min and 5 h 15 min. The increase in rate of protein accumulation at these points was close to a doubling. This segmented, linear model gave a much better fit to the calculated data than the exponential model. The residual sum of squares of the exponential fit was 5.7 times that of the best segmented linear model.

The overall doubling time of calculated protein content during the synchronous culture is the same as the cell doubling time. The 2 rate-change points in the segmented, linear model both occur at the same stage, 0.2, of the cell cycle. A possible rate change early in the first cycle was not considered, as there would be too few points to establish the initial rate.

It is clear that the calculated points for protein accumulation do not show a sharp rate change, but curve round from one linear rate to another. But DNA synthesis is not an instantaneous process; if gene replication controls the rate-doubling, the curve for total protein accumulation will be composed of many individual curves, with rate changes at slightly different times. These in total will show a smooth transition from one linear rate to the next. Imperfections of synchrony will also tend to make the rate-change gradual rather than sharp.