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relA-Dependent RNA Polymerase Activity in *Escherichia coli*

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Parameters relating to RNA synthesis were measured after a temperature shift from 30 to 42°C, in a *relA*$^+$ and *relA*$^-$ isogenic pair of *Escherichia coli* strains containing a temperature-sensitive valyl tRNA synthetase. The following results were obtained: (i) the rRNA chain growth rate increased 2-fold in both strains; (ii) newly synthesized rRNA became unstable in both strains; (iii) the stable RNA gene activity (rRNA and tRNA, measured as stable RNA synthesis rate relative to the total instantaneous rate of RNA synthesis) decreased 1.7-fold in the *relA*$^+$ strain and increased 1.9-fold in the *relA* mutant; and (iv) the RNA polymerase activity (measured by the percentage of total RNA polymerase enzyme active in transcription at any instant) decreased from 20 to 3.6% in the *relA*$^+$ strain and remained unchanged (or increased at most to 22%) in the *relA* mutant. It is suggested that both rRNA gene activity and the RNA polymerase activity depend on the intracellular concentration of guanosine tetraphosphate, whereas the altered chain elongation rate and stability of rRNA are temperature or amino acid starvation effects, respectively, without involvement of *relA* function.

In exponentially growing *Escherichia coli* bacteria, only 20 to 30% of the RNA polymerase is engaged in RNA chain elongation at any given time; the exact value depends on the growth medium (11, 23, 35). It is not known why so much of the RNA polymerase is inactive. The RNA synthesis is not limited by the concentration of DNA, as indicated by measurements of RNA synthesis in an *E. coli* strain with reduced DNA concentration due to a mutation in a gene controlling the initiation of chromosome replication (Churchward, Young, and Bremer, J. Bacteriol., in press). In the current work, the RNA polymerase activity in *E. coli* was found to decrease sixfold when wild-type (*relA*$^+$) bacteria were starved for an amino acid, whereas in *relA*$^-$ bacteria under these conditions, the polymerase activity increased slightly by about 10%. Since the *relA*$^+$ gene product catalyzes synthesis of guanosine tetraphosphate (ppGpp) during amino acid starvation (9, 17), it seems likely that the control of RNA polymerase activity observed here reflects an effect of ppGpp.

In these experiments, all factors determining the rate of rRNA accumulation were measured; i.e., rRNA stability, RNA polymerase activity, rRNA chain elongation, and the distribution of active RNA polymerase enzyme over rRNA and nonribosomal genes. The results remove some of the discrepancies that have previously been thought to exist between the extent of in vitro and in vivo effects of ppGpp on the synthesis of rRNA.

MATERIALS AND METHODS

Bacterial strains and growth conditions used. *E. coli* strains NC51, NC52, and NC3 were used in the physiological experiments. NC51 and NC52 are sibling strains obtained by P1Kc transduction of *fuc*$^+$ from K12W6 *met relA* to NC32 [*valS*(Ts) *lac hsr*], NC51 was a *fuc*$^+$ *rel*$^+$ transductant, and NC52 was a *fuc*$^+$ *relA* transductant (31). NF955, used for the preparation of λ d*ilv5*, DNA was kindly provided to us by N. Fiil.

Cultures were grown in medium C (18) supplemented with either 0.2% succinic acid or glucose. Growth was monitored as the increase in optical density at 460 nm (OD_{460}). Cultures were started by a 1:250 dilution of a fresh overnight culture.

Determination of RNA and protein. The accumulation of RNA and protein was determined as described previously (35). Briefly, this procedure involves precipitating 5-ml samples of culture with 1.0 ml of 3 M trichloroacetic acid at 0°C and then collecting the cells on glass fiber filters. The filters were dried and added to scintillation vials along with 2 ml of 0.2 M NaOH to hydrolyze RNA. After overnight incubation at room temperature, a 0.5-ml sample was removed for determination of protein by the Lowry assay, which was calibrated with bovine serum albumin; 1.5 ml of 0.5 M perchloric acid was added to the remaining 1.5 ml of hydrolysate to precipitate DNA and protein, which were removed by membrane filtration. The absorbance of the filtrate (RNA nucleotides) at 260 nm (A_{260}) was then determined, and the amount of RNA nucleotides per OD_{460} of culture was calculated as described previously (35).

Measurement of tRNA synthesis. Transfer RNA accumulation was determined as described previously (33). Exponentially growing cultures (10 ml) in succi-

nate minimal medium were radioactively labeled with 5 μCi of [5-^{3}H]uridine (28 Ci/mmol) at an OD_{460} of 0.1. After two doublings, essentially all of the label was exhausted from the medium and chased into stable nucleic acids. When the ^{3}H-labeled culture reached an OD_{460} of 0.4, [2-^{14}C]uracil (0.1 μCi/ml; 58 mCi/mmol) was added, and the labeling at 30°C was continued for 15 min. At this time (zero time) a 1.0-ml sample was removed into ethanol-phenol stopping solution (7.5 ml of ethanol, 2.1 ml of 0.1 M sodium acetate [pH 7.5], 0.2 ml of phenol, 0.2 ml of disodium EDTA), and half of the culture was shifted to 42°C. Further samples were taken at various times after the shift from either culture, and the cells were harvested by centrifugation, suspended, and lysed in 1 ml of boiling electrophoresis sample solution (0.2× TEB [1× TEB is 0.9 M Tris, 90 mM disodium EDTA, 0.9 M boric acid], 1% sodium dodecyl sulfate, 10% glycerol, and 0.001% bromophenol blue); the samples were kept at 100°C for 45 s and then stored at room temperature. The small-molecular-weight RNA species were separated on a 12% acrylamide-bisacrylamide (19:1)–0.1% sodium dodecyl sulfate gel (10 cm long, 2 mm thick) with a 5% acrylamide-bisacrylamide (19:1) stacking gel (5 cm long). Sample lysates (100 μl) were added to each slot and electrophoresed at 10 V/cm for 6 to 7 h in 1× TEB–0.1% sodium dodecyl sulfate buffer. The RNA bands were located by fluorography as described by Bonner and Laskey (2). The gels were dehydrated by soaking in 600 ml of dimethyl sulfoxide for 1 h for three successive times. The gel was then soaked for 3 h in 120 ml of 20% (wt/wt) of the scintillation dye 2,5-diphenyloxazole in dimethyl sulfoxide. This solution was poured off, and the gel was soaked in distilled water for 1 h with several changes to remove the dimethyl sulfoxide. The gel was dried on Whatman 3mm filter paper and autoradiographed, i.e., fluorographed, for 6 days with Kodak RP-Royal X-matic film. The bands containing the 4S tRNA and 5S rRNA were sliced out of the gel and incubated in 1.0 ml of 0.2 M NaOH at 37°C overnight. Radioactivity in the hydrolyzed RNA was determined in a liquid scintillation counter after the addition of 10 ml of toluene-based scintillation fluid containing 15% (vol/vol) Bio-Solv BBS-3 (Beckman Instruments, Inc.)

To ascertain the stability of RNA made during amino acid starvation, 10 ml of the ^{3}H-labeled culture was shifted to 42°C at an OD_{460} of 0.4, and simultaneously 1.0 μCi of [2-^{14}C]uridine (58 mCi/mmol) was added. After 15 min, the culture received rifampicin (200 μg/ml). Samples (1 ml) were taken before and after the addition of rifampicin and treated as described above, except that the acrylamide concentration in the stacking gel was 3.5% rather than 5% and that, in addition to 4S and 5S RNA, also the 16S and 23S bands were cut out of the gel and analyzed.

Determination of the rRNA chain growth rate. An exponentially growing culture (25 ml) in succinate minimal medium at 30°C was labeled with 2.5 μCi of [2-^{14}C]uridine, beginning at an OD_{460} of 0.1, and further incubated until the OD_{460} was about 0.4. At this time half the culture was placed into a 42°C waterbath. Five minutes after the temperature shift 60 μCi of [5-^{3}H]uridine was added along the rifampicin to give a final concentration of 250 μg/ml. Samples (1 ml) were taken at various times and treated as described above. Immediately after the last sample point the experiment was repeated for the culture that remained at 30°C. The isotope ratio ^{3}H/^{14}C in tRNA was determined and was plotted as a function of time (see Fig. 6), and the results were evaluated as described previously (26, 34).

The NC51 strain was found to be partially rifampicin resistant. To stop all RNA chain initiation within less than 5 s, the concentration of rifampicin had to be raised to 1.25 mg/ml for this strain. The high rifampicin concentration used caused a reduction in the time of residual tRNA synthesis after the addition of rifampicin (Fig. 1). Since most of the tRNA labeled in these experiments comes from tRNA genes located at the end of the rRNA transcriptional units (1, 34), we suspect that the excessively high rifampicin concentration caused some premature termination of rRNA precursor chains. Although this made it impossible to determine an absolute value for the rRNA chain elongation rate in NC51, the twofold change in the rRNA chain elongation rate after a shift in the growth temperature from 30 to 42°C is assumed to be correct since the same twofold change was found for NC52 and NC3 (see Table 3). Since at 30°C the rates of stable RNA and of mRNA synthesis were the same in NC51 and NC52, there was no reason to assume different chain growth rates in these two strains.

Determination of the fractional synthesis rate of stable RNA (r_s/r_t). The fractional synthesis rate of stable RNA was determined by a modification of the method of Shepherd et al. (35). Bacteria were grown exponentially at 30°C to an OD_{460} of 0.5. At this time, 5 min before the temperature shift, a 0.5-ml sample was removed and added to 5.0 μCi of [5-^{3}H]uridine. For amino acid-starved NC51, 12.5 μCi was used. After 60 s, labeling was stopped by the addition of 0.5 ml of ice-cold ethanol-phenol stopping solution (see above). After 1 min on ice the cells were harvested by centrifugation (10,000 × g for 7 min) and suspended in 0.5 ml of ice-cold medium C. The cells were then lysed by the addition of 0.5 ml of boiling sodium dodecyl sulfate lysing medium (7), boiled for 45 s, then cooled to and stored at room temperature.

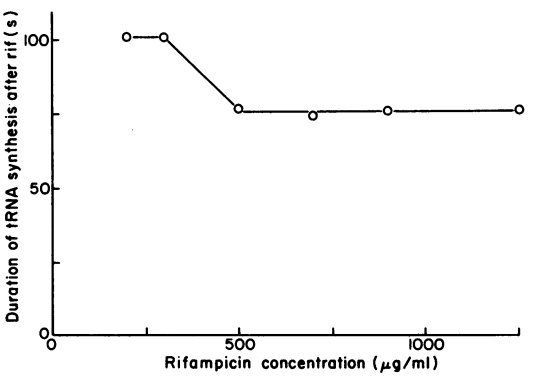

FIG. 1. Effect of high concentrations of rifampicin on the residual ("runout") tRNA synthesis in a culture of NC3 growing exponentially in succinate minimal medium at 30°C (experiment as in Fig. 6; see text for details). The reason for the drop in tRNA accumulation at very high concentrations of rifampicin is not understood.

The fraction of total RNA that was stable RNA (rRNA plus tRNA) was determined by RNA-DNA hybridization. Denatured λ *dilv5* DNA (10 μg per filter) was bound to nitrocellulose membrane filters (Bact-T-Flex; Schleicher and Schuell Co.; 0.45-μm pore size) after alkaline denaturation of the DNA as previously described (35). The DNA was prepared from λ *dilv5* phage (10) purified by two successive CsCl centrifugations (24). Nonspecific hybridization was measured by RNA retention to filters containing 10 μg of denatured DNA of phage T5.

Hybridization was carried out for 18 h at 67°C in vials containing 1.0 ml of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate 0.05 μg of ^{3}H-pulse-labeled RNA, and 0.005 μg of purified ^{14}C-labeled 16S and 23S rRNA (13). Each vial contained two λ *dilv5* filters and one T5 DNA filter. Further treatment was as described previously (35). The hybridization efficiency ranged from 60 to 80%, and hybridization to the T5 filter was indistinguishable from binding to blank filters.

RESULTS

RNA turnover during amino acid starvation. Amino acid starvation was induced by a temperature shift from 30 to 42°C in an isogenic pair of strains, *relA*$^{+}$ and *relA*$^{-}$, containing a temperature-sensitive valyl tRNA synthetase, growing in succinate minimal medium (for choice of medium, see below). After the shift, the accumulation of RNA (mainly rRNA) ceased in the *relA*$^{+}$ culture, whereas it was about twofold stimulated in the *relA*$^{-}$ culture (Fig. 2, Table 1). Using the accumulation of tRNA as a measure for the synthesis of rRNA (33) (Fig. 3 and 4; Table 2) it can be inferred that rRNA synthesis decreased to 22% of the preshift rate in the

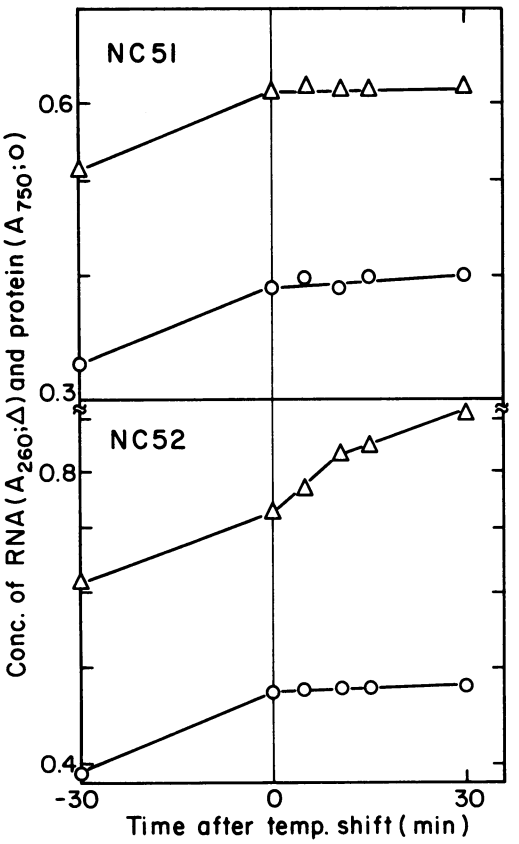

FIG. 2. Accumulation of RNA and protein in NC51 and NC52 growing exponentially in succinate minimal medium at 30°C and after a shift to 42°C (same as experiment 1 in Table 1). Symbols: △, RNA; ○, protein.

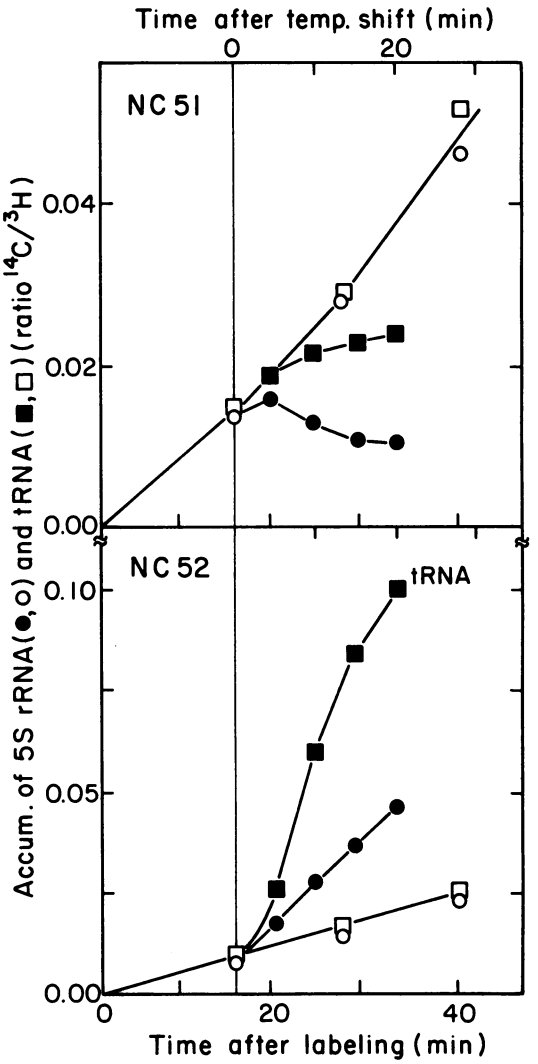

FIG. 3. Accumulation of tRNA (■, □) and 5S rRNA (●, ○) in NC51 and NC52 at 30°C (□, ○) or after a shift to 42°C (■, ●). The bottom abscissa uses an expanded time scale to correct for the growth of the culture at 30°C (only for open symbols); the top abscissa is linear (assuming no growth at 42°C; only for closed symbols). See experiment no. 1 of Table 2 for further details.

TABLE 1. Stable RNA accumulation in NC51 and NC52 growing in succinate minimal medium at 30°C and after a temperature shift to 42°C

Strain	Expt no.	Doubling time at 30°C[a] (min)	Mass concn[b] (OD_{460})	Total RNA[c] (A_{260}/OD_{460})	Stable RNA rate at 30°C[d] (nucleotides/s/OD_{460} unit)	Relative rate at 42°C[e]
NC51 *rel*$^+$	1	147	0.33	1.1	4.7×10^{12}	0
	2	135	0.32	1.1	5.2×10^{12}	0
	3	126	0.49	1.0	5.0×10^{12}	0
NC52 *rel*$^-$	1	139	0.56	1.2	5.5×10^{12}	2.1
	2	160	0.31	1.3	5.2×10^{12}	
	3	135	0.45	1.0	4.7×10^{12}	1.8
	4	114	0.46	1.0	5.6×10^{12}	2.1

[a] Culture doubling time at 30°C, determined from the increase in cell mass; growth was exponential for at least 1.5 doublings before RNA samples were taken.

[b] Mass concentration (OD_{460}) of culture at which first samples for RNA determination were taken (30 min before temperature shift).

[c] Total RNA measured as A_{260} units of RNA hydrolysate (at acid pH) per OD_{460} unit of culture; average from four samples, two which were taken at the OD_{460} indicated and two which were taken 30 min later.

[d] Rate of stable RNA accumulation in nucleotide residues per second per OD_{460} unit of culture, using the formula $(dR_s/dt)/OD_{460} = (A_{260}/OD_{460}) \times \varepsilon^{-1} \times 0.97 \times (\ln 2/\tau)$, where ε is the molar extinction coefficient of *E. coli* stable RNA at pH2 ($1/\varepsilon = 5.6 \times 10^{16}$ RNA nucleotides per A_{260} unit); 0.97 is the fraction of total RNA that is stable RNA (assuming 3% of total RNA is mRNA), and τ is the doubling time in seconds at 30°C.

[e] Relative rate of stable RNA accumulation at 42°C; preshift rate set at 1.0.

relA$^+$ strain and was stimulated five- to sevenfold in the *relA*$^-$ strain. The difference between synthesis and accumulation in both strains reflects the instability of rRNA made in the absence of protein synthesis.

The turnover of rRNA in the strains NC51 and NC52 was measured more directly by first labeling RNA for 15 min with [^{14}C]uridine and then adding rifampicin to inhibit all further synthesis of RNA (Fig. 5). Unstable RNA made during the first 15 min before rifampicin will become acid soluble under these conditions. The data in Fig. 5 show that tRNA remained stable under any conditions; also rRNA made at 30°C remained stable in both NC51 and NC52, but rRNA made at 42°C was degraded in either strain. The experiments of Fig. 5 show three curves, for the specific radioactivity of tRNA, 5S rRNA, and 16S plus 23S rRNA; the last curve included mRNA and nascent rRNA in the same molecular weight range and probably some labeled DNA that entered the electrophoresis gel. (There is an experimental difficulty in separating 4S and 5S RNA, which requires a hard gel, and simultaneously separating 16S and 23S from the origin [containing most of the DNA], which requires a very soft gel). Mainly because of the contamination with mRNA, the 16S plus 23S rRNA curve increased initially with a greater slope than the 4S and 5S curve; this reflects the fact that any unstable compound, like mRNA, is pulse-labeled with a higher specific activity than stable compounds (since the denominator in the ratio activity/amount [specific activity] is reduced by turnover). Most of this excess specific activity in the initial (16S plus 23S) curve disappeared after the addition of rifampicin due to the degradation of mRNA. At 30°C, however, there remained an excess (about 15%) even 30 min after the addition of rifampicin. This is assumed to reflect contamination by radioactive DNA. ([^{14}C]uridine labels DNA with a higher specific activity than [^{3}H]uridine in the prelabel because ^{3}H in [5-^{3}H]uridine is removed during thymidylate synthesis.) These complications were absent for 5S rRNA. The fact that at 30°C 5S rRNA and tRNA were initially labeled with the same specific radioactivity suggests that 5S rRNA and tRNA are made in the same relative proportions before and after the temperature shift and that 5S rRNA synthesized during exponential growth in succinate medium is essentially stable. Since the three species of rRNA (5S, 16S, and 23S) are contranscribed, all three species must be initially labeled with the same specific activity, i.e., 5S rRNA is representative for all rRNA. At 42°C the final specific activity of rRNA was only 30% of the specific activity of tRNA, indicating that 70% of the rRNA synthesized from 0 to 15 min after the shift to 42°C is degraded, independent of *relA* function.

The result that rRNA synthesized during exponential growth is essentially stable is in contrast to a report by Gausing (16), who estimated the turnover of "stable" RNA by measuring the difference "instantaneous rate of rRNA synthesis minus rate of accumulation of rRNA" and inferred that about 20% of the rRNA made

TABLE 2. Change in the rate of tRNA accumulation after a temperature shift from 30 to 42°C (t = 0) in NC51 and NC52 growing in succinate minimal medium

Strain	Expt no.[a]	Radioactive tRNA[b]													Ratio of slopes[d] (Avg)
		30°C (unshifted portion)							42°C (shifted portion)						
		t = 0 min			t = 25 min				t = 10 min			t = 20 min			
		^{14}C cpm	^{3}H cpm	$^{14}C/^{3}H$	^{14}C cpm	^{3}H cpm	$^{14}C/^{3}H$	Slope[c]	^{14}C cpm	^{3}H cpm	$^{14}C/^{3}H$	^{14}C cpm	^{3}H cpm	$^{14}C/^{3}H$	Slope[c]	
NC51 *rel*$^{+}$	1	358	27,977	0.0128	716	15,372	0.0466	0.00135	648	30,706	0.0211	751	31,581	0.0238	0.00027	0.20 } (0.22)
	2	443	32,850	0.0135	944	18,753	0.0503	0.00147	593	25,505	0.0232	780	29,381	0.0266	0.00034	0.23 }
NC52 *rel*$^{-}$	1	214	26,800	0.00799	380	14,076	0.0270	0.00076	851	14,449	0.0589	2,287	21,408	0.107	0.0048	6.3 }
	2	546	61,435	0.00889	1,232	22,481	0.0548	0.00184	5,561	57,482	0.0967	10,018	53,885	0.186	0.0089	4.9 } (5.2)
	3	439	53,449	0.00821	1,514	26,575	0.0570	0.00195	6,170	57,187	0.1079	10,682	55,705	0.192	0.0084	4.3 }

[a] Experiments no. 1 same as experiments in Fig. 3 and curve ○ of Fig. 4; experiments no. 2 and 3 same as curves △ and □ in Fig. 4.

[b] Cultures were prelabeled with [^{3}H]uridine (between A_{460} of 0.1 and 0.2) and labeled again with [^{14}C]uridine 15 min before the temperature shift at A_{460} = 0.4. At the times indicated samples were taken, and the ^{3}H and ^{14}C radioactivity in tRNA was determined as described in the text.

[c] Slope is the relative rate of tRNA accumulation: $[(^{14}C/^{3}H)_2 - (^{14}C/^{3}H)_1]/(t_2 - t_1)$. The subscripts refer to the two time points, 25 and 0 min at 30°C and 20 and 10 min at 42°, respectively.

[d] Ratio of slopes (slope at 42°C/slope at 30°C) corresponds to the *n*-fold change in the rate of tRNA (and thus also of rRNA) synthesis induced by the temperature shift.

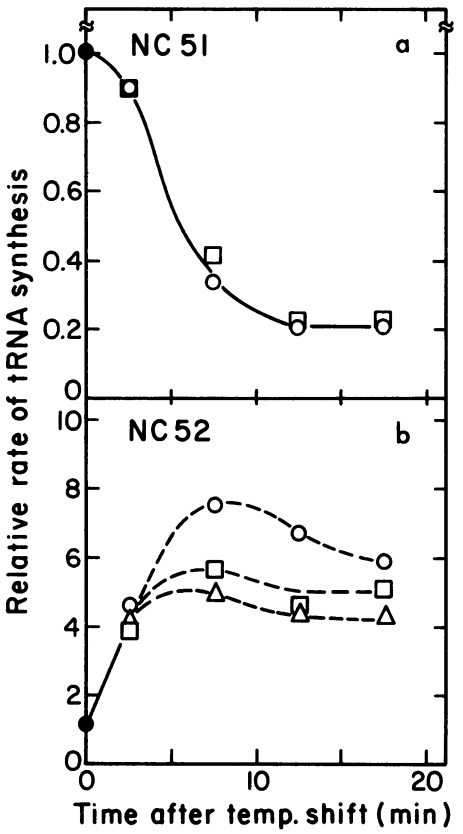

FIG. 4. Rate of tRNA synthesis in NC51 and NC52 after a temperature shift from 30 to 42°C. The points represent average rates in the time intervals 0 to 5, 5 to 10, 10 to 15, and 15 to 20 min, plotted over the interval midpoints and normalized to the zero time rate at 30°C. Symbols: ●, reference (preshift) value set at 1.0; ○, △, □, three different experiments for NC52, two experiments for NC51; ○, experiments of Fig. 3. See Table 2 for further details.

during exponential growth, at the growth rate of 0.4 doublings per h, is unstable.

Temperature dependence of rRNA chain growth. The temperature shift produced a twofold increase in the rate of rRNA chain elongation in both *relA*$^+$ and *relA*$^-$ strains (Fig. 6; Table 3). Thus, about half of the temperature-induced stimulation of rRNA and tRNA synthesis in the *relA*$^-$ strain (Fig. 4b) was due to an increased chain growth rate, whereas the remaining (about threefold) stimulation must be due to an increased fraction of RNA polymerase synthesizing stable RNA. Conversely, the reduction in rRNA and tRNA synthesis in the *relA*$^+$ strain due to the temperature shift (Fig. 4a) must have resulted from an even greater reduction in the fraction of polymerase synthesizing stable RNA, partly compensated by increased chain growth.

The rRNA chain growth rates in NC51 appeared to be about 30% higher than in NC52 (Table 3). Most likely this reflects an artifact of the method due to the extremely high concentration of rifampicin that had to be used to quickly stop RNA synthesis in NC51 (Fig. 1; see above).

Changes in rRNA and tRNA gene activity. The fraction (r_s/r_t) of the total instantaneous rate of RNA synthesis that corresponds to rRNA and tRNA synthesis (including spacers in the rRNA and tRNA precursors) was used to measure stable RNA gene activity. This parameter is independent of nonspecific variations in the RNA polymerase activity and in the RNA chain elongation rate and therefore reflects mainly the control specific for rRNA and tRNA genes. This fraction was determined from radioactively pulse-labeled RNA by measuring the label in rRNA and correcting for the synthesis of tRNA (footnote *g* of Table 4). At 30°C, for NC51 and NC52, 35% (observed range, 31 to 37%) of the total RNA synthesized at any instant was stable RNA (Table 4). After a shift to 42°C, this fraction increased from 35 to 65% (range, 64 to 66%) in the *relA*$^-$ strain and decreased to 21% (range 20 to 22%) in the *relA*$^+$ strain (Table 4). The value of 21% appears to be a minimum, since amino acid starvation of NC51 growing in glucose amino acids medium produced the same value (Fig. 7).

Changes in RNA polymerase activity. By combining the data from Fig. 2 through 7 and Tables 1 through 4, the number of growing RNA chains (per cell mass) was calculated for *relA*$^+$ and *relA*$^-$ bacteria before and after amino acid starvation (Table 5). The results indicate an inactivation of RNA polymerase as a result of amino acid starvation in the *relA*$^+$ strain and a slight stimulation of the polymerase activity in the *relA*$^-$ strain. By using measurements of total RNA polymerase enzyme (from reference 35), the absolute polymerase activities were estimated to vary between a minimum of 3.6% (starvation of *relA*$^+$) and a maximum of 22% (percentage of total enzyme active in transcription; starvation of *relA*$^-$), corresponding to sixfold differences in enzyme activity.

DISCUSSION

Amino acid starvation versus temperature effects. Since amino acid starvation was brought about by heat inactivation of valyl-tRNA synthetase, all observed changes in RNA accumulation were due to either the temperature shift alone, starvation alone, or both. The twofold increase in the rRNA chain elongation rate after a temperature shift from 30 to 42°C was independent of the *relA*$^+$ or *relA*$^-$ allele and independent of the temperature sensitivity of valyl-

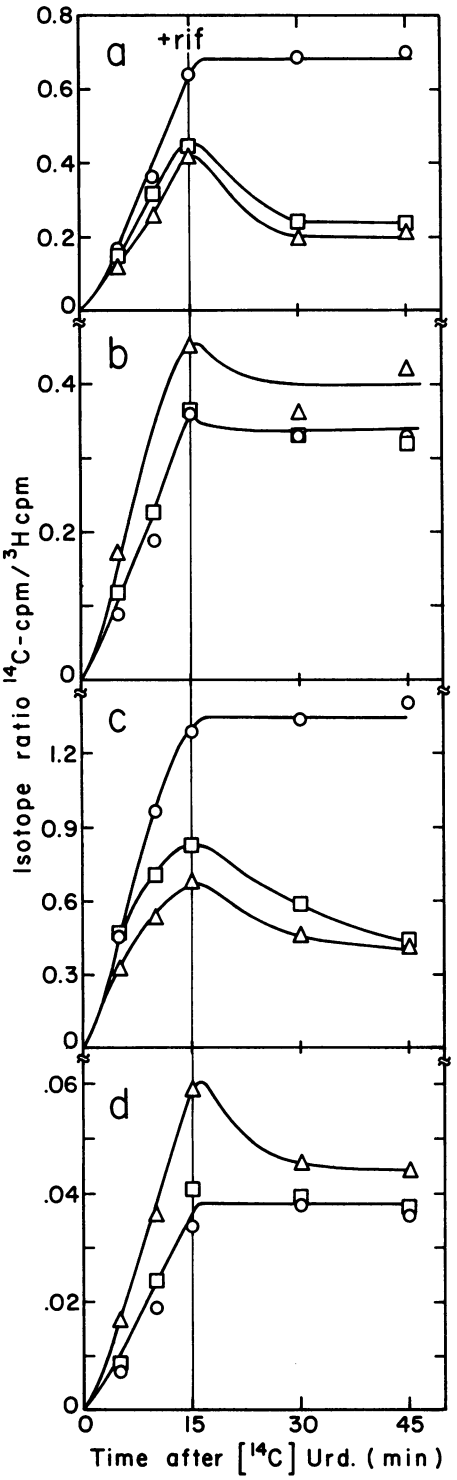

FIG. 5. Stability of newly synthesized tRNA (□) and 5S rRNA (○) and 16S plus 23S rRNA (△) in NC51 (*relA*$^+$; panels a and b) and NC52 (*relA*$^-$; panels c and d) at 30°C (panels b and d) and 42°C (panels a and c). The $^{14}C/^{3}H$ isotope ratio is a measure for the specific radioactivity of the RNA; ^{3}H is a prelabel, corresponding to RNA synthesized before the shift and which remains stable after the shift; ^{14}C is RNA labeled from zero time, the time of the temperature shift 30 to 42°C (only panels a and c).

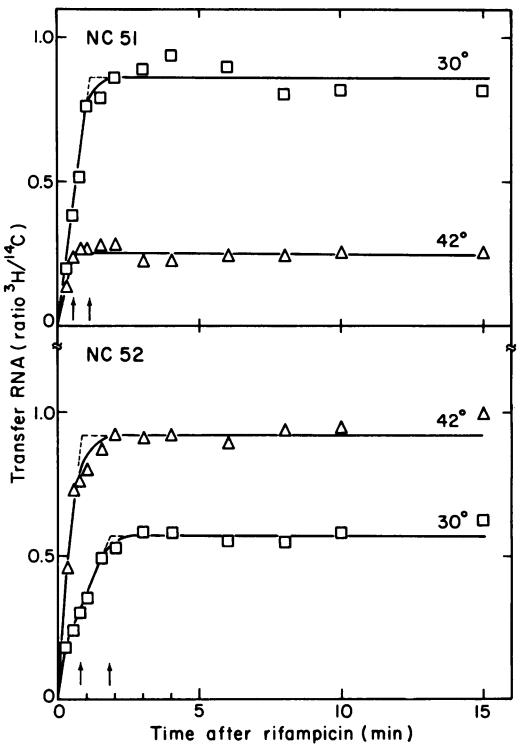

FIG. 6. Accumulation of tRNA in a rifampicin runout experiment for NC51 and NC52 at 30°C (□) and 42°C (△) (experiment no. 1 of Table 3; see text for details). The arrows indicate the estimated times of the break in the kinetics, assumed to correspond to the time it takes an RNA polymerase molecule to transcribe 6,300 nucleotides of an rRNA precursor from its 5′ beginning to the section near the 3′ terminus containing two tRNA molecules. The shorter runout times observed with NC51 are assumed to be an artifact due to the extremely high rifampicin concentration that had to be used to instantaneously stop RNA synthesis in this strain (see text and Fig. 1).

tRNA synthetase (Table 3). Therefore it is presumed that the chain elongation effect was due to temperature alone and that amino acid starvation, if brought about without a change in temperature, would not affect the RNA chain elongation rate. This conclusion is consistent with a previous finding that a chloramphenicol-induced stimulation of RNA synthesis in *E. coli* at 37°C (constant temperature) did not involve a change in the RNA chain growth rate (34). This also implies that amino acid starvation alone (without a temperature shift) would cause a

TABLE 3. rRNA chain growth rate in NC51 [*relA*$^+$ *valS*(Ts)], NC52 [*relA*$^-$ *valS*(Ts)], and NC3 (*relA*$^+$ *valS*$^+$) at 30 and 42°C

Strain	Expt no.	Concn of rifampicin (μg/ml)	Duration of tRNA synthesis[a]				Chain growth rate[b] (nucleotides/s)	
			30°C		42°C		30°C	42°C
			s	Avg	s	Avg		
NC51 *rel*$^+$	1	1,250[c]	66	64	30	31	73[d]	151[d]
	2	1,250	62		32			
NC52 *rel*$^-$	1	250	115	111	48	49	56	127
	2	250	108		50			
NC3 *rel*$^+$	1	250	115	113	52	51	55	122
	2	250	111		50			

[a] Duration of tRNA synthesis after the addition of rifampicin to a culture growing in succinate minimal medium; see Fig. 6 for details.

[b] Chain growth rate is 6,250 nucleotides per rRNA precursor per duration of tRNA synthesis after rifampicin.

[c] For NC51 the rifampicin concentration had to be increased to 1,250 μg/ml because of partial rifampicin resistance of this strain (see text).

[d] The value given for NC51 has been corrected for the higher concentration of rifampicin by multiplication with 0.75 (see text and Fig. 1). The corrected value may still be an overestimate (see text).

twofold greater reduction in the stable RNA synthesis rate in the *relA*$^+$ strain than observed in these experiments (Fig. 4; 0.11-fold instead of 0.22-fold) or a 2-fold lesser stimulation of stable RNA synthesis in the *relA*$^-$ strain (Table 2; 2.6-fold instead of 5.2-fold).

The instability of rRNA (Fig. 5) is likely to have resulted from amino acid starvation alone, i.e., from the lack of ribosomal protein to protect newly synthesized rRNA. Likewise, the changes in RNA polymerase activity and in stable RNA gene activity, which depend on the function of the *relA* gene product, are assumed to be mainly starvation rather than temperature effects, because at 30°C, the number of nascent RNA chains in NC51 and NC52 was the same (Table 5; 4.2×10^{11} chains per OD_{460} unit of culture) as was previously observed for wild-type *E. coli* B/r growing at 37°C in the same (succinate minimal) medium (Fig. 5 of reference 35; 400 nascent RNA chains per cell and 1 OD_{460} unit per 10^9 cells, corresponding to 4.0×10^{11} chains per OD_{460} unit). Further, a temperature shift from 30 to 42°C in a *valS*$^+$ *relA*$^+$ strain did not cause a change in the relative rate of stable RNA synthesis (r_s/r_t, observed in succiniate medium; data not shown).

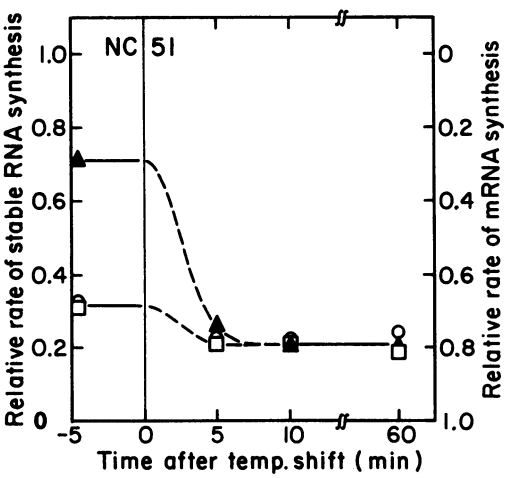

FIG. 7. Changes in the relative rate of stable RNA synthesis in NC51 after a temperature shift from 30 to 42°C. Cultures were labeled in succinate minimal medium (○, □) or glucose medium supplemented with Casamino Acids (▲). The cultures were pulse-labeled at the times indicated, and the relative rate of stable RNA synthesis (r_s/r_t, fraction of the instantaneous rate of RNA synthesis that is rRNA and tRNA, including unstable spacers in the rRNA and tRNA precursors) was determined as described in the text. The complement fraction ($1 - r_s/r_t$; right ordinate) is the relative rate of mRNA synthesis.

Comparison of chloramphenicol and amino acid starvation effects. Chloramphenicol produced an activation of RNA polymerase in wild-type *E. coli* B/r growing at 37°C, and this activation was greatest (twofold) for bacteria growing in succinate minimal medium (33). This was the reason for the use of succinate minimal medium in the current experiments, i.e., to maximize effects which were presumed to be small. With the *relA*$^-$ strain we observed only 10% (rather than twofold) stimulation of RNA polymerase activity (Table 5). The difference might be due to either different temperature (42°C rather than 37°C) or differences in the intracellular concentration of ppGpp; i.e., chloramphenicol may reduce the level of ppGpp more than amino acid starvation of a *relA*$^-$ strain (20, 33). The latter

TABLE 4. Relative rate of stable RNA synthesis in NC51 (*rel*$^+$) and NC52 (*rel*$^-$) before and after a temperature shift from 30 to 42°C

Strain	Expt no.	Before temperature shift[a]									After temperature shift[b]								
		^{3}H cpm[c]		^{14}C cpm[d]		Total cpm[e]		l_r/l_t[f]	r_s/r_t[g]	r_s/r_t avg	^{3}H cpm[c]		^{14}C cpm[d]		Total cpm[e]		l_r/l_t[f]	r_s/r_t[g]	r_s/r_t avg
		λ *ilv*	T5	λ *dilv5*	T5	^{3}H	^{14}C				λ *ilv*	T5	λ *dilv5*	T5	^{3}H	^{14}C			
NC51 *rel*$^+$	1	996	73	271	0	4,858	373	0.24	0.31	0.32	382	43	280	0	2,385	373	0.17	0.22	0.22
		1,037	73	274	0			0.25	0.32		323	35	254	0			0.16	0.21	
		990	50	261	0			0.26	0.34		336	43	248	0			0.16	0.21	
		946	79	255	0			0.24	0.31		366	29	259	1			0.19	0.25	
	2	851	59	285	0	4,218	382	0.23	0.30	0.31	302	23	273	0	2,150	382	0.17	0.22	0.21
		811	71	280	0			0.22	0.29		274	24	262	0			0.15	0.20	
		946	56	294	0			0.26	0.34		305	20	280	0			0.17	0.22	
		873	79	281	0			0.23	0.30		333	58	250	2			0.16	0.21	
	3	1,469	70	252	0	6,655	360	0.29	0.37	0.37	205	22	196	0	2,069	360	0.14	0.18	0.20
		1,542	49	275	0			0.29	0.37		188	21	184	0			0.14	0.18	
		1,535	55	262	0			0.29	0.37		219	14	211	0			0.16	0.21	
		1,427	52	251	0			0.28	0.36		228	13	205	0			0.17	0.22	
	4	1,382	25	264	0	5,956	358	0.30	0.38	0.37	294	11	268	0	2,349	358	0.15	0.20	0.20
		1,281	17	250	0			0.30	0.38		267	14	249	0			0.15	0.20	
		1,281	20	287	0			0.26	0.34		286	16	272	0			0.14	0.18	
		1,352	26	264	0			0.29	0.37		332	26	285	0			0.15	0.20	
NC52 *rel*$^-$	1	1,327	106	245	0	5,687	360	0.29	0.37	0.37	3,815	88	247	0	10,162	360	0.52	0.64	0.66
		1,299	113	245	0			0.28	0.36		3,834	98	241	0			0.53	0.65	
		1,199	86	225	0			0.29	0.37		4,109	70	246	0			0.57	0.70	
		1,321	95	247	0			0.29	0.37		3,644	80	235	0			0.53	0.65	
	2	920	20	254	0	4,596	360	0.27	0.35	0.34	3,183	151	187	0	11,361	360	0.49	0.61	0.64
		816	18	229	0			0.27	0.35		4,493	156	241	0			0.55	0.67	
		883	23	266	0			0.25	0.32										
		845	12	240	0			0.27	0.35										
	3	952	12	210	0	5,649	360	0.28	0.36	0.36	4,456	71	232	0	11,637	360	0.58	0.71	0.66
		1,049	18	227	0			0.28	0.36		4,535	76	270	0			0.50	0.62	
		1,261	15	268	0			0.29	0.37		4,398	62	237	0			0.56	0.68	
		1,078	8	239	0			0.28	0.36		4,286	95	249	0			0.51	0.63	

[a] One milliliter of culture growing exponentially at 30°C in succinate minimal medium was pulse-labeled with [^{3}H]uridine for 1 min at an A_{460} of 0.4 to 0.5.

[b] Pulse-labeling (see footnote *a*) was done 10 min after the culture was shifted to 42°C.

[c] ^{3}H cpm, Pulse-labeled RNA bound to filters with either λ *dilv*15 DNA (hybridized RNA, sum of two filters per vial) or T5 DNA (background, one filter per vial). All radioactivity values are corrected for spillover and background.

[d] ^{14}C cpm, Purified [^{14}C]rRNA added to the hybridization mixture to determine the efficiency of hybridization for rRNA.

[e] Total trichloroacetic acid-precipitable radioactivity per vial.

[f] l_r/l_t, Fraction of total labeled RNA that is rRNA: [(^{3}H/^{14}C) hybridized]/[^{3}H/^{14}C) total].

[g] r_s/r_t, Relative rate of stable RNA synthesis calculated from l_r/l_t by using the formula $r_s/r_t = \{(1 + [0.86/(l_r/l_t) - 1] \times 0.86)\}^{-1}$. This calculation corrects for the presence of tRNA (assumed to be 14% of total stable RNA) and the different mole fractions of pyrimidines in stable and mRNA (0.43 and 0.50, respectively). The synthesis of stable RNA, r_s, includes the synthesis of unstable spacer in the rRNA precursor (1).

TABLE 5. Change in RNA polymerase activity in NC51 and NC52 after a temperature shift from 30 to 42°C[a]

Strain	Temp (°C)	Stable RNA synthesis		mRNA synthesis		Total RNA synthesis[b]		Functioning RNA polymerase[c]	
		Nucleotides/ s/OD_{460} unit[d]	No. of nascent chains/ OD_{460} unit[e]	Nucleotides/ s/OD_{460} unit[f]	No. of nascent chains/ OD_{460} unit[g]	Nucleotides/ s/OD_{460} unit	No. of nascent chains/ OD_{460} unit	Relative (%)	Absolute (%)
NC51 *rel*$^+$	30	6.2×10^{12}	1.1×10^{11}	1.1×10^{13}	3.1×10^{11}	1.8×10^{13}	4.2×10^{11}	100	20
	42	1.4×10^{12}	1.1×10^{10}	5.3×10^{12}	6.4×10^{10}	6.7×10^{12}	7.5×10^{10}	18	3.6
NC52 *rel*$^-$	30	6.2×10^{12}	1.1×10^{11}	1.1×10^{13}	3.1×10^{11}	1.8×10^{13}	4.2×10^{11}	100	20
	42	3.2×10^{13}	2.6×10^{11}	1.7×10^{13}	2.0×10^{11}	4.9×10^{13}	4.6×10^{11}	110	22

[a] Evaluation of data from Tables 1 through 4.

[b] Total RNA synthesis is the sum of the corresponding stable RNA and mRNA values.

[c] The relative percent is the total number of nascent RNA chains at 30°C, set at 100%. For the absolute percent, in succinate medium the number of core RNA polymerase enzyme molecules is 5 times greater than the number of nascent RNA chains (35), i.e., at 30°C only 20% of the RNA polymerase was assumed to be active (see text).

[d] Stable RNA synthesis rate in nucleotide residues per second per OD_{460} unit of culture. At 30°C, the average rate of stable RNA accumulation from both NC51 and NC52 was 5.2×10^{12} nucleotide residues per s per A_{460} unit (Table 1). This value was multiplied by 1.2 to take into account unstable spacers in the stable RNA precursors (rRNA precursor, 6,295 nucleotides [1]; this includes six mature stable RNA species [23S, 16S, and 5S rRNA, three tRNAs] whose combined weights are about 5,300 nucleotides [1]. For 42°C values, the 30°C value was multiplied by 0.22 (NC51) or 5.2 (NC52) (from Table 2, change in tRNA accumulation after a temperature shift).

[e] Number of nascent stable RNA chains per OD_{460} unit of culture obtained from the stable RNA synthesis rate by division by the chain elongation rate, assumed to be 56 and 125 nucleotide residues per s per chain at 30 and 42°C, respectively (Table 2, average from NC52 and NC3). The same values were assumed for NC51 (see text).

[f] mRNA synthesis rate in nucleotide residues per second OD_{460} unit of culture, obtained from the stable RNA synthesis rate by multiplication with $\{[1/(r_s/r_t)] - 1\}$; r_s/r_t was 0.35 for NC51 and NC52 at 30°C, 0.21 for NC51 at 42°C, and 0.65 for NC52 at 42°C (Table 4).

[g] Number of nascent mRNA chains calculated analogously as the number of stable RNA chains (footnote *b* above) assuming mRNA chain growth rates of 37 and 83 nucleotide residues per s per chain at 30 and 42°C, respectively, i.e., two-thirds of the stable RNA chain growth rates.

idea is consistent with the observation that r_s/r_t, assumed to be a function of the level of ppGpp, was also increased more by chloramphenicol (Table 1 of reference 34; from 0.35 to 0.84) than by starvation (Table 4; from 0.35 to 0.65).

relA-dependent changes in stable RNA gene activity. The fraction r_s/r_t is independent of nonspecific changes in the RNA chain elongation rate or RNA polymerase activity and is therefore used here as a measure for the stable RNA gene activities. rRNA and tRNA genes are assumed to be coregulated (33).

For both NC51 and NC52 growing at 30°C in succinate minimal medium, 35% of the instantaneous rate of RNA synthesis was found to be stable RNA (rRNA and tRNA; Table 4; r_s/r_t, 0.31 to 0.37). Since the stable RNA chain growth rate is about 50% greater than the mRNA chain growth rate, the fraction of total functioning RNA polymerase molecules engaged in stable RNA synthesis (which we denote by the symbol ψ_s) is somewhat less than 35% (i.e., 27%; for conversion see reference 4).

In the *relA*$^-$ strain, amino acid starvation produced a 1.8-fold increase in r_s/r_t (Table 4; from 0.35 [ψ_s = 0.27] to 0.65 [ψ_s = 0.55]). Qualitatively, this is consistent with the observed increase in stable RNA accumulation (Fig. 4) and with the expectation from the reported decrease in the level of ppGpp in amino acid-starved *relA* strains (20). This result removes some of the apparent discrepancies between in vivo and in vitro ppGpp effects: in vitro, investigators have reported only "rather weak preferential effects, on the order of a factor of two, which are too small to account fully for the stringent control of ribosomal RNA synthesis in vivo" (quoted from reference 15). The differences in stable RNA gene activities observed here, which were less than twofold, are consistent with the extent of rRNA gene control observed in vitro (37, 40). Thus, the much larger differences in stable RNA accumulation seen in NC51 and NC52 after amino acid starvation were only partly due to differences in stable RNA gene control.

relA-dependent RNA polymerase activity. The RNA polymerase activity, as defined here, refers to the fraction of total enzyme that is active in transcription at any given instant rather than

to the instantaneous reaction rate catalyzed by the enzyme. (The latter depends on the RNA chain elongation rate which is a function of temperature and is irrelevant for the activity in the sense as this term is used here.) The about sixfold differences in RNA polymerase activity (Table 5) were found to depend on amino acid starvation and on *relA* function. It is therefore likely that the RNA polymerase activity is controlled by the intracellular concentration of ppGpp. Evidently, the differences in RNA polymerase activity do not parallel the changes in the stable RNA gene activity (r_s/r_t), which did not exceed a factor of two. In vitro RNA synthesis catalyzed by purified *E. coli* RNA polymerase is also inhibited by ppGpp (37, 40), although to a lesser extent than observed here in vivo.

In connection with the control of RNA polymerase activity, two questions arise. (i) Why is so much (Table 5, 78 to 96%) RNA polymerase inactive? (ii) How might ppGpp control RNA polymerase activity?

In regard to the first question, many instances of in vitro RNA polymerase inactivity have been reported that would also be plausible causes for in vivo inactivity; for example, RNA polymerase is inactivated by binding to tRNA (6, 36); the bound tRNA may be exchanged by another tRNA molecule (5); RNA polymerase that is active on superhelical DNA may become inactive by binding to relaxed DNA of the same kind (32); some RNA polymerase ("early quitter" polymerase) is active for only a few minutes (28); this polymerase does not reinitiate in "high-salt reaction mixtures" (3) when other RNA polymerase molecules are capable of continuously terminating and re-initiating RNA chains for several hours (22, 25). Finally, core RNA polymerase is known to bind non-specifically to DNA and to dissociate slowly (19, 27), such that under conditions of fast chain growth (i.e., in vivo) a substantial fraction of the core RNA polymerase molecules might be expected to be non-specifically bound to DNA before binding to sigma factor and initiating an RNA chain. The inactivity of RNA polymerase observed in vivo might be related to any one or a combination of these phenomena observed in vitro.

The two effects of *relA* function observed here, one on the stable RNA gene activity (r_s/r_t) and the other on the activity of RNA polymerase, might reflect two different mechanisms. It has been suggested that RNA polymerase has a binding site for ppGpp, perhaps involving the σ-subunit (39), that controls the conformation of the enzyme (12) and thereby its promoter specificity (37, 38). This mechanism might be responsible for the observed changes in r_s/r_t (Table 4). Cashel (8) found that ppGpp inhibits in vitro synthesis of RNA chains starting with pppG, presumably by binding to the site for the chain-initiating nucleotide. It is conceivable that this effect is responsible for the in vivo changes in RNA polymerase activity observed during amino acid starvation of the *relA*$^+$ strain.

Comparison with previous results. Winslow and Lazzarini (41) reported that the number of nascent RNA chains was unaffected by amino acid starvation in both *rel*$^+$ and *rel*$^-$ strains, and that the starvation-induced changes in RNA accumulation were due to changes in the RNA chain elongation rate. These conclusions are contrary to our findings (Table 5). Lazzarini and Dahlberg (21) reported an about threefold reduction in the instantaneous (total) rate of RNA synthesis upon amino acid starvation of a *rel*$^+$ strain. This appears to be similar to our observation of a 2.7-fold reduction (Table 5; NC51, 1.8×10^{13} to 6.7×10^{12} nucleotides per s per OD_{460} unit of culture); however, if one takes into account the twofold increase in the RNA chain elongation rate due to the increased temperature in our experiments, then the reduction in RNA synthesis due to starvation is about 5-fold in our experiments. A threefold reduction including the temperature effect was also found by Nierlich (29), using guanosine rather than uridine labeling.

Pederson (30) observed a decrease in r_s/r_t during amino acid starvation of an *rel*$^+$ strain from 0.45 to 0.09, in qualitative agreement with our data. However, the value of 0.09 is below our 21% minimum. The difference may reflect that his data were not corrected to include tRNA and his cells were pulse-labeled after concentration and storage on ice for 75 min.

A turnover of nascent rRNA during amino acid starvation has been inferred earlier by Donini (14). The experiments presented here (Fig. 5) support Donini's proposition. Nierlich (29) observed that 90% of the RNA synthesized during starvation of an *rel*$^+$ strain was unstable, in agreement with our data, but with his technique he was not able to distinguish between unstable mRNA and unstable rRNA.

By including in our study measurements of rRNA chain elongation rates and of the absolute rate of rRNA synthesis, we were able to analyze the stringent and relaxed response in greater detail than has been possible in the past; in particular we were able to assess changes in the total RNA polymerase activity which had not been previously evaluated.

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