Regulation of Ribosomal Protein Synthesis in *Escherichia coli*

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The kinetics of synthesis of ribosomal, nonribosomal, and total protein, and of ribosomal ribonucleic acid (RNA), were measured in *Escherichia coli* during a shift-up involving a doubling of specific growth rate. The increase in ribosomal protein synthesis was not accompanied by a corresponding decrease in non-ribosomal protein synthesis. Thus, the average rate of protein synthesis per ribosome increased after the shift. The increase did not occur by an increase in polypeptide chain growth rates. The average growth periods of ribosomal and nonribosomal proteins did not change after a shift, and were constant in cells in balanced growth over a wide range of specific growth rates. The average growth period for ribosomal protein was twice that for nonribosomal protein. The fraction of ribosomes in polyribosomes was found to increase after the shift, accounting for the increase in ribosomal protein synthesis and in protein synthesis per ribosome. These results show that ribosomal protein synthesis is regulated by control of initiation of either transcription or translation of ribosomal protein messenger RNA, by some means other than availability of free ribosomes. If the messenger for ribosomal protein is ribosomal RNA, the conclusions can be extended to apply to regulation of ribosomal RNA synthesis. In support of this, it is shown that ribosomal RNA and ribosomal protein synthesis are closely coordinated during a shift.

The ratio of ribosomes to protein in bacterial cells in balanced growth is approximately a linear function of specific growth rate (2, 7). This observation implies that the rate of protein synthesis per ribosome is constant and independent of the specific growth rate of the cell, and thus of the total rate of protein synthesis. Thus, during a shift to a higher specific growth rate, a cell increases its content of ribosomes, permitting an increase in its rate of protein synthesis. The increase in the rate of synthesis of ribonucleic acid (RNA; 8) and mature ribosomes (13) occurs within 1 to 2 min after the shift. Since the pool of ribosomal protein is small (23), the rate of synthesis of ribosomal protein must also increase in parallel with the increase in ribosomal RNA (rRNA). The majority of the RNA synthesized during a shift is rRNA (16), and the expected increase in the rate of ribosomal protein synthesis has been shown to occur (17, 23). However, no direct measurement of the kinetics of rRNA and ribosomal protein synthesis during a shift have been made.

Such measurements are presented here, to-gether with measurements of the kinetics of synthesis of nonribosomal and total protein, and can be interpreted to provide information on the general nature of the mechanism of regulation of ribosomal protein synthesis. After a shift, ribosomal protein synthesis can amount to 30 to 40% of total protein synthesis. Here it is shown that this increase occurs without a corresponding decrease in the rate of synthesis of nonribosomal protein. The total rate of protein synthesis increases, because of an increase in the proportion of ribosomes engaged in protein synthesis. There is no detectable increase in polypeptide chain growth rates.

MATERIALS AND METHODS

Growth conditions. *Escherichia coli* ML30 was grown in a medium containing (in grams per liter): glycerol, 2.0; NaCl, 1.0; KH_2PO_4, 4.2; K_2HPO_4, 8.5; $FeSO_4 \cdot 7H_2O$, 0.00025; $CaCl_2$, 0.005; $MgCl_2 \cdot 6H_2O$, 0.025; Na_2SO_4, 0.1; and NH_4Cl, 0.8. The pH of the medium is 6.9 to 7.0. This will be referred to as "minimal medium." "Supplemented medium" is minimal medium plus (in milligrams per liter): L-as-partic acid and L-glutamic acid, 50.0; L-leucine, 10.0; 18 other L-amino acids, each 20.0; adenosine, guano-sine, thymidine, and cytidine, each 20.0; uracil or

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uridine, 10.0; p-aminobenzoic acid, calcium panto-thenate, thiamine, niacinamide, pyridoxine, and folic acid, each 1.0; biotin, 0.1; and vitamin B_{12}, 0.01.

Cultures were grown under forced aeration at 30 C. Balanced growth was obtained by maintaining cultures in exponential growth for 10 to 15 generations at a cell density of less than 100 μg (dry weight) per ml.

Shifts were performed on cultures in balanced growth in glycerol minimal medium by addition of the supplements listed above; just prior to the shift, these were mixed, and the mixture was brought to pH 7 with sodium hydroxide and warmed to 30 C.

Bacterial dry weights were determined by measurement of optical density (OD) at 600 nm in a 1-cm cuvette by use of a Beckman DU spectrophotometer. A concentration of 1.0 mg/ml (dry weight) corresponds to an OD at 600 nm of 2.62. Protein was estimated by the method of Lowry et al. (12). Protein in whole cells was measured after overnight hydrolysis in 0.1 N NaOH at 0 to 5 C. RNA was determined by the orcinol method (5).

Radioactivity measurements. Tritium and ^{14}C were determined with a Packard scintillation spectrometer. The counting fluid contained: xylene, 5 volumes; 1,4-dioxane, 5 volumes; absolute ethyl alcohol, 3 volumes; naphthalene, 8%; 2,5-diphenyloxazole, 0.5%; and 1,4-bis-2-(5-phenyloxazolyl)-benzene, 0.005%.

For measurement of radioactivity in cell extracts, up to 0.5 ml of extract was added to 15 ml of counting fluid. Bacterial cells were suspended in cold 5% trichloroacetic acid and then were deposited on a 25-mm membrane filter (Millipore Corp., Bedford, Mass.) and washed two times with 5% trichloroacetic acid and two times with water; the filter was then dissolved in 15 ml of counting fluid. Counts were corrected for the slight quenching effect of the filter. In double-label experiments, tritium was counted with 20% and ^{14}C with 67% efficiency. Overlap of ^{14}C and tritium was measured with each set of samples counted.

Preparation of cell fractions. Samples of cultures (100 to 150 ml, containing 40 to 100 μg of cells/ml) were poured over crushed ice containing 2,4-dinitro-phenol (final concentration, 10^{-4} M) or sodium azide (final concentration, 0.02 M). After centrifugation at 6,000 × g for 5 min, the cells were washed four times in 40 ml of 10^{-3} M tris(hydroxymethyl)aminomethane chloride buffer, pH 7.5, containing 10^{-2} M $MgCl_2$ (TM buffer), by centrifuging at 6,000 × g. The cells were suspended in 5 ml of TM buffer, and were broken by 4-min treatment at 0 to 10 C with a Mullard MSE sonic oscillator. After dilution to 15 ml with TM buffer, the extracts were centrifuged at 17,000 × g for 30 min, and the pellet was discarded. The supernatant liquid (total extract) was centrifuged at 100,000 × g for 2 hr (Spinco 40 rotor at 40,000 rev/min).

The nonribosomal protein fraction was obtained by gently removing the upper 5 to 7 ml of the supernatant liquid with a hypodermic syringe and was further purified as follows. Biogel P-30 (Calbiochem, Los Angeles, Calif.; molecular weight exclusion limit, 30,000) was added (0.2 g, dry weight), and the mix-

ture was stirred frequently during 3 hr. The gel was removed by filtration; the excluded protein was precipitated by treatment with 10% trichloroacetic acid at 80 to 90 C for 15 min, collected by centrifugation, washed once more with hot trichloroacetic acid, centrifuged, dissolved in 0.5 ml of 0.1 N NaOH, and made to 5 ml with water.

The remaining supernatant liquid from the 100,000 × g centrifugation was discarded, and the walls of the tube were wiped dry. The pellet, containing the ribosomal fraction, was suspended in 1 ml of a puromycin solution [puromycin, 10^{-3} M; reduced glutathione, 0.02 M; KCl, 0.05 M; $MgCl_2$, 0.01 M; guanosine triphosphate, 10^{-4} M; tris(hydroxymethyl)-aminomethane chloride, 0.01 M; pH 7.5] and incubated at 37 C for 1 hr. This treatment was shown to remove 85 to 90% of the radioactivity associated with ribosomes prepared from cells labeled for 15 sec with a ^{14}C-amino acid mix or with ^3H-leucine; thus, more than 85% of the nascent proteins are removed from the ribosomes. After dilution to 10 ml with TM buffer, the suspension was centrifuged at 17,000 × g for 30 min, the pellet was discarded, and the supernatant liquid was centrifuged at 100,000 × g for 2 hr. The supernatant liquid was discarded, the walls of the tube were wiped dry, and the clear ribosomal pellet was rinsed twice with 1 ml of TM buffer and suspended in TM buffer. The suspension was subjected to a single ammonium sulfate fractionation (10), and the ribosomal protein was extracted with 67% acetic acid (27). The acetic acid was completely removed by evaporation under vacuum; the protein was dissolved in 0.25 ml of 8 M urea plus 0.1 ml of 1 M NaOH and made to 5 ml with water.

Measurement of specific growth rates. Specific growth rates were calculated from the equation:

$$k = (\ln x - \ln x_0)/(t - t_0) \qquad (1)$$

in which k is the specific growth rate, hr^{-1}, and x and x_0 are the values of the measured extensive parameter of the population at times t and t_0.

Dry weight specific growth rates were determined by measurement of OD at 600 nm. Specific rates of increase of total protein and total RNA were determined colorimetrically, and by measurement of specific radioactivities as described below. Specific rates of increase of nonribosomal protein, rRNA, and ribosomal protein were determined from specific radioactivities.

If a known precursor of a cell component is supplied in radioactive form, measurement of the specific radioactivity of the cell component as a function of time leads to a determination of its relative increase:

$$x = x_0 \cdot s_I/(S_I - S) \qquad (2)$$

in which x and x_0 are as defined in equation 1, S is the specific radioactivity (counts per minute per microgram of x) at the time of measurement, and S_I is the specific radioactivity after an infinite period of labeling.

The compounds to be added in radioactive form were added in nonradioactive form at least one doubling before beginning measurements, and were

omitted from the supplement if a shift was to be per-formed. The radioactive compounds were added 5 min before beginning measurements. At intervals, samples were taken for colorimetric measurement of total protein and RNA, for measurement of radio-activity in cells, and for preparations of cell fractions. Specific radioactivities of total protein and RNA were measured on w*ole cells and total extracts. For meas-urement of specific radioactivities of nonribosomal protein, the Biogel P-30 treatment was omitted. Specific radioactivities of ribosomal protein and rRNA were determined on purified ribosomes, with omission of the puromycin treatment, the ammonium sulfate fractionation, and subsequent steps. Further purifica-tion of ribosomal protein by precipitating twice with hot 10% trichloroacetic acid produced no change in the specific activities. The value of S_I was determined empirically in each experiment for total protein by plotting radioactive leucine incorporation per milli-liter of culture versus total protein per milliliter of culture, determined colorimetrically. The slope of such a plot is then equal to S_I. The value of S_I for total RNA was determined from a plot of radioactive uracil or uridine incorporation versus total RNA per milliliter, determined colorimetrically on whole cells. Such plots were linear in all cases. The values of S_I for ribosomal and nonribosomal protein would be expected to differ from the value determined for total protein, because of known differences in amino acid composition and possible differences in extinction of the different proteins. For similar reasons, the value of S_I for rRNA is not likely to be equal to that deter-mined for total RNA. To determine the relative values of S_I for ribosomal and nonribosomal protein and for rRNA, and to determine the accuracy of the method, measurements were made on a culture grow-ing in glycerol-minimal medium plus leucine, cytosine, and uracil, each 10 μg/ml.

For this purpose, it was necessary to show that the culture was in a state of balanced growth. This was demonstrated by the following observations: (i) the specific growth rate was constant; (ii) measured on whole cells, the ratios of total protein and RNA to dry weight of cells were constant; and (iii) in cell-free extracts, the proportions of total RNA, soluble RNA, and rRNA, and of total, ribosomal, and nonribosomal protein, remained constant.

The culture was divided into two parts. One was labeled with ^{14}C-leucine and the other with ^{14}C-uracil, and measurements were made as described above. The specific growth rates and their standard errors were computed from the experimental points by linear regression according to equation 1. Results are shown in Table 1. The specific growth rates for dry weight, total protein, and RNA, measured by the various methods, are not significantly different. The standard error of specific growth rates measured from specific radioactivities is satisfactorily small, between 1 and 2%.

The values of S_I for the various cell components could be determined, since it was shown that the culture was in balanced growth. Consequently, the specific growth rates of all cell components are equal and identical with the dry weight specific growth rate. From the measurements of specific radioactivity of a component and its known specific growth rate, the value of S_I for the component can be determined by inspection. Values thus determined for ribosomal and nonribosomal protein were, respectively, 0.92 and 1.01 times the value of S_I for total protein. For rRNA, S_I was found to be 1.2 times the value for total RNA.

The corrected values of S_I were used to compute the specific growth rates of these components shown in Table 1. The correction factors for S_I of the various components determined in this experiment were used in all experiments reported in this paper.

The method of measurement of specific growth rates by measurement of specific radioactivities is thus both precise and accurate. The precision decreases with increasing specific activity; thus, precise measure-ments are obtained only over two doublings of a com-ponent at most. Some theoretical difficulties which could be raised do not, in practice, appear to lead to any detectable error. The specific activity of ribosomal protein, for instance, probably includes a contribution from nascent proteins bound to the ribosomes. The rapid labeling of messenger RNA could likewise lead to errors in the measured kinetics of RNA synthesis. However, if the labeled precursors are added at least 5 min before beginning measurements, both of these will contribute a constant addition to specific activity during the experimental period and can be readily corrected for if necessary.

Calculation of hypothetical kinetics of synthesis of rRNA, ribosomal protein, and nonribosomal protein. Since the ratio of ribosomes to deoxyribonucleic acid

TABLE 1. *Specific growth rates of cell components measured in balanced growth*

Component	Method of measurement	k (hr^{-1})
Dry weight	OD at 600 nm	0.518 ± 0.0031
Total protein	Colorimetric	0.518 ± 0.0066
	SA[a], whole cells	0.519 ± 0.0046
Nonribosomal protein[b]	SA	0.515 ± 0.0250
Ribosomal protein[b]	SA	0.522 ± 0.0185
Total RNA	Colorimetric	0.515 ± 0.0139
	SA, whole cells	0.517 ± 0.0090
Ribosomal RNA[b]	SA	0.514 ± 0.0119

[a] Specific activity.
[b] Calculated with corrected values of S_I as described in the text.

(DNA) increases approximately linearly with specific growth rate (7), the rates of synthesis of ribosomal protein and rRNA per genome must increase as the square of specific growth rate. Thus,

$$dRP/dt = \alpha_1 \cdot k^2 \cdot DNA \qquad (3)$$

in which RP is ribosomal protein and α_1 is a constant. The ratio of protein to DNA is approximately independent of specific growth rate (7). Thus, the assumption that the ratio of nonribosomal protein to DNA is constant will be a reasonable approximation. Then, from equation 3,

$$dRP/dt = \alpha_2 \cdot k^2 \cdot P \qquad (4)$$

and, similarly,

$$dR/dt = \alpha_3 \cdot k^2 \cdot P \qquad (5)$$

in which P is nonribosomal protein, R is rRNA, α_2 is a constant equal to $\alpha_1 \cdot DNA/P$, and α_3 is a constant equal to $\alpha_2 \cdot R/RP$.

Assume now that both the polypeptide chain growth rate and the fraction of ribosomes engaged in nonribosomal protein synthesis are independent of specific growth rate and constant. Then,

$$dP/dt = \beta_1 \cdot RP \qquad (6)$$
$$= \beta_2 \cdot R \qquad (7)$$

where β_1 and β_2 are constants.

Equations 4 and 6 together, and equations 5 and 7 together, form examples of Hinshelwood's (4) "autosynthetic" system and can readily be solved for RP, R, and P. The values of the constants involved in the solutions were obtained from the measurements of RNA and protein contents of cells in balanced growth in the various media used in this work. Solutions for the experimental shifts are represented by the solid lines in Fig. 2, 3, and 4. If the conditions of equations 6 and 7 are modified to assume that the fraction of ribosomes engaged in *total* protein synthesis is constant, then the dashed lines in Fig. 3 and 4 are obtained.

Measurement of average polypeptide chain growth periods. The method used was based on that of Lacroute and Stent (11), in which the polypeptide chain growth period is defined as the time elapsed between the laying down of the NH$_2$-terminal amino acid of a polypeptide and the appearance of that polypeptide in completed protein. ^{14}C-L-Alanine (0.5 μg/ml, 0.2 μc/μg) was added to a culture growing at a cell density of 90 to 100 μg/ml. Samples were taken for the preparation of cell fractions at intervals during the period from 50 to 200 sec after addition of ^{14}C-alanine. Alanine was chosen as the label because it forms a major fraction of the NH$_2$-terminal amino acids of both ribosomal and nonribosomal protein in *E. coli* (26, 27). Heavier labeling of NH$_2$-termini could have been obtained by using ^{14}C-methionine, but the results would have been confused by the existence of two pools of methionyl-transfer RNA, one specifically involved in polypeptide chain initiation (15). The addition of L-alanine to cultures in balanced growth was found to produce no disturbance of the

steady state. "Completed" ribosomal and nonribosomal proteins were defined in this study as those obtained in the complete fractionation protocols described above. In the case of ribosomal proteins, omission of the ammonium sulfate fractionation and subsequent steps produced no significant change in the results obtained. The time of laying down of the first ^{14}C-labeled NH$_2$-terminal alanine was taken as the time at which a plot of specific radioactivity (counts per minute per microgram of protein) of total ^{14}C-alanine in ribosomal and nonribosomal protein extrapolated to zero. The time of appearance of the first ^{14}C-labeled NH$_2$-terminal alanine in completed protein was determined in the same way from a plot of specific radioactivity of NH$_2$-terminal ^{14}C-alanine (NH$_2$-terminal counts per minute per microgram of protein). It can be seen from equation 2 that a plot of specific radioactivity versus time should be very close to linear during a period of 200 sec after addition of label. The NH$_2$-terminal specific radioactivity might be expected to deviate from a linear function of time due to the undoubtedly heterogeneous chain lengths of the proteins of the ribosomal and nonribosomal fraction. However, it can be demonstrated that, for a sample of proteins having a distribution of growth periods with a standard deviation equal to or less than half the mean growth period, specific radioactivity of NH$_2$-terminal amino acids will increase as a linear (within 1%) function of time after a time equal to approximately twice the mean growth period. The linear function extrapolates to zero specific radioactivity at a time equal to the mean growth period.

Thus linear regression of specific radioactivities of total and NH$_2$-terminal ^{14}C-alanine, measured on samples taken between 50 and 200 sec after addition of label, will estimate the times of initiation and completion of the protein of mean molecular weight. Thus, the mean polypeptide growth period and its standard deviation can be estimated.

The NH$_2$-terminal amino acids were isolated as dinitrophenyl (DNP) derivatives (21). To 4 ml of the purified ribosomal or nonribosomal protein sample in a 50-ml centrifuge tube, 1 ml of 2% DL-alanine and 1 mg of bovine serum albumin were added as carriers. The protein was precipitated by addition of 1.5 ml of 50% trichloroacetic acid and was collected by centrifugation at 12,000 × g for 10 min. After resuspension in 10 ml of acetone, the precipitate was collected again by centrifugation and was dissolved in 1 ml of 8 M urea, 0.015 ml of 1 N NaOH, and 0.2 ml of 5% NaHCO$_3$. Fluorodinitrobenzene (2 ml of a 5% solution in absolute ethyl alcohol) was added, and the mixture was shaken at 37 C for 2 hr. The DNP-proteins were precipitated with 0.2 ml of concentrated HCl and 7 ml of acetone, transferred quantitatively to a 15-ml conical centrifuge tube, collected by centrifugation at 7,000 × g for 15 min, and washed twice more with 10 ml of acetone plus 0.1 ml of concentrated HCl. After being dissolved in 0.5 ml of concentrated HCl, the DNP-proteins were hydrolyzed by heating at 100 C for 4 hr, the tube being capped with a marble. DNP-alanine (2.5 μg) and 1.5 ml of water were added, and the DNP-amino acids were extracted with 3 × 5

ml of ether. The combined ether extracts were washed twice with 1 ml of 0.01 N HCl, transferred to scintillation vials, evaporated to dryness, and counted. Recovery of total alanine after hydrolysis was measured in all samples, and was between 50 and 60%. Quenching by DNP was measured on some samples, and was always less than 1%.

Estimation of the fraction of ribosomes in polyribosomes. For this purpose, cell suspensions were prepared and lysed by the method of Godson and Sinsheimer (3), followed by five cycles of freezing and thawing. Their lysis mixture was modified to contain 0.06 M KCl (final concentration). The fraction of ribosomes in polyribosomes was estimated as described by Schaechter et al. (22), with the use of a Beckman model E analytical ultracentrifuge equipped with schlieren optics. To 0.5 ml of lysate, 0.05 ml of TM buffer containing 10 μg of pancreatic ribonuclease per ml was added. To a second 0.5-ml sample was added 0.05 ml of TM buffer alone. The samples were immediately loaded into 12-mm cells equipped with 4° Kel-F centerpieces, one with a 2° wedge window, and centrifuged simultaneously at 21,740 rev/min at 8 C. The areas under the peaks corresponding to 50S plus 70S particles were measured from tracings of the schlieren diagrams, and the difference between the ribonuclease-treated and untreated samples was taken as giving the amount of polyribosomes. These estimates agreed within 2 to 5% of estimates based on the relative amounts of material sedimenting at rates corresponding to 70S and greater than 70S. Estimates from different times during the run also agreed to within 2 to 5%.

RESULTS

Characteristics of the shift. Increases in dry weight, total RNA, and total protein during a typical shift from glycerol-minimal to glycerol-supplemented medium are shown in Fig. 1. Table 2 shows steady-state specific growth rates before and after the shifts in three experiments, and the radioactive compounds used in each case.

Ribosomal protein and rRNA synthesis during the shift. Immediately after the shift, the specific rates of synthesis of ribosomal protein and rRNA both increased by three to four times, and then slowly decreased to the value characteristic of growth in glycerol-supplemented medium (Fig. 2). The kinetics of increase of ribosomal protein and rRNA are consistent with results reported by others (6, 16, 23). The striking feature of the results is the closely coordinated synthesis of ribosomal protein and rRNA.

Nonribosomal and total protein synthesis. If the rate of protein synthesis per ribosome remains constant, the specific rate of nonribosomal protein synthesis will decrease immediately after the shift, because of the increase in ribosomal protein synthesis. The specific rate of total protein synthesis will in this case increase only in proportion to the increase in the ratio of ribo-

somes to protein. The kinetics of nonribosomal and total protein synthesis expected on this basis are shown as curves A in Fig. 3 and 4.

If the rate of protein synthesis per ribosome increases after the shift sufficiently to produce the increase in ribosomal protein synthesis, the kinetics of increase of nonribosomal protein will be the same as described for total protein above. This is shown as curves B in Fig. 3. The specific rate of total protein synthesis will then increase immediately after the shift (Fig. 4, curves B).

The results in Fig. 3 and 4 show clearly that the increase in rate of ribosomal protein synthesis does not occur at the expense of a decrease in rate of nonribosomal protein synthesis. Likelihood ratios for the two cases were calculated for the results of each experiment, and values of 10^{10} to 10^{20} were obtained in favor of this interpretation. The rate of protein synthesis per ribosome must therefore increase almost immediately after the shift.

Polypeptide chain growth periods. This increase does not occur by an increase in the polypeptide chain growth rate of either ribosomal or nonribosomal protein. The mean polypeptide growth periods are not significantly different when measured during balanced growth in glycerol-minimal medium, 10 min after a shift from glycerol-minimal to glycerol-supplemented medium, or during balanced growth in glycerol-supplemented medium (Fig. 5, Table 3).

Polypeptide growth periods measured during balanced growth in glucose-minimal and glucose-supplemented medium were not significantly different from each other or from those measured in the glycerol medium (Table 3). The polypeptide chain growth rates of both ribosomal and nonribosomal protein are thus independent of the rate of total protein synthesis within the limits of error of the method used for measurement, both in balanced growth and during a shift.

Fraction of ribosomes in polyribosomes. The rate of protein synthesis per ribosome could increase by an increase in the fraction of ribosomes engaged in protein synthesis. It seems reasonable to assume that this can be measured, at least approximately, by measurement of the fraction of ribosomes in polyribosomes. Two experiments were performed in which a culture in balanced growth in glycerol-minimal medium was sampled for polyribosome measurement just before shifting to glycerol-supplemented medium, and 5 min after the shift in one experiment and 10 min after in the second. Under the conditions used for lysis and analysis (0.06 M K$^+$, 0.01 M Mg^{++}), free ribosomes should sediment mainly as 70S particles (9, 18), whatever their state in vivo.

This is shown by the tracings of typical schlieren diagrams in Fig. 6. The sedimentation coefficient of the major peak, corrected to 20 C neglecting the effects of solutes, was 60 to 65S, and is assumed to contain the 70S ribosomal particles. The 50S particles were resolved from the trailing edge of the 70S peak on further centrifugation, and amounted to less than 10% of the total ribosomal material. All the material sedimenting at >70S was degraded by 1 μg of ribonuclease per
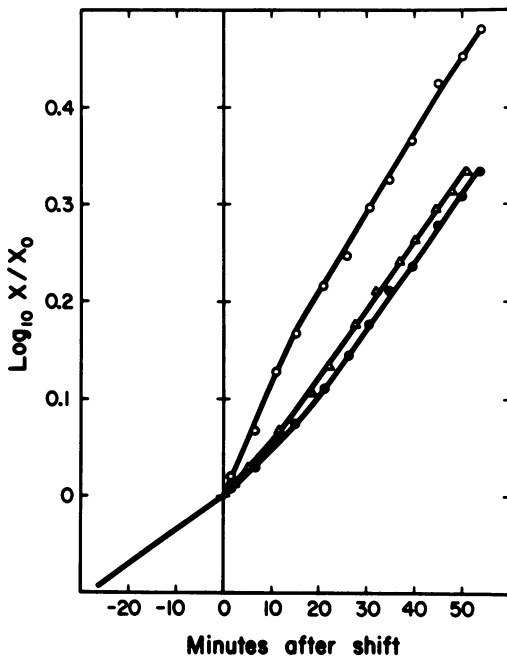

FIG. 1. *Kinetics of dry weight* (△), *total protein* (●), *and total RNA* (○) *increase after a shift from balanced growth in glycerol-minimal medium to glycerol-supplemented medium. Protein and RNA were determined colorimetrically. Shift was made at 0 min. Data from experiment II(Table 2).*

TABLE 2. *Details of shifts from glycerol-minimal medium to glycerol-supplemented medium*

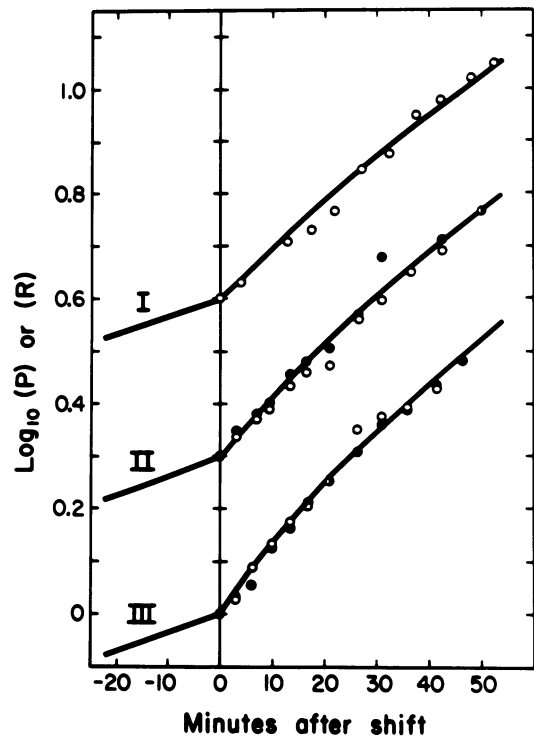| Expt.	Dry wt specific growth rates (hr^{-1})		Radioactive compounds added
	Glycerol-minimal medium	Glycerol-supplemented medium	
I	0.45	0.89	^{14}C-leucine
II	0.49	0.97	^{14}C-leucine ^{3}H-uridine
III	0.49	1.06	^{3}H-leucine ^{14}C-uracil

FIG. 2. *Synthesis of ribosomal protein* (○) *and ribosomal RNA* (●) *after a shift from glycerol-minimal to glycerol-supplemented medium, measured by increase in specific radioactivity. The Roman numerals refer to the three experiments listed in Table 2. The solid lines are not drawn to fit the experimental points, but are hypothetical functions whose derivation is explained in the text.*

ml and appeared as 70S particles, since the total area under the peaks sedimenting at >50S was the same in the ribonuclease-treated and untreated samples, and no significant change occurred in the 50S peak. Thus, the material sedimenting at >70S must be polyribosomes.

Just before the shift, during balanced growth in glycerol minimal medium, the fraction of ribosomes in polyribosomes was 38 ± 2% in one experiment 35 ± 2% in the other. At 5 min after the shift, 57 ± 2% of the ribosomes were in polyribosomes; 10 min after, the value was 55 ± 1%. Thus, within 5 min after the shift, the fraction of ribosomes in polyribosomes increased by 50%.

DISCUSSION

It is shown here that after a shift-up the rate of ribosomal protein synthesis almost immediately increases, without a corresponding decrease in

the rate of synthesis of nonribosomal proteins. The average rate of protein synthesis per ribosome therefore increases. The increase occurs by an increase in the number of growing polypeptide chains rather than by an increase in polypeptide chain growth rate, since the mean polypeptide chain growth periods for both ribosomal and nonribosomal proteins do not change after the shift, whereas the fraction of ribosomes engaged in protein synthesis increases by about 50% within 5 min after the shift.

Kjeldgaard (6) concluded that during a shift the rate of protein synthesis was at all times proportional to the concentration of ribosomes, apparently contradicting the results presented here. However, the increase in the rate of total protein synthesis per ribosome is small, and measurements of total protein synthesis alone could be interpreted as supporting either conclusion.

The fraction of ribosomes in polyribosomes in *E. coli* has been found to increase with increasing
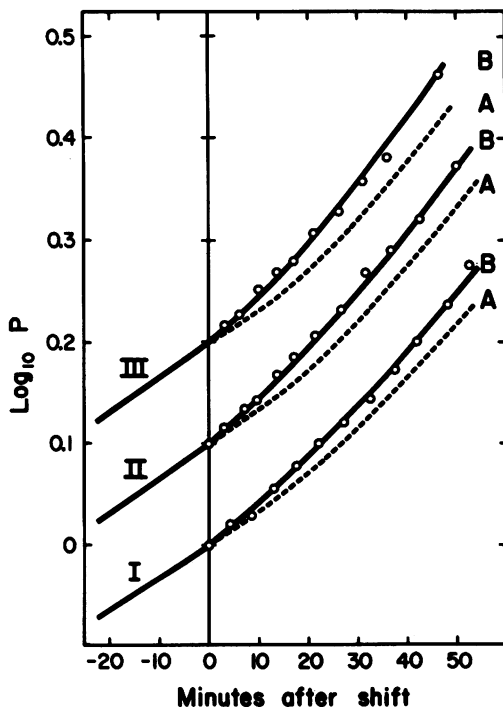

FIG. 3. *Synthesis of nonribosomal protein after a shift from glycerol-minimal to glycerol-supplemented medium, measured by increase in specific radioactivity. The Roman numerals refer to the three experiments listed in Table 2. The dashed and solid lines labeled A and B are not drawn to fit the experimental points (O), but are hypothetical functions whose derivation is explained in the text.*
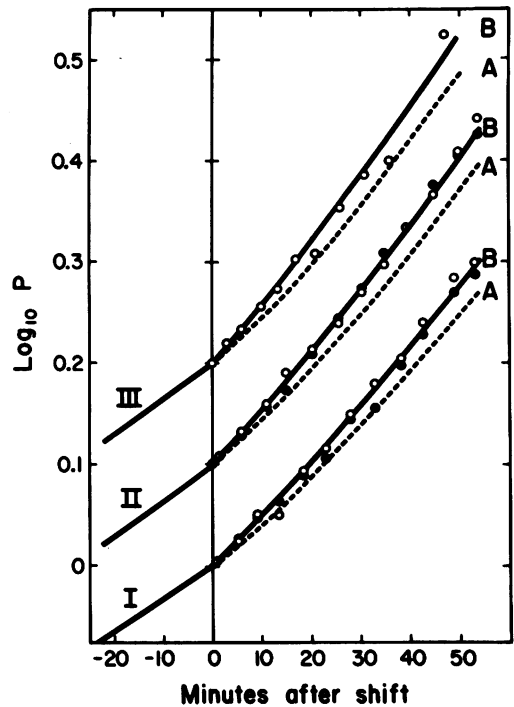

FIG. 4. *Total protein synthesis after a shift from glycerol-minimal to glycerol-supplemented medium, measured by increase in specific radioactivity (O), and colorimetrically (●). The significance of the Roman numerals and the dashed and solid lines is as in Fig. 3.*

specific growth rate by Godson and Sinsheimer (3). Their values agree closely with those determined here measured at similar specific growth rates. These results indicate that under most conditions a considerable fraction of the ribosomes of the cell are not engaged in protein synthesis. The observation that the fraction of ribosomes in polyribosomes rapidly increases after a shift-up suggests, however, that these idle ribosomes are competent to engage in protein synthesis. Thus, the total rate of protein synthesis is not limited by the concentration of ribosomes in the cell, though it may be roughly proportional to it. Maaløe and Kjeldgaard's (13) conclusion that the rate of protein synthesis per ribosome is constant, maximal, and independent of the rate of protein synthesis cannot be supported. Other measurements of the ratios of rRNA to protein as a function of specific growth rate have been made (20, 25), and agree with the conclusions drawn from the present work.

Maaløe and Kjeldgaard (13) extended the hypothesis of constant rate of protein synthesis per ribosome to conclude that the rate of growth of polypeptide chains was constant and inde-
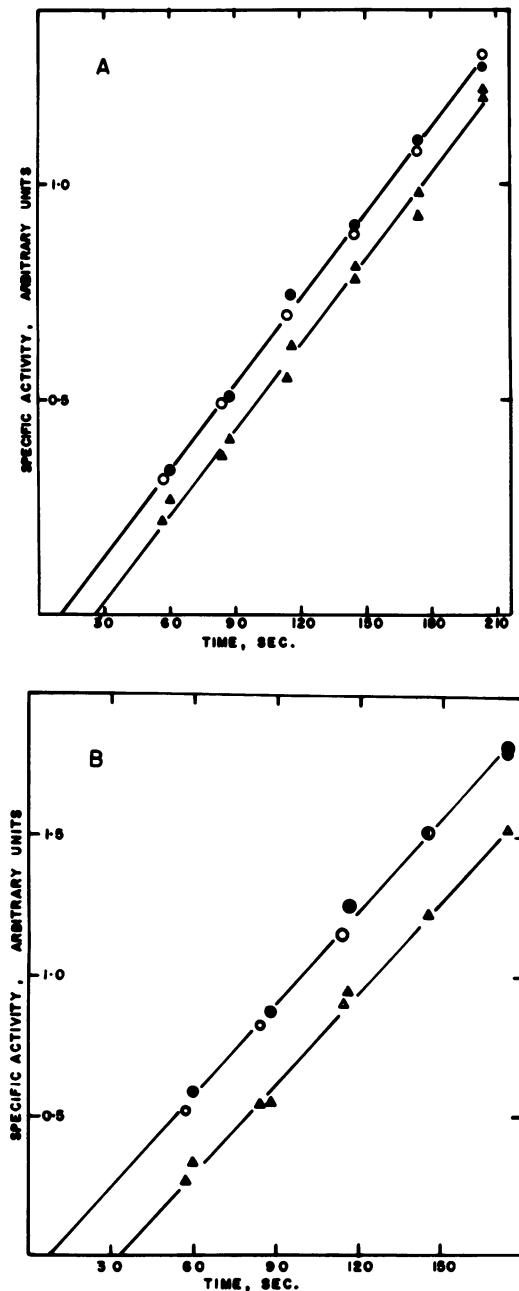
pendent of the total rate of protein synthesis. This hypothesis is confirmed by the measurements of polypeptide chain growth periods presented here. The average growth periods were 14 and 28 sec for nonribosomal and ribosomal protein, respectively. It is difficult to draw exact conclusions regarding polypeptide chain growth rates from these data, since the average molecular weights of the proteins having alanine as N-terminus are unknown. Waller (26) and Waller and Harris (27) obtained average molecular weights for the ribosomal and nonribosomal protein of *E. coli* B of 25,000 and 50,000, respectively. For nonribosomal proteins, the percentage having alanine as N-terminus was quite uniform among fractions from a diethylaminoethyl cellulose column (26). If these figures are taken as representing the average molecular weights of ribosomal and nonribosomal proteins of *E. coli* ML30 having alanine as N-terminus, the average polypeptide chain growth rates are 7 amino acids per second for ribosomal proteins, and 28 amino acids per second for nonribosomal protein; i.e., the rate of polypeptide chain elongation of nonribosomal protein is apparently four times faster than that of ribosomal protein. This is roughly consistent with the extent of the increase in the fraction of ribosomes in polyribosomes observed after the shift, if, as the maintenance of the preshift rate of nonribosomal protein synthesis per ribosome suggests, all of the increase is due to ribosomes engaging in ribosomal protein synthesis. It must be emphasized again that these conclusions are based in part on doubtful assumptions regarding the average molecular weights of the protein species involved; nevertheless, the conclusion that the polypeptide chain growth rates for ribosomal and nonribosomal proteins are quite different is difficult to avoid.

Comparison of the values for polypeptide chain growth rates measured here with those measured or calculated by others is made uncertain by the fact that most other published values are based on measurements made at 37 C, whereas the present measurements were at 30 C. A factor of 1.3 will give a rough conversion, however. The average polypeptide growth rate measured here is about 20 amino acids per second for all species,

FIG. 5. *Average polypeptide chain growth periods before and after a shift-up from glycerol-minimal to glycerol-supplemented medium. (A) Non-ribosomal protein; (B) ribosomal protein. Symbols: circles, total* ¹⁴C-alanine specific radioactivity in ribosomal or non-ribosomal protein; triangles, N-terminal ¹⁴C-alanine specific radioactivity; closed symbols, balanced growth in glycerol-minimal medium before shift; open symbols, 10 min after shift. Specific radioactivities normalized to give identical slopes. Net ¹⁴C-alanine counts per

minute per 4 ml in the final samples were as follows. Ribosomal protein, preshift: total, 1.8 × 10⁴; N-terminal, 158. Ribosomal protein, postshift: total, 1.3 × 10⁴; N-terminal, 136. Nonribosomal protein, preshift: total, 8.8 × 10⁴; N-terminal, 435. Nonribosomal protein, postshift: total, 4.8 × 10⁴; N-terminal, 251. Protein concentrations in samples: ribosomal protein, 40 to 60 µg/ml; nonribosomal protein, 180 to 250 µg/ml. Net counts per minute in samples taken at zero time (not shown) were zero in all ca ses.

TABLE 3. *Average polypeptide growth periods of ribosomal and nonribosomal proteins in Escherichia coli*

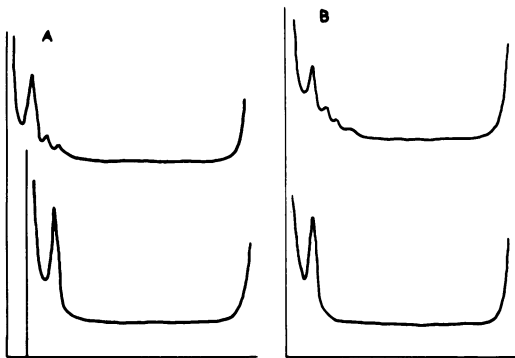| Medium	No. of measure-ments	Specific growth rate (hr⁻¹)	Polypeptide growth periods (sec)			
			Non-ribosomal protein	SD	Ribosomal protein	SD
Glycerol-minimal..........................	2	0.48	13	4	27	7
Glucose-minimal...........................	2	0.76	13	6	29	7
Glycerol-supplemented....................	1	0.9	15	5	31	7
Glycerol-supplemented, 10 min after shift from glycerol-minimal.................	1	0.82	16	3	26	4
Glucose-supplemented....................	1	1.17	13	2	26	3

FIG. 6. *Tracings of schlieren diagrams of lysates prepared before (A), and 10 min after (B), a shift from glycerol-minimal to glycerol-supplemented medium. Both photographed 16 min after reaching speed. Lower traces, 1 μg of ribonuclease per ml; upper, untreated.*

at 30 C; this corresponds to about 26 amino acids per second at 37 C. The calculations of Maaløe and Kjeldgaard (13) and Schlief (23) give values of 13 to 15 amino acids per second at 37 C. However, these calculations assumed 100% of the ribosomes to be engaged in protein synthesis. When the fraction of idle ribosomes is taken into account, these values agree with the present results. Good agreement is also found between the present results and Maaløe and Kjeldgaard's (13) estimate, from the data of McQuillen et al. (14), of the average polypeptide growth period in *E. coli* B as 15 sec at 37 C. However, there is not good agreement with the results of Lacroute and Stent (11), who from the growth period of β-galactosidase obtained a value of 15 amino acids per second at 37 C.

Regulation of ribosomal protein and rRNA synthesis. The data presented in this paper allow some conclusions to be drawn regarding the general nature of the mechanism of regulation of ribosomal protein and rRNA synthesis. First, it is necessary to show that during a shift these regula-

tory mechanisms do operate. It is possible, for instance, that after a shift the rates of ribosomal protein and rRNA synthesis are maximal and unregulated.

The functions represented by the solid lines of Fig. 2 are calculated on the assumption that the rates of ribosomal protein and rRNA synthesis per genome characteristic of growth in the post-shift medium are attained immediately after the shift and maintained thereafter. The experimental points fit these functions within the limits of error. Thus, there seems little doubt that the rate of synthesis of ribosomal protein and rRNA is regulated during the shift.

The mechanism of regulation of ribosomal protein operates to control the number of growing chains, rather than their rate of elongation. It must therefore operate through control of initiation of either transcription or translation of the ribosomal protein messenger RNA, modulated by some cytoplasmic factor other than the concentration of free ribosomes. Various mechanisms operating within this definition could be proposed, such as the direct regulation of initiation of transcription by the RNA polymerase factor of Burgess et al. (1) or regulation of initiation of translation by the control of dissociation of 70S ribosomes to subunits (9). Other possibilities could be suggested, but the present results allow no distinction to be made between them.

The results presented here allow some conclusions to be drawn regarding the regulation of RNA synthesis. First, Stent's (24) model cannot be generally correct. Though the coupling of RNA and protein synthesis undoubtedly occurs, the presence of a large number of idle ribosomes makes it difficult to see how the coupling could have a primary regulatory function. Translation could regulate transcription only if it were itself regulated through some control of ribosome function. However, this conclusion is true only if it is assumed that all ribosomes are functionally identical. It could be proposed that at least two

functional classes of ribosomes exist, one specifically engaged in the synthesis of ribosomal protein and present in excess, except possibly at the maximal growth rate. The rate of transcription and translation of the messenger RNA molecules for nonribosomal protein could then be determined by the concentration of the particular class of ribosomes involved. This proposal is consistent with the results presented here, but there is no real evidence in favor of it.

The general conclusions regarding regulation of ribosomal protein synthesis can be extended to the regulation of rRNA synthesis, if it is assumed (19) that rRNA acts during its synthesis as the messenger for ribosomal protein. With the coupling of transcription and translation, a single control would then regulate the synthesis of both.

If so, the synthesis of ribosomal protein and rRNA should be closely coordinated, not only in balanced growth but also during a shift-up. This prediction is confirmed by the results presented here.

ACKNOWLEDGMENT

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