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Control of RNA Synthesis in *Escherichia coli* After a Shift to Higher Temperature

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Parameters of RNA synthesis were measured after a temperature upshift in a pair of *Escherichia coli* B/r strains that are isogenic except for having *relA* and *relA*$^+$ loci, to examine the cause for a reported anomaly in the correlation between guanosine tetraphosphate (ppGpp) and stable RNA (rRNA, tRNA) synthesis under such conditions. Two main results were: (i) the specific stable RNA gene activity (stable RNA per total RNA synthesis) correlated in the conventionally expected fashion with the level of ppGpp but was obscured by a nonspecific increase in the RNA chain elongation rate due to the higher temperature; (ii) the temperature upshift caused a transient reduction in the RNA polymerase activity (transcribing per total enzyme) that accounts for the previously observed oscillating RNA synthesis rate after a temperature shift.

When grown exponentially at different (physiological) temperatures, the macromolecular composition of *Escherichia coli* or *Salmonella* bacteria remains unaltered (18, 19). However, a number of transient effects occur after a culture is shifted from a lower to a higher growth temperature; the rates of protein and RNA synthesis oscillate during the initial 30 min (4, 8, 12, 26, 27), the synthesis of certain polypeptides relative to total protein synthesis temporarily increases or decreases (12, 26, 27), and the cells may show symptoms of amino acid deprivation, accompanied by an accumulation of guanosine tetraphosphate (ppGpp) (4, 8).

When subjected to amino acid deprivation at constant temperature, wild-type (Rel$^+$) *E. coli* respond with an accumulation of ppGpp and a strong curtailment of RNA accumulation ("stringent" response; 3, 23). Under the same conditions, strains with a mutation in the *relA* locus fail to accumulate ppGpp, and RNA accumulation continues (3, 23). Therefore, it was proposed that ppGpp is an effector for the control of stable RNA (rRNA and tRNA) synthesis (3). In the case of a temperature upshift, however, RNA accumulation continues at the preshift rate or may even be stimulated rather than inhibited, despite an accumulation of ppGpp in *rel*$^+$ bacteria (4, 8). This latter observation, which appears to be inconsistent with the idea that ppGpp is directly involved in the control of stable RNA synthesis, was the starting point for the current investigation.

We have studied the effects of a temperature upshift on RNA synthesis and found that in *rel*$^+$ bacteria the specific stable RNA gene activity (transcription of rRNA and tRNA genes relative to total transcription) is reduced after the temperature shift to a minimum value similar to that observed during amino acid starvation (17). However, this specific reduction in stable RNA gene activity was obscured in the RNA accumulation kinetics by a nonspecific stimulation of total RNA synthesis resulting from an increased RNA chain elongation rate, a consequence of the higher temperature. These results, therefore, are consistent with the idea that ppGpp is an effector in the control of stable RNA gene activity. In addition, we observed a temporary reduction in the RNA polymerase activity immediately after the temperature shift (also not specific for rRNA and tRNA) that accounts for the oscillation in RNA accumulation observed previously (see above).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used were RL331 (*relA*$^+$) and RL332 (*relA*), which were derived by transduction of *fuc*$^+$ from NC52 (*relA fuc*$^+$; 16) into LEE123, a *phe thr* derivative of *E. coli* B/r.

Cultures were grown in Medium C (10), supplemented with 0.2% glucose and 50 μg of phenylalanine and threonine per ml or with 0.2% glucose and 0.8% Casamino Acids (experiment of Fig. 8). Growth was followed as mass increase (optical density at 460-nm wavelength [OD_{460}]).

Determination of RNA and protein. The amounts of RNA and protein were determined as described previously (2) by precipitating the cells in 5-ml samples of culture with 1 ml of 3 M trichloroacetic acid. After the cells were collected, washed, and dried on glass fiber filters, nucleic acids and protein were solubilized, and

the RNA was hydrolyzed by incubating the filters overnight in 2 ml of 0.2 M NaOH at 25°C. Part of this hydrolysate (0.5 ml) was used for protein determination by the method of Lowry et al. (13); the remainder was used to measure RNA as UV absorbence at 260 nm (A_{260}), after DNA and protein were precipitated and removed. One A_{260} unit of RNA (at acid pH) was assumed to be equal to 5.45×10^{16} nucleotides (20).

Determination of tRNA. Details of the method for determining tRNA have been described previously (17, 21). Briefly, exponentially growing cultures were radioactively labeled with [^{3}H]uridine, beginning at OD_{460} = 0.1, until the label was exhausted from the medium and chased into stable nucleic acids. At an OD_{460} of 0.4, [^{14}C]uracil was added. After 15 min, half of the culture was shifted from 20 to 40°C (zero time). Samples (1 ml) were taken immediately before and after zero time from both cultures. The samples were treated with ethanol-phenol "stopping solution" (18); the cells were harvested by centrifugation, resuspended, and lysed in 1 ml of boiling sodium dodecyl sulfate-electrophoresis sample solution (18). After electrophoresis, the RNA bands were located by fluorography, and the bands containing the 4S tRNA were sliced out of the gel. The RNA in the gel was solubilized with 0.2 M NaOH, and the radioactivity (isotope ratio) in these hydrolysates was determined (for further evaluation see below).

Fractional synthesis rate of stable RNA. Details of the method used to determine the fractional synthesis rate of stable RNA have been described (17, 18, 20). Cultures grown exponentially at 20°C to an OD_{460} of 0.5 to 0.6 were shifted to 40°C (zero time). Samples (0.5 ml) were pulse-labeled with [^{3}H]uridine for 1 min at different times before and after zero time, as indicated. The samples were treated and the cells were lysed as described above. The fraction of the total pulse label that was rRNA (including unstable "spacers") was determined by hybridization to denatured λ *dilv5* DNA bound to nitrocellulose filters. The hybridization efficiency was monitored by including purified ^{14}C-labeled rRNA in the hybridization mixture (the hybridization efficiency ranged from 60 to 90%; for further evaluation and formulas see below).

Measurement of ppGpp. At the indicated times before and after the temperature shifts from 20 to 40°C, 10-ml samples of cultures were added to 1 ml of 1.9% formaldehyde and kept on ice for 15 to 20 min. The cells were harvested by centrifugation and suspended in 0.5 ml of 0.1 M KOH. After incubation for 30 min at 0°C, the KOH was neutralized by the addition of 5 μl of 44% phosphoric acid, and 0.5 ml of HPLC buffer (0.03 M KH_2PO_4, 0.01 M tetrabutylammonium phosphate, 14.8% acetonitrile, adjusted to pH 3.25 with phosphoric acid) was added. The samples were centrifuged at 30,000 × *g* for 45 min to remove debris; the supernatants were retained for high-pressure liquid chromatography analysis.

Portions (0.2 ml) were fractionated by ion-pair reversed-phase high-pressure liquid chromatography on an Ultrasphere IP-C18 column (Altex, Berkeley, Calif.) with HPLC buffer at a flow rate of 1 ml/min. The A_{254} of the eluate was continuously monitored, and the area under the peak corresponding to ppGpp was determined and converted to picomoles per OD_{460} unit of culture as described previously (18).

Determination of the relative RNA chain elongation rate. The rate of decrease in the residual rate of labeling of RNA after the addition of 200 μg of rifampin per ml was taken as a relative measure for the RNA chain elongation rate. At an OD_{460} of 0.5, a 0.5-ml sample was added to 5 μl of [5-^{3}H]uridine (zero time). The labeling was stopped after 30 s by the addition of an equal volume of ice-cold ethanol-phenol stopping solution. Immediately after the labeling of this sample, 200 μg of rifampin was added per ml of the remaining culture, and samples were labeled at various times afterward and treated like the zero-time sample. Cells were harvested by centrifugation, suspended in 0.5 ml of Medium C, lysed by the addition of 0.5 ml of boiling sodium dodecyl sulfate lysis buffer, and kept at 100°C for 30 s. Radioactivity in 0.1-ml samples was determined by precipitation with cold trichloroacetic acid, collection of the precipitate on glass fiber filters, and counting in a liquid scintillation counter.

Calculations. (i) Amount of stable RNA. The amount of stable RNA (Fig. 3) was calculated from the isotope ratio in tRNA (Fig. 2). A constant, *C*, was calculated from the following equation:

$$[C + I(12.5)]/[C + I(0)] = 2^{12.5/\tau}$$

where I(12.5) and I(0) are the observed isotope ratios at 12.5 and 0 min in the (unshifted) control culture (Fig. 2) and τ is the doubling time at 20°C. The amount of stable RNA, normalized to 1.0 at zero time, was calculated by the formula:

$$\text{amount}(t) = [C + I(t)]/[C + I(0)]$$

where $I(t)$ is the observed isotope ratio at the time t (from Fig. 2).

(ii) Relative rate of stable RNA synthesis. The relative rate of stable RNA synthesis (Fig. 4) was calculated from the amounts of RNA in Fig. 3. The rate in the time interval t_1 to t_2 (interval midpoint) was found from the formula:

$$\text{Rate} = \{[\text{amount}(t_2) - \text{amount}(t_1)]/[t_2 - t_1]\}/(\ln 2/\tau)$$

(iii) Absolute rate of stable RNA synthesis. The absolute rate of stable RNA synthesis (Fig. 9a and c) was calculated from the relative rate (Fig. 4). The conversion factor, *f*, was found from the A_{260} in RNA observed at zero time (Fig. 1):

$$f = (A_{260}/OD_{460}) \times (\ln 2/\tau) \times (5.6 \times 10^{16} \text{ nucleotides}/A_{260})$$

The absolute rate per OD_{460} unit of culture is then given by

$$r_s/OD_{460} = f \times \text{relative rate}$$

(iv) mRNA synthesis rate. The mRNA synthesis rate, r_m (Fig. 9a and c), was calculated from the absolute rate of stable RNA synthesis, r_s (see above), and from the observed ratio r_s/r_t (Fig. 6):

$$r_m/OD_{460} = (r_s/OD_{460}) \times [1/(r_s/r_t) - 1]$$

Note that r_s/r_t is obtained from a hybridization assay as the fraction of pulse label corresponding to rRNA and tRNA, not from measurements of the actual rates r_s and r_t.

(v) Instantaneous rate of total RNA synthesis. The instantaneous rate of total RNA synthesis (Fig. 9a, c,

and e) was found from the sum:

$$r_t/OD_{460} = r_s/OD_{460} + r_m/OD_{460}$$

This is identical to the relationship:

$$r_t/OD_{460} = (r_s/OD_{460})/(r_s/r_t)$$

In the latter relationship, numerator and denominator are observed, i.e., the absolute instantaneous rate of total RNA synthesis is obtained from the absolute rate of stable RNA synthesis and the relative fraction of total RNA synthesis that is stable RNA.

(vi) Nascent RNA chains. The number of nascent RNA chains (Fig. 9b, d, and f) was obtained from the rates of RNA synthesis (r_s and r_m; Fig. 9a, c, and e) and from the RNA chain growth rates (c_s for stable RNA, from reference 18; c_m for mRNA, assuming $c_m = 0.6 \times c_s$, from reference 18):

$$\text{Number of chains} = (r_s/c_s) + (r_m/c_m)$$

This is the total number (Fig. 9f); the numbers of stable RNA chains and of mRNA chains are given by the first and second term, respectively.

RESULTS

RNA, protein, and mass accumulation. In both the *relA*$^+$ and *relA* mutant strains, a shift in growth temperature from 20 to 40°C resulted in increased rates of accumulation of mass (OD_{460}), protein, and RNA (Fig. 1). For the *relA*$^+$ strain, an initial about threefold stimulation was followed by a decreased rate, equal to or below the initial value. The minimum rate was observed between 10 and 20 min after the shift and was followed by a threefold increase until around 30 min, when the rates increased exponentially with the final postshift doubling time (46 min; preshift value, 150 min). The *relA* mutant showed similar kinetics, but the oscillations were less pronounced. This behavior was similar to that observed previously (see Introduction).

For several hours after the shift, the doubling time in the *relA* mutant was only 120 min until the final doubling time of 46 min (same as in *relA*$^+$) was established (this final phase is not illustrated in Fig. 1).

Stable RNA synthesis. During conditions of amino acid starvation, rRNA is unstable (6, 17). Therefore, to measure the rate of stable RNA synthesis after the temperature shift, which is also a condition under which rRNA might be unstable, the synthesis of tRNA was measured. This provides a reliable measure for the synthesis of both tRNA and rRNA since tRNA is synthesized in constant proportions with rRNA and remains stable during amino acid starvation (17, 21).

The bacterial RNA was first homogeneously labeled with ^3H during exponential growth at 20°C; then, 15 min before the temperature shift to 40°C, the RNA was labeled with ^{14}C. Samples were taken immediately before and after the shift, as indicated, and the isotope ratios in tRNA were determined (Fig. 2; two independent experiments with each strain, *relA*$^+$ and *relA*). These isotope ratios were evaluated to obtain the amounts of tRNA, normalized to the amounts at the shift time (Fig. 3). Only the kinetic changes in the amounts of tRNA can be compared with the accumulation of total RNA in Fig. 1. The kinetics of tRNA accumulation after the temperature shift (Fig. 3) quantitatively resembled those for the RNA accumulation in Fig. 1, indicating that no significant turnover of stable RNA occurred after the temperature shift.

The kinetic changes in the rate of tRNA synthesis and thus of stable RNA synthesis were calculated from the slopes of the amount curves (Fig. 4). The rate of stable RNA synthesis oscillated equally, independent of the *relA* allele. The initial stimulation in the rate of stable RNA synthesis was threefold (Fig. 4), equal to the expected change in the stable RNA chain elongation rate as previously determined during steady-state growth at these temperatures (18).

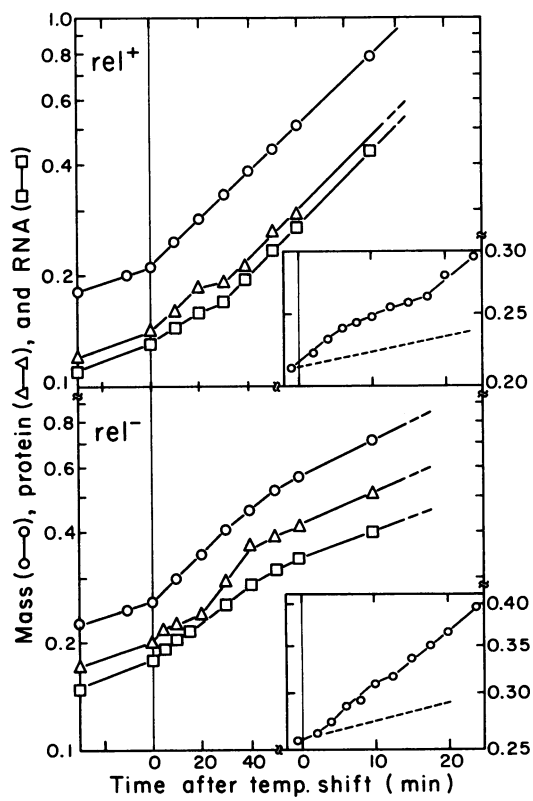

FIG. 1. Accumulation of mass, protein, and RNA in RL331 (*relA*$^+$) and RL332 (*relA*) after a shift in growth temperature from 20 to 40°C in glucose minimal medium. Inserts: details of mass accumulation during the first 20 min, showing oscillating rate.

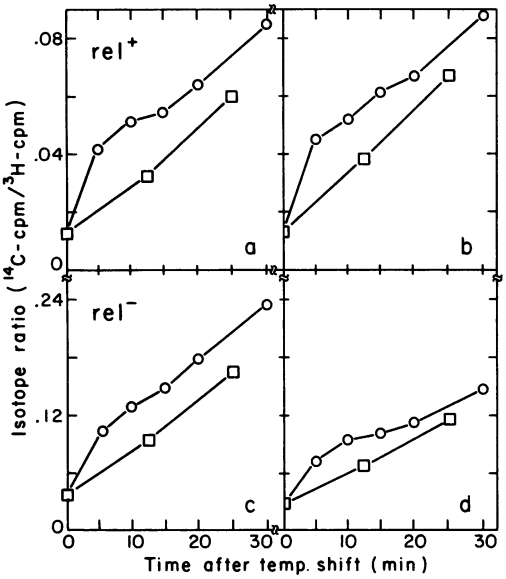

FIG. 2. Radioactive labeling of tRNA after a temperature shift (same conditions as for Fig. 1); two repeats of the same experiment with each strain. (a and b) *rel*$^+$; (c and d) *relA*. Symbols: ○, Shifted culture; □, unshifted control.

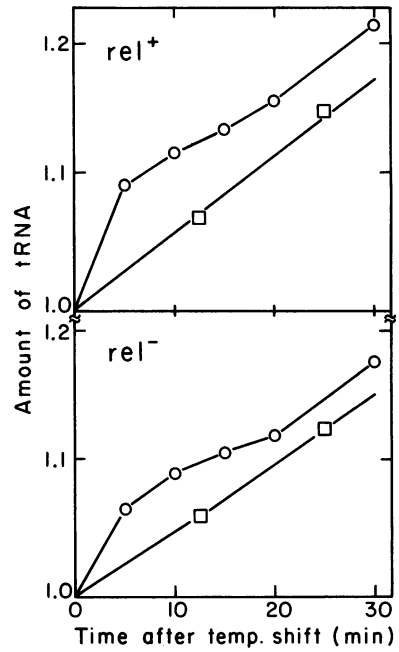

FIG. 3. Accumulation of tRNA after a temperature shift (○), calculated from the data in Fig. 2. The data in Fig. 2a and b and 2c and d, respectively, were averaged and normalized to 1.0 at zero time and to the doubling time of the unshifted control (□) (see the text).

ppGpp accumulation. After the temperature shift, ppGpp accumulated to about 30-fold the basal level in the *rel*$^+$ strain; after 10 min, it began to decrease again and reached the final postshift level for 40°C (18) after 60 min (Fig. 5). In the *rel* mutant the ppGpp level gradually increased to the postshift steady-state level over a period of 60 min. The final postshift level was the same in both strains and higher than the steady-state level at the lower temperature, in agreement with previous observations (18).

Relative proportions of stable and mRNA synthesis. The fractions of the instantaneous rate of RNA synthesis that corresponded to stable RNA (r_s/r_t) or mRNA ($r_m/r_t = 1 - r_s/r_t$) were used as a specific measure for stable RNA gene regulation (see below). This parameter was obtained by radioactively pulse-labeling RNA and determining the fraction of pulse label in rRNA and tRNA by a hybridization assay. In this fraction, r_s includes the synthesis of unstable spacers in the stable RNA precursors. (Note that determination of r_s/r_t does not require knowledge of the individual rates r_s and r_t.)

After the temperature shift, r_s/r_t transiently decreased in the *rel*$^+$ strain and increased in the *rel* mutant (Fig. 6). By 60 min, r_s/r_t had returned to the preshift value, which was the same for both strains, in agreement with previous measurements of r_s/r_t during steady-state growth at different temperatures (18).

The reduction in r_s/r_t in the *relA*$^+$ strain and the increase in r_s/r_t in the *relA* mutant are consistent with the normal correlation between stable RNA gene activity and ppGpp levels (see below).

RNA chain elongation rate. The rRNA chain

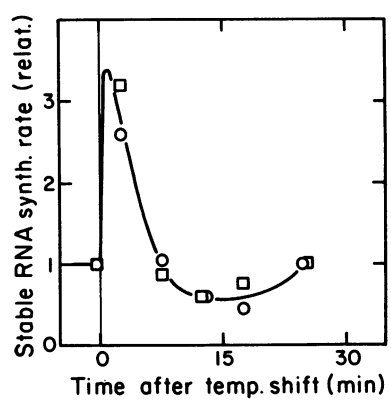

FIG. 4. Rate of tRNA accumulation after a temperature shift, calculated from the slopes of the amount curves in Fig. 3. The rate was determined for each time interval between two adjacent points in Fig. 3, normalized to the zero time rate, and plotted over the interval midpoint. Symbols: ○, *relA*$^+$; □, *relA*.

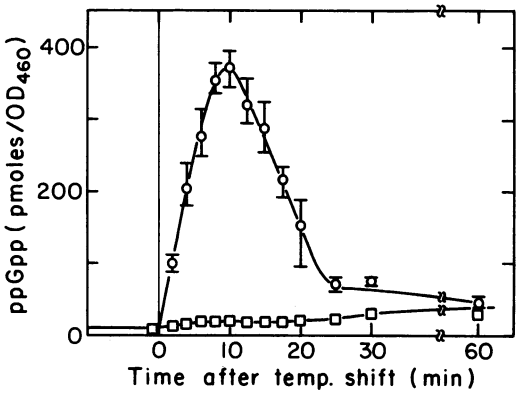

FIG. 5. Accumulation of ppGpp after a temperature shift in RL331 (*relA*$^+$, ○) and RL332 (*relA*, □). Conditions were the same as for the experiment shown in Fig. 1. The data shown are from three independent experiments for each strain; the vertical bars reflect the variation between the values obtained.

elongation rate was previously measured during steady-state exponential growth at different temperatures (18). Although the chain growth rate of tRNA had not been directly measured, it was assumed that it is equal to the rRNA chain growth rate (stable RNA chain growth rate, c_s), and that the chain growth rate of mRNA was proportional to, but less than, the stable RNA chain growth rate ($c_m = 0.6 \times c_s$). Observed data were consistent with these assumptions (18). Here the time required for the RNA synthesis rate to drop to 1% of its initial value after the addition of the drug rifampin was used to obtain a relative value for all RNA chain elongation rates (proportional to the reciprocal of this time). This time was measured in the *rel*$^+$ strain immediately before the temperature shift, 10 min after the shift when the rate of stable RNA synthesis was at a minimum (Fig. 4), and 60 min after the shift (Fig. 7). The RNA chain elongation rate at 10 and 60 min after the shift was seen to be threefold higher than before the shift. This difference in the rate of RNA chain growth is the same as previously observed during exponential growth at different temperatures (18). These results are therefore consistent with the assumption that the temperature upshift produces a stepwise increase in the RNA chain elongation rate, without oscillations, since the rate remained at the same higher level, even when RNA synthesis was at a minimum.

Temperature shift in the presence of amino acids. When the *rel*$^+$ strain was shifted to higher temperature in the presence of amino acids, the level of ppGpp increased only 10-fold, rather than 30-fold (Fig. 8a), and only a slight reduction in the specific stable RNA gene activity occurred (r_s/r_t; Fig. 8b). However, the kinetics of stable RNA accumulation still showed the oscillating rate (Fig. 8c) observed after a temperature shift in the absence of amino acids (Fig. 1 and 4).

DISCUSSION

Control of stable RNA gene activity. To correlate the accumulation of ppGpp with the control of stable RNA synthesis in exponentially growing cultures, the amount of RNA per genome (11), or of RNA per milligram of protein (15), has previously been used as a measure for the stable RNA gene activity. These parameters reflect not only the control of stable RNA synthesis, but also the control of the reference units used, DNA or protein. This explains why apparent departures from the normal correlation between ppGpp and stable RNA gene activity were found in mutants with abnormal control of DNA replication or with defects in ribosome assembly or function (8, 15).

After perturbations of exponential growth, changes in the rate of stable RNA accumulation have been used as a measure for the changes in stable RNA gene activity. However, changes in the accumulation rate are not uniquely related to the ratios of the amounts of RNA per genome or RNA per unit of protein, nor do they exclude nonspecific effects due to changes in RNA polymerase activity or in the RNA chain elongation rate. Furthermore, when rRNA is unstable, e.g., after amino acid starvation (6, 17), the rate of RNA accumulation is not a suitable measure for stable RNA gene activity. Such complications can be avoided if the fraction of the total rate of RNA synthesis that is stable RNA, r_s/r_t, is used as a specific measure for the control of rRNA

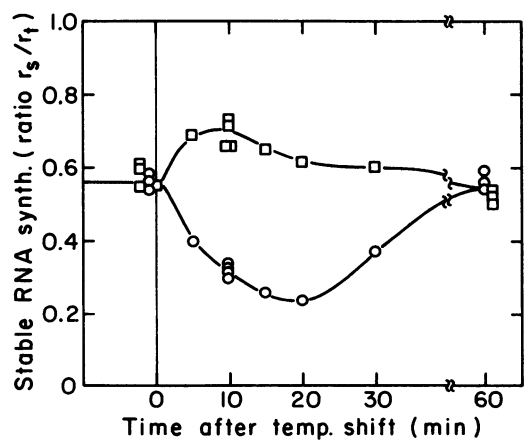

FIG. 6. Relative rate of stable RNA synthesis (r_s/r_t = fraction of the instantaneous rate that is rRNA and tRNA) after a temperature shift in RL331 (○) and RL332 (□). Samples were taken from the same cultures used for the experiment shown in Fig. 5.

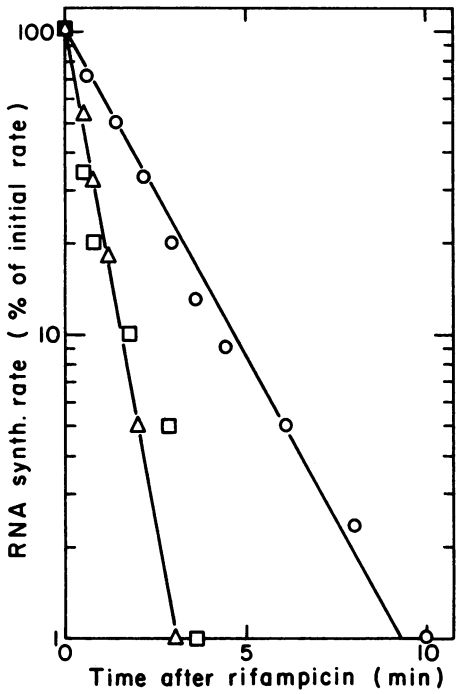

FIG. 7. Decrease in the residual rate of RNA synthesis after treatment of RL331 (*relA*$^+$) growing in glucose minimal medium with rifampin at 20°C before (○), or 10 min (□) or 60 min (△) after, a temperature shift to 40°C. The reciprocal of the time required for the RNA synthesis rate to drop to 1% of its initial value (intersection of curves with abscissa) was used as a measure for the RNA chain elongation rate.

and tRNA gene activities, as is generally done for in vitro studies of factors controlling the transcription of rRNA genes (24, 25). The parameter r_s/r_t is not affected by irrelevant reference units or by factors affecting total RNA synthesis such as changes in RNA chain elongation rate or in RNA polymerase activity, and it is equally well suited to express the stable RNA gene activities during exponential growth and during the non-steady-state conditions following perturbations of growth. Using r_s/r_t as a specific indicator for the control of stable RNA gene activity, the reduction in r_s/r_t in the *relA*$^+$ strain and the increase in r_s/r_t in the *relA* mutant after the temperature shift (Fig. 6) show a correlation with ppGpp levels that agrees both qualitatively and quantitatively with in vitro effects of ppGpp on RNA synthesis. In addition, the values of r_s/r_t are similar to those obtained when *relA*$^+$ and *relA* mutant strains were subjected to amino acid starvation (17).

Previously, an anomalous correlation between ppGpp and stable RNA accumulation was reported to exist after a similar temperature shift (8). This interpretation relied on the rate of RNA accumulation as a measure for stable RNA gene activity and failed to distinguish between nonspecific effects of the RNA chain elongation rate (Fig. 7) and RNA polymerase activity (Fig. 9) on total RNA synthesis and effects specific for stable RNA. Thus, by a more thorough analysis, this anomaly disappears. Other situations in which apparent anomalies between ppGpp and stable RNA gene activity were reported to exist (9, 14, 22) might similarly be explained by a more thorough examination of all factors affecting the rate of RNA accumulation.

Although the results obtained here do not prove that ppGpp is an effector for stable RNA gene control as in vitro experiments have suggested (24, 25), they do remove the inconsistencies that existed when this idea was applied to in vivo experiments (8).

Transient changes in RNA polymerase activity after a temperature shift. From the rate of stable RNA synthesis (Fig. 4) and the relative proportions of stable to total RNA (Fig. 6), the post-shift kinetic changes in the absolute rates of

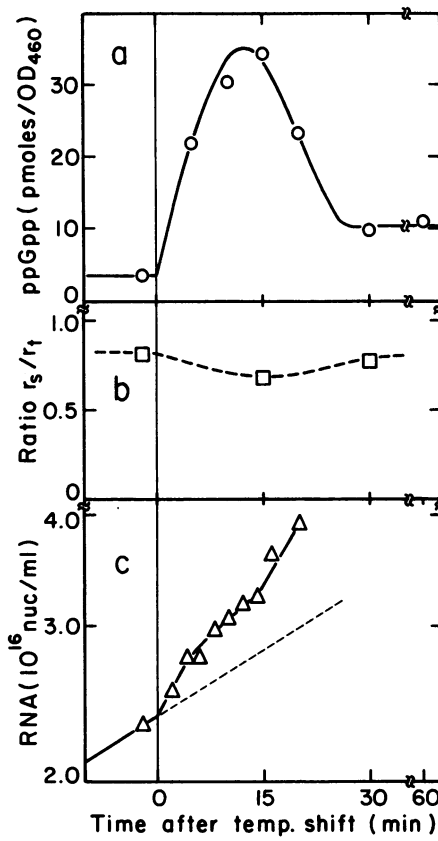

FIG. 8. Temperature shift-induced changes in the accumulation of ppGpp, in r_s/r_t, and in the accumulation of total RNA in RL331 (*relA*$^+$) growing in glucose-amino Acids medium.

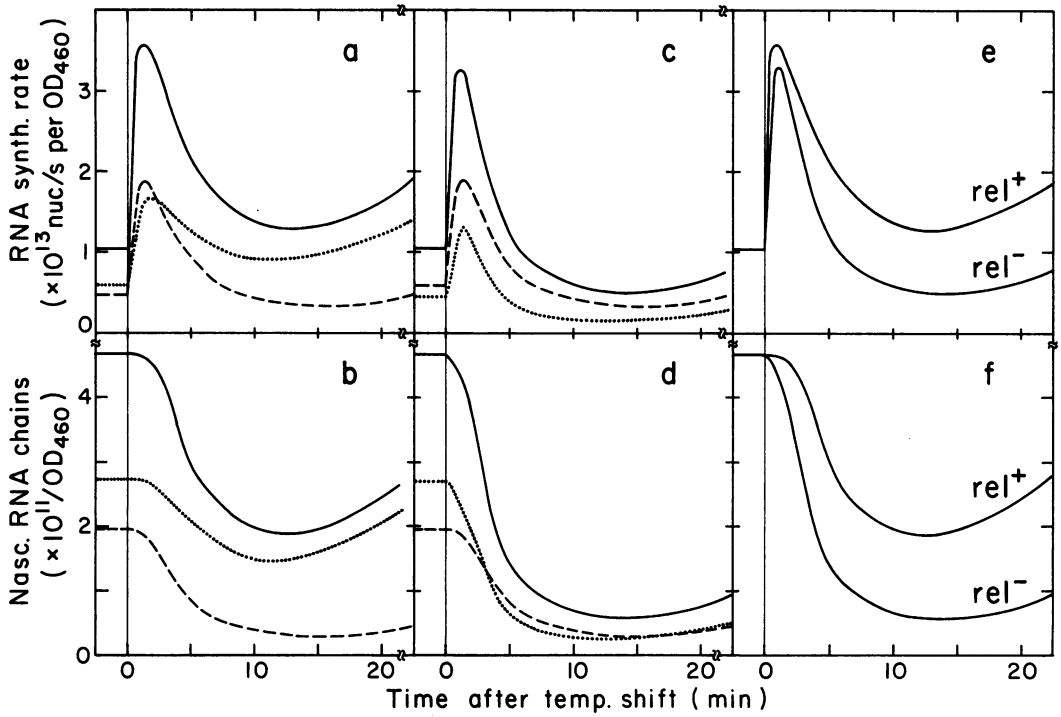

FIG. 9. Calculated changes in the instantaneous rates of total (——), messenger (....), and stable (---) RNA synthesis (a, c, e) and in the number of growing RNA chains (b, d, f; RNA polymerase activity, see text) during a temperature shift (see the text for details).

RNA synthesis were calculated (Fig. 9a and c). Independent of the *relA* allele, the rates of synthesis of stable RNA, mRNA, and total RNA rapidly increased after the temperature shift, then decreased to a minimum around 10 min postshift time, and finally increased again. These oscillating changes in the RNA synthesis rate are the result of two superimposed effects: a stepwise threefold increase in the RNA chain elongation rate (Fig. 7) and a temporary threefold (*rel*$^+$) to eightfold (*relA*) decrease in the number of nascent RNA chains (Fig. 9b and d). Since RNA synthesis in *E. coli* is not limited by the availability of free promoters (5), the transient reduction in the number of growing RNA chains is interpreted as a change in RNA polymerase activity. This drop in RNA polymerase activity occurred independently of *relA* function or of the presence or absence of exogenous amino acids, and its causes are not known.

Figure 9e and f shows a *relA*-dependent quantitative difference in the transient drop of RNA polymerase activity. This difference only affects RNA polymerase engaged in mRNA synthesis, but not enzyme engaged in stable RNA synthesis, since changes in the rate of stable RNA synthesis are the same in both strains (Fig. 4). The reason for this specific effect on mRNA synthesis is also not known (see below).

Relationship between stable RNA synthesis and growth. A striking result obtained here is the equality of the kinetic changes in the rate of stable RNA synthesis after a temperature shift in the *relA*$^+$ and *relA* mutant strains (Fig. 4), despite great differences in ppGpp concentration and in r_s/r_t. This result does not imply that ppGpp is irrelevant for the control of stable RNA synthesis. The formal reason for the equality of the stable RNA synthesis in Fig. 4 is that the difference in r_s/r_t in the two strains (at 10 min: 0.3 in *rel*$^+$, 0.7 in *relA*) is compensated for by a difference in the RNA polymerase activity (threefold decrease in *rel*$^+$, sevenfold decrease in *relA*; Fig. 9f). Although the exact compensation might be a coincidence, it is more likely that the equality results from a control of the rate of stable RNA synthesis that adjusts this rate to some other growth-limiting reaction which is the same in both strains.

The transient drop in the stable RNA synthesis rate (which is the same in both strains despite differences in the effects that produce it) might be explained by the following hypothetical model. According to this model, the RNA polymerase activity depends both on factors independent of the *relA* allele (associated with the temperature shift) and on ppGpp; the temperature factor causes a drop in activity which is

modulated by the different concentrations of ppGpp in the two strains. If ppGpp affects the conformation of the RNA polymerase enzyme (24), then a different response of the different enzyme conformations to the inactivating factors might be plausible. Furthermore, if the *relA*-independent synthesis of ppGpp (1, 7) is controlled via changes in amino acid supply and consumption, then the levels of ppGpp and r_s/r_t could be adjusted to the capacity of the cell to synthesize proteins. The growth-limiting reaction that provides the signal for the control of ppGpp synthesis by the *relA*-independent system could be the charging of tRNA with amino acids, as has been proposed previously in an analysis of a nutritional shift-up (20). For example, if the rate of ribosome synthesis were "too low," amino acid consumption by ribosomes would be lower than amino acid supply, and an "internal nutritional shift-up" would ensue. As a result, the level of ppGpp would go down and r_s/r_t would go up until all relevant parameters were in equilibrium.

ACKNOWLEDGMENT

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