# Synthesis and Function of Ribonucleic Acid Polymerase and Ribosomes in *Escherichia coli* B/r After a Nutritional Shift-Up

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The syntheses of stable ribosomal ribonucleic acid (RNA) and transfer RNA in bacteria depend on the concentration and activity of RNA polymerase and on the fraction of active RNA polymerase synthesizing stable RNA. These parameters were measured in *Escherichia coli* B/r after a nutritional shift-up from succinate-minimal to glucose-amino acids medium and were found to change in complex patterns during a 1- to 2-h period after the shift-up before reaching a final steady-state level characteristic for the postshift growth medium. The combined effect of these changes was an immediate, one-step increase in the exponential rate of stable RNA synthesis and thus of ribosome synthesis. This suggests that the distribution of transcribing RNA polymerase over ribosomal and nonribosomal genes and the polymerase activity are continuously adjusted during postshift growth to some growth-limiting reaction whose rate increases exponentially. It is proposed that this reaction is the production of amino-acylated transfer RNA and that its exponentially increasing rate results in part from a gradually increasing concentration of aminoacyl transfer RNA synthetases after a shift-up. This idea was tested and is supported by a computer simulation of a nutritional shift-up.

When bacteria are shifted from a nutritionally poor to a rich medium, their growth accelerates, which involves an increased synthesis of ribosomes (27, 28). According to current ideas (35), one of the early physiological effects of such a nutritional shift-up is a greater extent of tRNA charging with amino acids which reduces ribosome idling and thus synthesis of guanosine tetraphosphate (17, 18). A low concentration of this nucleotide is presumed to increase the expression of rRNA and ribosomal protein genes (6, 16, 25), thereby stimulating ribosome synthesis. The extent of tRNA charging depends on several factors which include the concentrations of amino acids, ATP, charging enzymes, and tRNA and protein synthesis which regenerates uncharged tRNA. Since these factors respond with different time constants to a perturbation and mutually affect one another, one should expect the synthesis of ribosomes after a nutritional shift to follow complex kinetic patterns; but, contrary to this expectation, ribosomes accumulate immediately after the shift at their final postshift exponential rate (5, 27). Here we have investigated the reasons for this one-step

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kinetic response of ribosome synthesis to a change in growth medium.

Starting point for this study was a previous theoretical analysis of the relations between RNA polymerase, ribosome synthesis, and the growth rate (2). Since the rate of rRNA synthesis after a shift-up is so rapidly established at its final level, it had been thought that all factors affecting rRNA synthesis assume their final postshift values after an essentially stepwise adjustment shortly after the shift of medium. The theory then showed that RNA polymerase and ribosomes must be made in fixed proportions before and after the shift-up (2), an idea which appeared to be supported by the finding that RNA polymerase genes are cotranscribed with ribosomal protein genes (26, 34, 45). But despite this cotranscription, synthesis of RNA polymerase relative to that of ribosomes was found to be a function of growth rate (21, 22, 30 42). The contradiction between theory and observation is resolved here by the observation that two parameters affecting rRNA synthesis, i.e., the RNA polymerase activity and the relative rate of stable RNA synthesis (relative to total RNA synthesis), are actually not rapidly responding in a one-step manner, but are changing over a period of more than 1 h after the shift-up before they reach a final level characteristic for the

postshift growth medium. This suggested that these parameters are continuously adjusted during postshift growth to some other reaction whose rate increases exponentially after a shift-up. It is proposed that this reaction is the production of aminoacyl tRNA.

Based on known and presumed properties of the tRNA charging reaction and relations between tRNA charging, levels of guanosine tetraphosphate, and rRNA synthesis, a theoretical model is derived to explain the observed shift-up response of ribosome synthesis. With respect to ribosome, RNA polymerase, and protein synthesis, the behavior of this model system in a computer simulation closely resembles the observed behavior of a bacterial culture subjected to a nutritional shift-up. The model predicts that the extent of tRNA charging after a shift-up should increase with biphasic kinetics, the final postshift level being reached only after several hours; this prediction may be testable in the future.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The bacterial strain used for each of the four nutritional shift-up experiments was *Escherichia coli* B/r (ATCC 12407). Cultures were grown at 37°C in supplemented medium C (19), and growth was monitored as the increase in the concentration of cell mass (absorbance at 470 nm, [$A_{470}$]). For all shift-up experiments, the exponential growth of the bacteria in minimal medium supplemented with a final concentration of 0.2% succinic acid was monitored for at least 2 h until the $A_{470}$ was between 0.35 and 0.51. At this time ($t = 0$), the culture was diluted approximately twofold by the addition of fresh, prewarmed medium C supplemented with glucose (final concentration, 0.2%) and a mixture of 20 synthetic L-amino acids (each in proportion to the molar concentration in *E. coli* protein, all >50 μg/ml) (43), and culture growth was continued. At various times the culture was again diluted (approximately twofold) with prewarmed medium to keep the culture between an $A_{470}$ of 0.25 and 0.85. Exact values of the dilution factors were obtained from the changes in $A_{470}$ as described previously (5). The growth curves for an undiluted culture were then reconstructed as shown in the panels labeled "mass" in Fig. 1, 3, and 4. The postshift doubling times were determined from the RNA accumulation curves.

**Determination of RNA, protein, and RNA polymerase content.** At both pre- and postshift times, duplicate 5-ml samples were removed for determination of RNA (as $A_{260}$ of acid-precipitable, alkali-labile material) and protein (by Lowry assay) as described previously (5). For RNA polymerase determination, 150-ml samples were removed and prepared for electrophoresis as described (42). The lysates from all samples of a given shift-up experiment were coelectrophoresed in separate sample wells on at least four sodium dodecyl sulfate-polyacrylamide slab gels. A quantitative method of Coomassie brilliant blue stain-

ing on sodium dodecyl sulfate-polyacrylamide gels was used to determine the amount of RNA polymerase $\beta'$ subunit protein in each sample lysate (42). Total protein in each sample lysate was determined by the method of Lowry et al. as described previously (5).

**Determination of the fractional synthesis rate of stable RNA.** The fraction of pulse-labeled RNA that represents ribosomal and transfer RNA ($r_s/r_t$) was determined as described previously (42). Briefly, duplicate 0.5-ml samples were removed from the culture at an $A_{470}$ of 0.5, pulse-labeled with [5-³H]uridine for one minute, and lysed for 30 s with sodium dodecyl sulfate at 100°C. The fraction of total pulse label in rRNA was determined by hybridization to λ *dilv5* DNA (carrying a ribosomal RNA gene [23]), including purified [¹⁴C]rRNA as the hybridization standard in the mixture. The relative synthesis of stable RNA obtained in this manner includes the unstable spacer material in the rRNA precursor.

**Calculations.** Normalized postshift kinetics of protein accumulation (heavy line in panels labeled "Prot." in Fig. 1, 4, and 5) were calculated using the formula (5):

$$P = \frac{a}{\Delta \alpha_r} (2^{t/\tau} - 1) + 1$$

where for any given experiment, $\tau$ is the postshift doubling time (from RNA accumulation), $a$ is the zero-time intercept of the straight line portion of the RNA accumulation curve (normalized to 1.0 immediately before the shift), and $\Delta \alpha_r$ is the change in the relative synthesis rate of r-protein (Table 1); the symbol $\Delta$ is used ($\Delta \alpha_r$, $\Delta \alpha_p$) to indicate an n-fold change and not a difference. This idealized equation assumes that postshift ribosome accumulation is exponential and that the rate of protein synthesis per average ribosome increases abruptly to its final value after the shift.

The normalized postshift kinetics of RNA polymerase accumulation per unit volume of culture (Fig. 1, 4) were obtained from the accumulation of protein and from the increase in $\alpha_p$ (Table 1, $\Delta \alpha_p$), also assumed to occur stepwise at the time of the shift-up (see Fig. 2):

$$\text{RNA polymerase} = \frac{a \Delta \alpha_p}{\Delta \alpha_r} (2^{t/\tau} - 1) + 1$$

The normalized rate of stable RNA synthesis ($r_s$) after the shift-up was obtained from the pre- and postshift doubling times, $\tau_1$ and $\tau_2$, of total RNA:

$$r_s = a (\tau_1/\tau_2) \times 2^{t/\tau_2}$$

The rate of mRNA synthesis ($r_m$) was calculated from the fractional synthesis rate of stable RNA ($r_s/r_t$; see above) using the formula:

$$r_m = r_s \{[1/(r_s/r_t)] - 1\}$$

The fraction of active RNA polymerase engaged in synthesis of stable RNA, $\psi_s$, was obtained by using the formula (12):

$$\psi_s = 1/1.5 \{1 - [1 - 1/(r_s/r_t)]\}$$

where 1.5 represents the ratio of the stable and messenger RNA chain growth rates, assumed to be 75 and

TABLE 1. *Comparison of preshift and postshift parameters in four shift-up experiments (succinate minimal to glucose-amino acids)*

| Expt. | $\mu^a$ (d/h) | $\alpha_r^b$ (%) | $e_r^c$ (aa/s) | $\Delta\mu^d$ | $\Delta\alpha_r^e$ | $\Delta e_r^f$ | $\Delta\alpha_p^g$ |
|---|---|---|---|---|---|---|---|
| **Fig. 1** | | | | | | | |
| preshift | 0.53 | 7.4 | 9.5 | 4.5 | 2.7 | 1.7 | 1.6 |
| postshift | 2.40 | 20.0 | 16.0 | | | | |
| **Fig. 3** | | | | | | | |
| preshift | 0.90 | 8.1 | 14.8 | 2.7 | 2.6 | 1.0 | 1.6 |
| postshift | 2.40 | 21.1 | 15.1 | | | | |
| **Fig. 4** | | | | | | | |
| preshift | 0.66 | 10.1 | 8.7 | 3.4 | 2.6 | 1.3 | Not con- |
| postshift | 2.22 | 26.3 | 11.2 | | | | stant |
| **Fig. 7** | | | | | | | |
| preshift | 0.80 | 9.2 | 11.6 | 2.7 | 2.1 | 1.3 | $ND^h$ |
| postshift | 2.14 | 18.9 | 15.1 | | | | |

$^a$ $\mu$, Growth rate in doublings per hour. Preshift value was determined from the preshift exponential accumulation of $A_{460}$; postshift value was determined from the postshift exponential accumulation of RNA.

$^b$ $\alpha_r$, Relative synthesis rate of r-protein in percent of total protein synthesis. The preshift value was calculated from the RNA/protein ratio ($R/P$; $\alpha_r$ [%] = 117 $R/P$; see reference 42). The postshift value of $R/P$ was not established until several hours after the shift; therefore, it was obtained from the slope ($\Delta R/\Delta P$) in a plot of RNA versus protein (Fig. 2).

$^c$ $e_r$, Ribosome efficiency in amino acids per second per average ribosome calculated from the respective values of $\mu$ and $\alpha_r$ ($e_r = 133 \mu/\alpha_r$; see reference 13).

$^d$ $\Delta\mu$, Change in growth rate = $\mu$ (postshift)/$\mu$ (preshift).

$^e$ $\Delta\alpha_r$, Change in relative synthesis rate of r-protein = $\alpha_r$ (postshift)/$\alpha_r$ (preshift).

$^f$ $\Delta e_r$, Change in ribosome efficiency = $e_r$ (postshift)/$e_r$ (preshift).

$^g$ $\Delta\alpha_p$, Change in relative synthesis of RNA polymerase, determined as the slope in a plot of RNA polymerase versus protein (Fig. 2).

$^h$ ND, Not determined.

50 nucleotides per s (4, 11, 31, 39).

The number of functioning RNA polymerase molecules is defined as the number of growing RNA chains, equal to the quotient RNA synthesis rate divided by RNA chain growth rate:

$$\text{Number of RNA chains} = (r_s/75) + (r_m/50)$$

where $r_s$ and $r_m$ are the stable RNA and mRNA synthesis rates (in nucleotides$\cdot$s$^{-1}$) and 75 and 50 are the stable and mRNA chain growth rates (in nucleotides$\cdot$s$^{-1}\cdot$chain$^{-1}$), respectively.

## RESULTS

**Comparison of postshift RNA polymerase and ribosome synthesis.** The accumulations of mass ($A_{460}$), RNA (as a measure for ribosomes and tRNA), protein, and RNA polymerase $\beta$ and $\beta'$ subunits was followed before and after a nutritional shift-up from succinate minimal to glucose-amino acids medium. To keep growth exponential, the culture was periodically diluted. Figure 1 shows the measuring data after correction for this dilution and normalization to the zero time (shift time) values. Within minutes after the shift, RNA accumulated exponentially with a doubling time that remained constant during postshift growth (followed for 90 min, 10-fold increase in RNA).

Since this doubling time (24 min) was within the range of values observed as a culture doubling time during steady-state growth in glucose amino acids medium, it was presumed that the accumulation of RNA observed immediately after the shift-up was equal to (and thus determined) the final postshift doubling time of the culture, as has been concluded previously (5, 12, 27, 28). RNA polymerase approached this doubling time much later, and total protein approached this doubling time even later. This shows that, after the shift, the synthesis of RNA polymerase relative to total protein ($\alpha_p$) increased, but it decreased relative to that of ribosomes (assuming RNA to be representative for ribosomes). This is seen more clearly in the graph shown in Fig. 2, in which RNA and RNA polymerase from the experiment in Fig. 1 were plotted as a function of protein. The abrupt changes in the slopes of the curves at abscissa point $P = 1$ (i.e., at the time of the shift) indicate a stepwise increase in the relative rates of synthesis (relative to protein synthesis) of RNA polymerase ($\Delta\alpha_p$, 1.6-fold increase) and of ribosomes ($\Delta\alpha_r$, 2.7-fold increase; see reference 5 for details about the evaluation of this plot). Actually, $\alpha_p$ may oscillate for some time after the
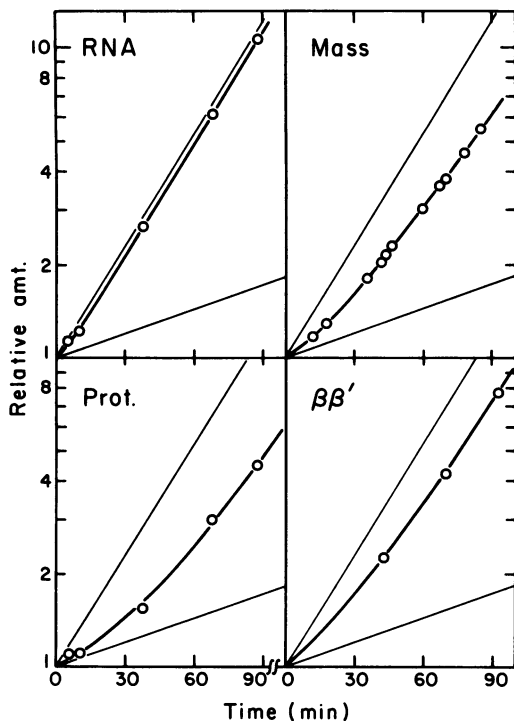
FIG. 1. *Shift-up experiment: accumulation of RNA, mass, protein, and RNA polymerase after the nutritional shift at t = 0. The experiment was performed as described in the text, with culture dilutions of 2.00-, 1.81-, and 1.79-fold at t = 0, 43, and 69 min, respectively. Each of the four parameters was determined from samples taken at the indicated times (○), all values being normalized to zero time. The preshift and postshift doubling times were 114 and 25 min, respectively (thin straight lines). The kinetic curves of protein and RNA polymerase accumulation were calculated as described in the text, using the value of a = 0.9, $\Delta\alpha_r$ = 2.7, and $\Delta\alpha_p$ = 1.6.*

perturbation caused by the shift-up, as has been observed for $\alpha_r$ (9), but such oscillations would be difficult to detect from measurements of amounts rather than synthesis rates.

**Changes in the concentration of RNA polymerase and in the RNA synthesis rate.** The observed amount of RNA polymerase protein per total protein (Fig. 3, curve labeled RNAP/P) can be used as a measure for the intracellular concentration of RNA polymerase, assuming that the protein content of a bacterium is a measure of its volume. We have verified this assumption by measuring, with an electronic particle-size analyzer, the average volume of cells growing at different rates, and with different DNA replication parameters that affect the cell volume. By combining the results with measurements of the average amount of protein per

cell, the intracellular concentation of protein was found to be equal to 0.3 (±10%) g/ml (data not shown). In succinate-grown *E. coli* B/r, 1% of total protein is RNA polymerase (42); therefore, the average intracellular concentration of total RNA polymerase (i.e., in DNA-bound and in free form) is about 3 mg/ml. After a shift-up to glucose amino acids medium (experiment of Fig. 1) the concentration of RNA polymerase gradually increased and reached a 1.6-fold higher value after a period of several hours (Fig. 3).

The rates of rRNA and tRNA synthesis were calculated from the observed accumulation of RNA, assuming that rRNA and tRNA are essentially stable and are made in constant proportions (10 tRNA molecules per 70S rRNA equivalent [8, 41]). With protein as a reference unit, the rate of synthesis of stable RNA species per total protein (Fig. 3, curve labeled $r_s/P$)
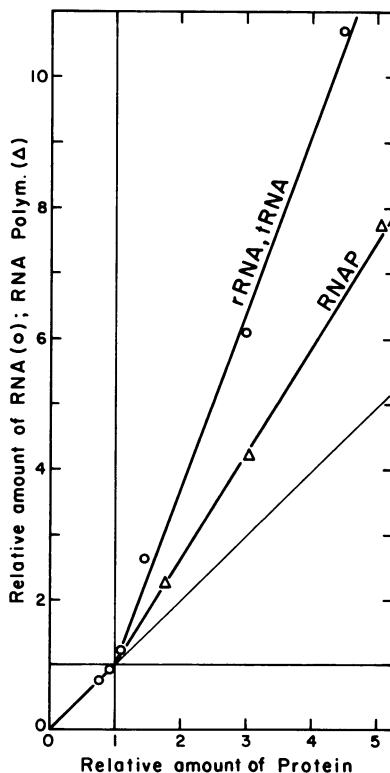


FIG. 2. *RNA (○) and RNA polymerase (Δ) in a shifted culture as a function of protein; replot of the data in Fig. 1. All zero-time values were set at 1.0; therefore, the line at 45° represents the preshift slope. The change in the slope of the RNA curve is equal to the change in the fractional synthesis of ribosomal protein ($\Delta\alpha_r$ = 2.7). Likewise, the change in the slope of the RNA polymerase curve is equal to the change in the fractional synthesis rate of RNA polymerase at the time of the shift-up ($\Delta\alpha_p$ = 1.6).*
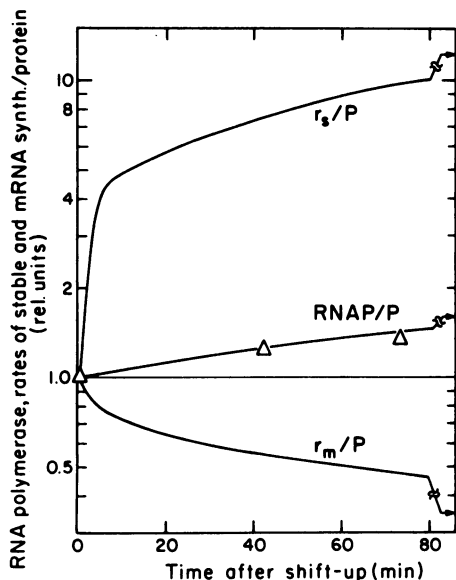
FIG. 3. *Changes in the amount of RNA polymerase per amount of protein (RNAP/P, as a measure for the intracellular concentration of RNA polymerase) and in the rates of synthesis of stable RNA ($r_s/P$) and mRNA ($r_m/P$) per amount of protein after a shift-up (for the significance of $r_s/P$, and $r_m/P$, see text); evaluation of the data in Fig. 1 (see text). All preshift values were set at 1.0. For the $r_m/P$ curve, data from Fig. 6a were also used; △, Observed β' protein per total protein.*

increased with biphasic kinetics to a final level that was 12 times higher than the preshift level. Since in *E. coli* the rRNA genes are clustered near the origin of DNA replication (1, 24), and since the amount of protein per origin shows little variation with growth rate (14), the number of rRNA genes per total protein is approximately constant and therefore $r_s/P$ is also a measure for the rate of transcription per rRNA gene. The rapid initial increase in rRNA gene transcription reflects mainly the shift of RNA polymerase from mRNA to stable RNA genes (see Fig. 6a); the subsequent slower increase in $r_s/P$ must reflect the combined effect of the gradual increase in RNA polymerase concentration and the further gradual shift of polymerase from the synthesis of mRNA to that of stable RNA (see Fig. 6a below and Discussion).

By combining the $r_s/P$ curve with observed values for the relative proportions of the instantaneous synthesis rates of mRNA and stable RNA (ratio $r_m/r_s$, see Fig. 6a), the rate of mRNA synthesis per total protein was obtained (Fig. 3, curve labeled $r_m/P$). Synthesis of mRNA per total protein decreased after the shift-up, which resulted from the fact that the ratio $r_m/r_s$ decreased with time more than the concentration

of polymerase increased. If DNA were not a limiting factor for the rate of transcription (i.e., if the rate of transcription were limited by the concentration of RNA polymerase), then $r_s/P$ and $r_m/P$ would reflect the concentration of free, functional RNA polymerase. The polymerase may exist in different comformations, one of which binds preferentially to rRNA (and tRNA and ribosomal protein) promoters, the other one of which binds to *lac* and bulk mRNA promoters (44). Thus, the decrease in $r_m/P$ suggests that the concentration of free RNA polymerase in the conformation that synthesizes bulk mRNA decreases after a shift-up, as has been concluded previously from estimates of the transcription of constitutive *lac* genes after a nutritional shift-up (7). Conversely, the increase in $r_s/P$ suggests an increase in the free enzyme of the "rRNA form". That DNA is not a limiting factor for RNA synthesis in bacteria is suggested by the 12-fold increase in the rate of transcription per rRNA gene (see above) and by observations with DNA-free minicells, which indicated that most of the RNA polymerase enzyme in *E. coli* is bound to DNA, and only a small fraction (about 15%) is free (N. Shepherd, Ph.D dissertation, University of Texas at Dallas, 1979). If DNA were limiting for RNA polymerase binding, a larger fraction of the polymerase should be in the free form. We have also measured RNA synthesis in a mutant derivative of *E. coli* B/r with an abnormal control of DNA replication in which the DNA concentration (DNA per unit of $A_{460}$) has about half the normal value of the B/r parent. In spite of the lower DNA concentration, RNA synthesis was normal in this strain (Churchward and Bremer, unpublished data), suggesting that the concentration of DNA does not limit RNA synthesis under physiological conditions.

**Variability in the shift-up response.** Several repeats of the experiment in Fig. 1 gave somewhat variable results: in one experiment the RNA curve showed a small initial overshoot (Fig. 4); in another experiment the curve showed an initial lag of about 10 min (Fig. 5). Most likely these differences are due to differences during preshift growth in succinate minimal medium (preshift doubling times were 114, 67, and 91 min in the experiments of Fig. 1, 4, and 5). These in turn reflect different values for the growth parameters, e.g., for the protein synthesis rate per ribosome (Table 1). Regardless of these variations, in all experiments the final postshift exponential increase in RNA accumulation was established rapidly, and RNA polymerase synthesis increased less than did ribosome synthesis after the shift-up.

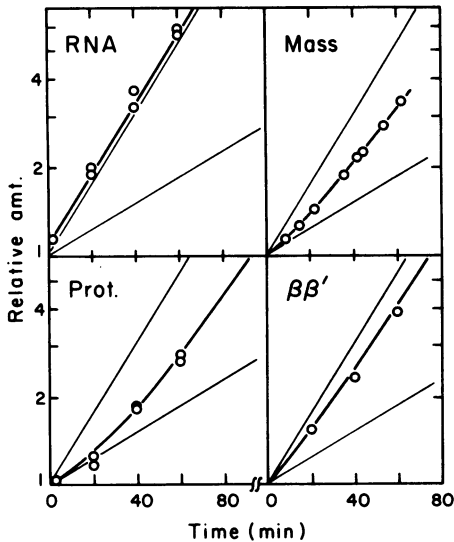**Postshift changes in RNA polymerase function and activity.** Since the synthesis of

FIG. 4. *Shift-up experiment as in Fig. 1, except culture dilutions were 1.22- and 2.00-fold at t = 2 and 18 min, respectively; pre- and postshift doubling times were 67 and 25 min, respectively, and a = 1.16, $\Delta\alpha_r$ = 2.6, and $\Delta\alpha_p$ = 1.6.*

RNA polymerase is stimulated less than that of ribosomes by the shift-up, some of the parameters affecting ribosome synthesis were expected to increase late after the shift to keep the accumulation of ribosomes exponential (see above). Pertinent parameters are the distribution of active RNA polymerase molecules over stable RNA and mRNA genes (fraction of functioning RNA polymerase engaged in the synthesis of rRNA and tRNA) and the RNA polymerase activity (fraction of total RNA polymerase engaged in RNA chain elongation at any given time). These were also measured in the experiments shown in Fig. 1, 4 and 5. The relative rate of stable RNA synthesis increased with biphasic kinetics over a period of 40 to 60 min (Fig. 6a): an initial (~5 min) phase of rapid increase was followed by a second longer phase of slow increase. Likewise, the RNA polymerase activity, given by the vertical distance between the total and functioning RNA polymerase curves in Fig. 7, changed continuously during the first 60 to 90 min of postshift growth. Thus, in spite of the rapid establishment of a final and constant exponential rate of ribosome accumulation, factors that determine this rate did not rapidly assume final, constant values. Therefore, the constancy of the postshift ribosome doubling time does not result from the constancy of these parameters; rather, it appears that these parameters are adjusted to some other reaction whose rate increases exponentially after the shiftup (see below).

**Protein synthesis per ribosome is not**

limited by the concentration of charged tRNA. The rate of protein synthesis per average ribosome is a growth-limiting parameter. It was reported to be independent of the growth rate (27), to be constant only at growth rates above 1.2 doublings per hour (13), or to increase and approach a constant value with increasing growth rate (5). (Apparently, it does not change very much with growth rate, which makes these changes difficult to measure.) In the experiment shown in Fig. 8, the protein synthesis rate per ribosome oscillated for the first 10 to 20 min after the shift-up and then stabilized at a value about 30% higher than the preshift value.

Figure 8c (triangular symbols) also shows the kinetic changes in the amount of RNA per protein. Since rRNA and tRNA are made in constant proportions (8, 41), the amount of RNA per total protein can be used as a measure for the intracellular concentrations of ribosomes and tRNA (total; i.e., both charged and uncharged tRNA). After the shift-up, the concentration of tRNA increased about twofold over a period of more than 2 h. Since the extent of tRNA charging did not decrease during this period (it is actually assumed to increase, see below), the concentration of charged tRNA must have increased at least as much as that of total tRNA; i.e., more than twofold during postshift growth. Evidently, the changes in the concentration of charged tRNA do not parallel the changes in the rate of protein synthesis per ribosome, suggesting that the rate of protein
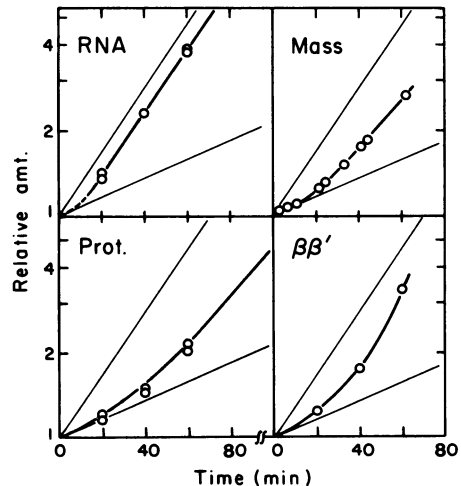


FIG. 5. *Shift-up experiment as in Fig. 1, except culture dilutions were 1.22- and 2.00-fold at t = 2 and 18 min, respectively; pre- and postshift doubling times were 91 and 27 min, respectively, and a = 0.83 and $\Delta\alpha_r$ = 2.6. Because $\Delta\alpha_p$ was not a constant, the postshift kinetics of RNA polymerase accumulation were simply drawn through the data points.*
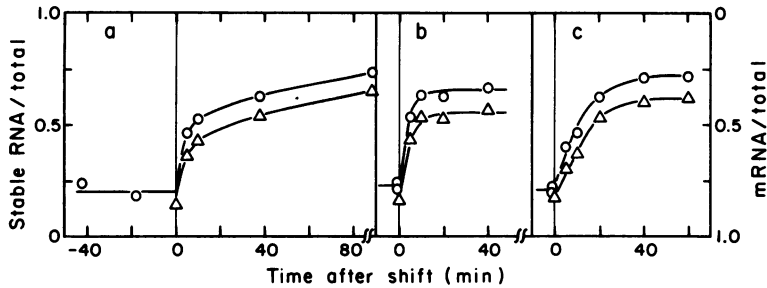
FIG. 6. *Fractional synthesis rate of stable RNA* ($r_s/r_t$; ○) *and fraction of functioning RNA polymerase engaged in the synthesis of stable RNA* ($\psi_s$, △) *as a function of time after shift-up; panels a, b, and c are from experiments in Fig. 1, 4, and 5, respectively. The values of the relative rate of stable RNA synthesis observed here are 20 to 30% lower than those that have been observed for exponential cultures (42). This might reflect a variation in culture growth or a systematic error in the hybridization procedure (estimate of hybridization efficiency). In the current context, this is not essential.*
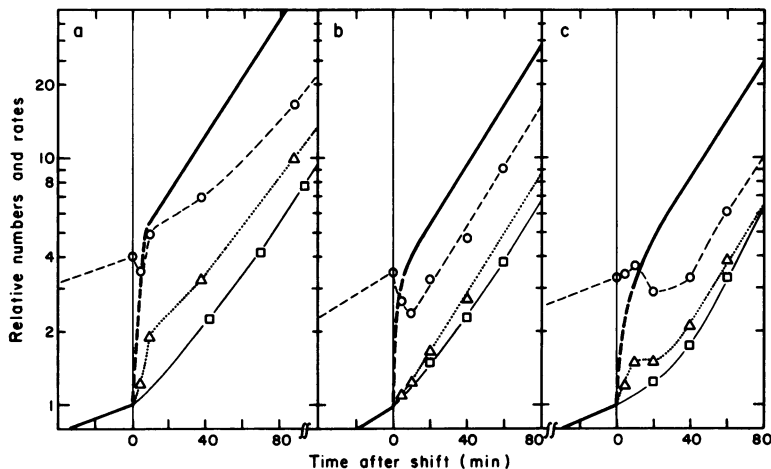


FIG. 7. *Kinetic changes in the rates of stable RNA* (——) *and mRNA* (○) *synthesis and in the number of functioning* (△) *and total* (——) *RNA polymerase molecules after a shift-up; evaluation of experiments in Fig. 1, 4, and 5 (panels a, b, and c). Total RNA polymerase kinetics are the same as those shown in Fig. 1, 4, and 5; the other parameters were calculated as described in the text. All parameter values were normalized to 1.0 at t = 0, except mRNA synthesis, which is given relative to stable RNA synthesis; e.g., a value of 4.0 for the zero-time rate of mRNA synthesis means that during preshift growth the rate of mRNA synthesis is four times greater than the rate of stable RNA synthesis. After the shift-up, the rate of mRNA synthesis became smaller than the rate of stable RNA synthesis (mRNA curve below stable RNA curve).*

synthesis per ribosome is not limited by the concentration of aminoacyl tRNAs. This implies that ribosome idling times (the times during which uncharged tRNA is bound to the ribosome) are short in comparison with the total step times of polypeptide chain growth. Therefore, the initial fluctuations in the rate of protein synthesis per ribosome and its final 30% increase are probably not due to changes in tRNA charging; rather, they might be the result of changing nucleotide (ATP and GTP) pools.

## DISCUSSION

**Control of RNA polymerase synthesis.** After a nutritional shift-up from succinate min-

imal to glucose-amino acids medium, the relative rates of accumulation of both ribosomes ($\alpha_r$) and RNA polymerase ($\alpha_p$) abruptly increased to their final levels (Fig. 2); the increases for ribosome synthesis and RNA polymerase synthesis were 2- to 3-fold and about 1.6-fold, respectively (Table 1). The stepwise increase in $\alpha_p$ must result, at least in part, from the induction of ribosome synthesis since the genes for RNA polymerase $\beta$ and $\beta'$ subunits *rpoB,C* are cotranscribed with ribosomal protein genes *rplJ,L* (26, 34, 45). RNA polymerase enzyme molecules are made in five- to sevenfold lower numbers than ribosomes (10, 42), which reflects a partial attenuation of transcription at the junction of the *rplL* and *rpoB*

genes (10). This attenuation has been shown to be controlled and dependent on the function of the *relA* gene (29, 37). Therefore, the different shift-up responses of RNA polymerase and ribosome synthesis, evident in the different slopes of the polymerase and RNA curves of Fig. 2, most likely reflect this control of transcription attenuation. Since the proportion of RNA polymerase to ribosomes decreases by the shift-up, and since this decrease occurs in a stepwise manner (Fig. 2), we suggest that the concentration or activity of a hypothetical factor that controls the extent of this read-through also changes in a stepwise manner. The activity of the control factor should increase if the control is negative (termination factor), or decrease if the control is positive (anti-termination factor).

It has been proposed that RNA polymerase in bacteria acts as a negative control factor in its own synthesis (15, 40). If this regulation were

operative during a nutritional shift-up, one would have to infer that the shift-up produces a sudden (about 1.5-fold [42]) increase in the concentration of RNA polymerase which would then cause the reduction in *rpoB,C* gene expression relative to ribosomal gene expression. Neither the concentration of total RNA polymerase (Fig. 3) nor the concentration of free RNA polymerase (see legend to Fig. 3) shows the stepwise 1.5-fold increase postulated for the change in the concentration of the hypothetical control factor. This is difficult to reconcile with the idea of an autogenous negative control as the major mechanism responsible for the growth rate-dependent changes in RNA polymerase synthesis. Whatever its nature, the factor that controls the expression of *rpoB,C* genes must either be unstable or be subject itself to a control of its activity to produce the sudden change in the proportions of the synthesis rates of ribosomes
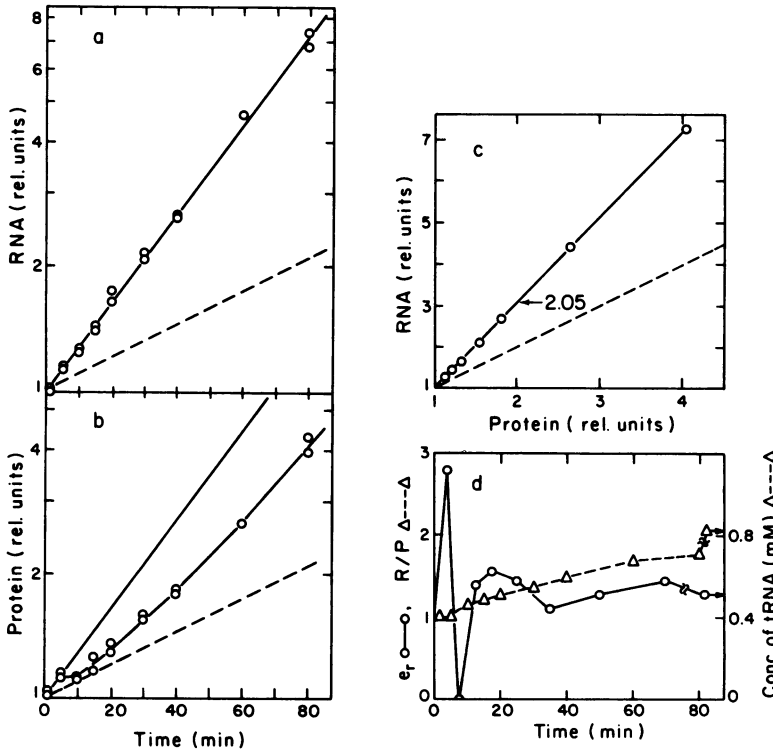


FIG. 8. *Protein synthesis per average ribosome ($e_r$) and RNA per protein (R/P, a measure for the concentrations of ribosomes and tRNA) as a function of postshift time (d), calculated from the observed accumulation of RNA (a) and protein (b). The experiment was similar to that shown in Fig. 1; the culture dilutions were 1.09-, 1.88-, and 1.96-fold at t = 0, 19, and 50 min, respectively; pre- and postshift doubling times were 75 and 28 min, respectively. In panel d, the relative rate of protein synthesis was determined by graphic differentiation between successive time points in panel b. The rate was then plotted at the midpoint of the respective time interval. The ordinate value of 1.0 corresponds to 11.6 amino acid residues per second per ribosome (Table 1), and to ribosomes and tRNA concentrations of 0.04 and 0.4 mM, respectively (for conversion into molar concentration, see legend to Fig. 3). Final levels ($\rightarrow$) for $e_r$ and R/P were obtained from the slope of the RNA versus the protein curve (=2.05) in panel c (see also footnotes b and c of Table 1).*

and polymerase observed after a nutritional shift-up.

**A model system to simulate the synthesis of ribosomes and RNA polymerase.** The rate of RNA polymerase synthesis is the product of $\alpha_p$ multiplied by the rate of protein synthesis, and the rate of protein synthesis is the product of the number of ribosomes multiplied by protein synthesis per ribosome. Using these relations, beginning with an arbitrary value for the number of ribosomes, and taking $\alpha_p$ and the protein synthesis per ribosome ($e_r$) observed for succinate medium (Table 1), we have calculated the rate of RNA polymerase synthesis, and from this rate, by multiplication by 1 min, the increment of RNA polymerase during a 1-min interval from $t = 0$ to $t = 1$ min. When this calculated increment is added to an arbitrary value for the number of RNA polymerase molecules at $t = 0$ min, the amount of RNA polymerase at $t = 1$ min is obtained. From the amount of RNA polymerase and from the observed values for the RNA polymerase activity ($\beta_p$), for the fraction of RNA polymerase engaged in rRNA synthesis ($\psi_r$) and for the rRNA chain elongation rate ($c_r$), the rate of rRNA synthesis and the 1-min increment in the number of ribosomes were calculated to give the ribosome number at $t = 1$ min. By reiteration of this process, the kinetics for the accumulation of ribosomes and RNA polymerase were obtained from observed values of $\alpha_p$, $e_r$, $\beta_p$, $\psi_r$ and $c_r$ (Fig. 9). Ribosomes and RNA polymerase initially accumulated at different rates, which depended on the arbitrarily assumed zero-time values for RNA polymerase and ribosomes. Gradually, however, the slopes for the ribosome and RNA polymerase curves became parallel and constant, corresponding to the 90-min doubling time characteristic for succinate medium. This shows that the bacterial growth rate is implied in or determined by the five observed parameters used for the calculation. (Previously, this had been shown mathematically by integration of the two rate equations for ribosome and polymerase synthesis [2]). Also, the final proportion of RNA polymerase per ribosome was independent of the arbitrary zero-time ratio: after several hours this proportion became equal to one RNA polymerase molecule per five ribosomes, as is typical for *E. coli* B/r in succinate medium (42).

From the rate of protein synthesis calculated above, the increment of protein during 1-min intervals and, again using an arbitrary zero time value, the kinetics for the accumulation of protein were obtained (Fig. 9). The protein doubling time also became 90 min; besides, the amounts of RNA polymerase and ribosomes per amount of protein (corresponding to $\alpha_p$ and $\alpha_r$) assumed
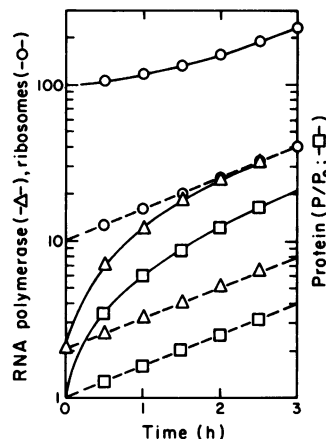


FIG. 9. *Accumulation of ribosomes ($N_r$, ○), RNA polymerase ($N_p$, △) and protein ($P$, □) in a simulated system calculated from parameters observed in E. coli B/r growing in succinate minimal medium. The parameters (and values) were: $\psi_s$, fraction of active RNA polymerase engaged in the synthesis of stable RNA (0.28); $\beta_p$, fraction of total RNA polymerase that is active (0.21); $c_r$, ribosomal RNA chain elongation rate (4,500 nucleotides per min); $\alpha_p$, fractional synthesis of RNA polymerase (0.01); $e_r$, protein synthesis rate per average ribosome (580 amino acids per min). For the solid curves, $N_r$ and $N_p$ for $t = 0$ were arbitrarily set at 100 and 2, respectively ($N_p/N_r = 0.02$). The simulated system reached "steady-state exponential growth" after several hours, with the correct (observed) $N_p/N_r$ value of 0.2. For the dashed curves, after the number of ribosomes in the simulated culture had reached a value of 1,000 (exponential growth), the system was "diluted" 100-fold (i.e., the values for $N_r$, $N_p$, and $P$ were divided by 100), and the time was reset to 0. Protein, ribosomes, and RNA polymerase continued to increase exponentially with the correct doubling time.*

the correct values for succinate medium. (This cannot be seen in Fig. 9, because instead of plotting absolute values for protein, relative values have been plotted by setting the zero-time amount of protein at 1.0.) When the curves for protein, ribosomes, and RNA polymerase become parallel, one has in fact simulated steady-state exponential growth of the model system. The simulated system may be "diluted" by dividing all values by the same factor and the time reset to zero: the system then continues to "grow exponentially" (Fig. 9, dashed curves).

The model may be extended to include DNA and cell division such that the amounts of DNA, RNA, protein, RNA polymerase, ribosomes, and mRNA, etc., can be given per cell rather than per unit volume of culture. For that purpose, one would have to introduce additional observed parameters (amount of protein per replication origin [14] and the so-called C- and D-period

[20]) and the known theoretical relations between these parameters and replication and division (3). However, since DNA concentration and cell division can be experimentally altered without affecting the rate of growth (36), DNA and cell division do not appear to be growth limiting and therefore were not included here. (In fact, the amounts of DNA per cell are irrelevant for the rate of growth; pertinent are the concentrations, which are independent of the cell size.)

The five observed input parameters may be substituted by other growth parameters; for example, $\psi_s$ is substituted below by parameters that relate to tRNA charging. Further, some of the parameters are directly regulated, like the ppGpp-dependent distribution of RNA polymerase activity over ribosomal ($\psi_s$) and nonribosomal ($\psi_m$) genes; other parameters are not directly controlled, like the rRNA chain elongation rate ($c_r$).

**Relation between tRNA charging and synthesis of stable RNA.** To quantitatively relate $\psi_s$ to tRNA charging, we have assumed that the fraction ($\psi_m$) of RNA polymerase engaged in the synthesis of mRNA ($\psi_m = 1 - \psi_s$) increases in proportion to the relative concentration of uncharged tRNA ($U/T$, uncharged/total tRNA); i.e., the more uncharged tRNA relative to total tRNA, the more ppGpp; the more ppGpp, the more mRNA and the less rRNA synthesis. Assuming that 70% of the tRNA is charged during growth in succinate medium ($U/T = 0.3$), then the relation $\psi_m = 2.4 \times (U/T)$ gives $\psi_m = 0.72$ (= $2.4 \times 0.3$) and $\psi_s = 0.28$ (= $1 - 0.72$), as observed. Obviously this relation is an approximation that cannot be valid if more than 40% of the tRNA is uncharged, since mRNA synthesis cannot exceed 100%.

The extent of tRNA charging can be related to protein synthesis and to the activity of charging enzymes as follows: ($U/T$) = $e_r/k[S]$, where [$S$] is the concentration of synthetase, and the activity factor $k$ is defined as the rate of aminoacylation of tRNA per amount of synthetase and per concentration of uncharged tRNA. The relation says that the more ribosome activity ($e_r$), the more uncharged tRNA, which reflects the fact that uncharged tRNA is the byproduct of protein synthesis; and the more synthetase activity, the less uncharged tRNA. The relation takes into account that tRNA is made in constant proportion to ribosomes (8, 41): the equation should logically contain the concentration of ribosomes in the numerator and the concentration of total tRNA in the denominator, but because of the constant relative proportions of ribosomes and tRNA these cancel with the exception of a factor of 10 that has been included

in $k$ (there are 10 tRNA molecules per ribosome).

The relation is further based on the unproven but plausible assumption that synthetase enzymes do not become saturated with uncharged tRNA in the physiological range of tRNA concentrations. Otherwise, the slightest reduction in ribosome function (e.g., if bacteria are subjected to a very low concentration of chloramphenicol) would immediately produce a nearly complete disappearance of uncharged tRNA. This seems unlikely since it would not allow the cell to finely adjust ribosome synthesis to small changes in the supply or consumption of amino acids.

Setting again the average charging of tRNA in succinate minimal medium equal to 70% ($U/T = 0.3$), the activity factor $k$ for succinate medium was estimated from the above relation by substituting values for $e_r$ (Table 1) and [$S$] (reference 38; [$S$] ~synthetase protein per total protein, $\alpha_s$, approximately equal to 1%; the exact value is not important; only the relative change after a nutritional shift-up will become important below). A simulation of exponential growth using these relations and values ($U/T = 0.3$; $\alpha_s$
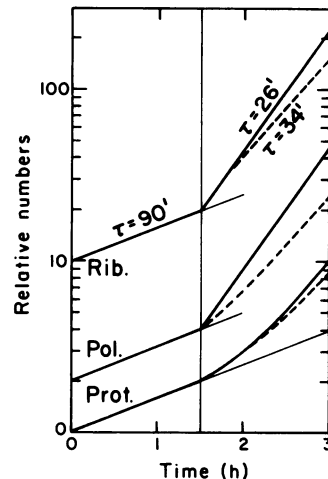


FIG. 10. Two simulated shift-up experiments, from succinate to glucose-amino acids, assuming only stepwise changes in the values of parameters (i.e., no late adjustments during postshift growth). Preshift as in Fig. 9; - - -, at 90 min., the values of $\psi_s$, $\beta_p$, $\alpha_p$ and $e_r$ increased stepwise (in amino acids per minute): $\psi_s$, 0.28 to 0.71; $\beta_p$, 0.21 to 0.30; $\alpha_p$, 0.010 to 0.015; $e_r$: 580 to 800. The accumulation of ribosomes was nonexponential, and the final postshift doubling time was 34 min, i.e., longer than observed; ———, same as above except that $\alpha_p$ was assumed to increase to 0.024 such that $\Delta\alpha_p = \Delta\alpha_r$. Postshift accumulation of ribosomes was exponential as observed, and final doubling time was 26 min, but $N_p/N_r$ remained unchanged (0.20), at variance with the observed decrease to 0.14.

= 0.01) instead of $\psi_s$ necessarily gives the same result as that shown in Fig. 9; the advantage, however, is that it allows prediction of the effect of a changing production of synthetase on the synthesis of ribosomes, RNA polymerase, and, finally, on the growth rate.

**Simulation of a nutritional shift-up.** As mentioned above, the rapid establishment of the new exponential rate of RNA and ribosome accumulation after a shift-up suggested that all parameters that affect RNA synthesis assume their final postshift values within minutes after the change in growth medium. However, a computer simulation of such a shift-up, using the observed preshift values for succinate medium and the postshift values for glucose amino acids

medium (see legend to Fig. 10), showed that an abrupt change in parameter values did not lead to an exponential accumulation of RNA or ribosomes (Fig. 10, broken lines). Only if the relative synthesis rate of ribosomes and RNA polymerase ($\alpha_r$ and $\alpha_p$) increased by the same factor did RNA accumulation immediately assume its final exponential rate (Fig. 10, solid lines), but this did not actually occur since it was found that $\alpha_p$ increased less than did $\alpha_r$ (Fig. 2). This implies that at least one of the factors affecting stable RNA synthesis must increase late after the shift-up to keep ribosome synthesis exponential.

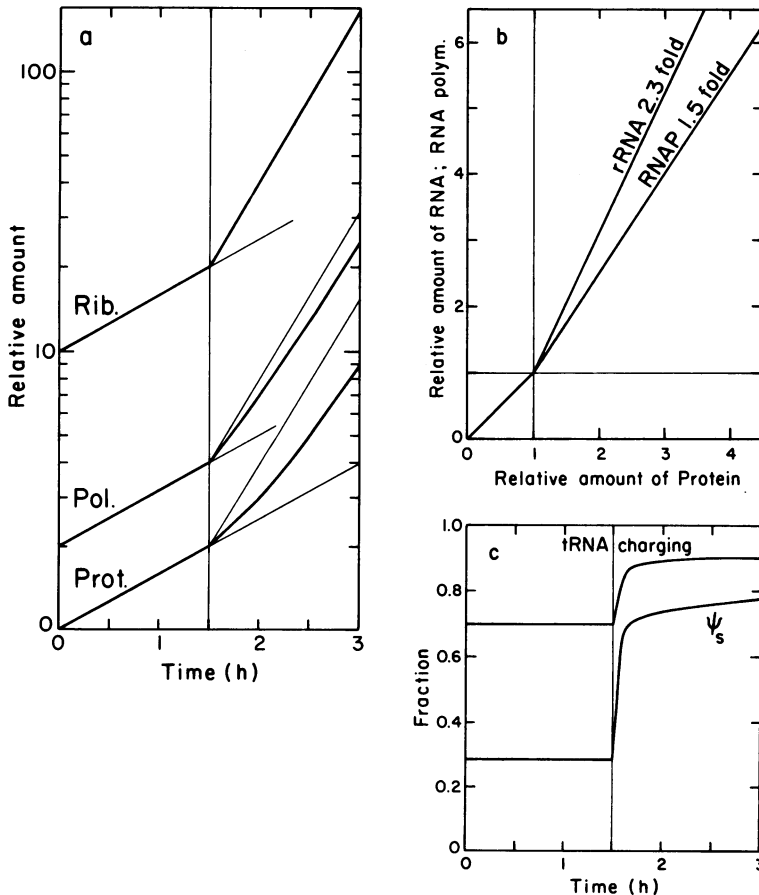Two of the relevant parameters were indeed found to change in complex patterns rather than



FIG. 11. *Simulated shift-up, succinate to glucose-amino acids medium, taking tRNA charging (U/T) and the production of aminoacyl tRNA synthetase (S) into account (see text). For the first 90 min (preshift) the following values were used: U/T, 0.3; $\alpha_s$, 0.01; k, 192,000. These values give a $\psi_s$ of 0.28 as observed for succinate medium. After 90 min the values for $\alpha_s$ and k were increased: $\alpha_s$, 0.010 to 0.015; k, 192,000 to 600,000; $\alpha_p$, $\beta_p$ and $c_p$ were increased as in Fig. 10, but no assumption about $\psi_s$ had to be made. (a) Accumulation of ribosomes, RNA polymerase, and protein (compare with Fig. 1). (b) Ribosomes and RNA polymerase versus protein (compare with Fig. 2). (c) Fraction of active RNA polymerase engaged in the synthesis of stable RNA, $\psi_s$ (compare with Fig. 6), and fraction of total tRNA that is charged (1 − U/T; predicted, not yet observed).*

stepwise after the shift-up: the RNA polymerase activity ($\beta_p$) and the fraction of total transcribing RNA polymerase that is engaged in ribosomal or stable RNA synthesis ($\psi_r$ or $\psi_s$, respectively). We suppose it is the control of the latter parameter, $\psi_s$, that keeps ribosome synthesis exponential; if the value of the product of RNA polymerase synthesis and activity ($\alpha_p \times \beta_p$) was not high enough to supply the amount of rRNA required for exponential ribosome synthesis, then more RNA polymerase was shifted from the synthesis of mRNA to the synthesis of stable RNA (Fig. 6). A gradually increased overall charging of tRNA during postshift growth is proposed to be responsible for the observed gradual increase in the relative rate of stable RNA synthesis. To test this idea, a shift-up was simulated, assuming that the initial rapid increase in $\psi_s$ immediately after the shift-up reflects a sudden increase in synthetase activity, i.e., in the value of the activity factor $k$ in the relation above, due to increased ATP and amino acid pools and that $k$ remains constant during further postshift growth (i.e., that the ATP and amino acid pools either remain constant or become saturating for synthetase function). The agreement of the simulated kinetics of $\psi_s$ and of ribosome and RNA polymerase accumulation with observed results (compare Fig. 11a, b, and c with Fig. 1, 2, and 6) suggests that the above assumptions about the synthetase activity may in fact explain certain features of the shift-up response of bacteria, in particular the late increase in $\psi_s$ during postshift growth.

According to this model, the reason for the exponential accumulation of ribosomes throughout the postshift transition period is the increase in the fractional synthesis of aminoacyl-tRNA synthetase ($\alpha_s$) by the shift-up. This can be seen from the relation given above between tRNA charging ($U/T$) and synthetase concentration [$S$]: without an increase in $\alpha_s$ ($dS/dP$) there would be no change in the concentration of synthetase ([$S$] ~ $S/P$), and hence no gradual increase in the fraction of charged tRNA. Therefore, $\psi_s$ would remain constant after the immediate increase due to the increased synthetase activity, $k$. The control of synthetase gene expression is not understood, but it has been shown that different synthetase genes respond differently to a shift-up, such that, on the average, $\alpha_s$ increases stepwise after the shift-up and somewhat less than $\alpha_r$ (32, 33, 38). For the simulated shift-up shown in Fig. 11, an average 1.5-fold increase in $\alpha_s$ was used (estimated from the data in reference 38).

## ACKNOWLEDGMENTS

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