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Transcription of Ribosomal Component Genes and *lac* in a *relA*$^+$/*relA* Pair of *Escherichia coli* Strains

R. LITTLE† AND H. BREMER*

Biology Programs, The University of Texas at Dallas, Richardson, Texas 75080

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To determine the stringent response, a repression of gene activity during amino acid starvation assumed to be mediated by the effector necleotide guanosine tetraphosphate (ppGpp), of metabolically regulated constitutive genes, we measured the transcription of ribosomal protein genes, the constitutive *lac* operon, and stable RNA genes in a variety of growth media and after amino acid starvation in a *relA*$^+$/*relA* pair of *Escherichia coli* B/r strains. For rRNA and tRNA (stable RNA) it has previously been shown that the distinction between stringent control and growth rate control is unfounded, as the function describing the stable RNA gene activities at different concentrations of guanosine tetraphosphate is independent of growth conditions (exponential growth or amino acid starvation) and of the *relA* allele present. Here, the results indicated that the stringent responses of ribosomal protein genes and *lac* differ from their metabolic control during exponential growth in different media. This can be explained by polarity and RNA polymerase sink effects during amino acid starvation which are irrelevant for stable RNA genes but which are superimposed on mRNA gene activities.

In *Escherichia coli* the relationship between stable RNA (rRNA, tRNA) gene activity, measured as the rate of stable RNA synthesis relative to the rate of total RNA synthesis (r_s/r_t), and the intracellular concentration of guanosine tetraphosphate (ppGpp) can be described by a single function which applies to all growth media and to exponential growth or amino acid starvation conditions and which is independent of the *relA* allele (35). The single-function relationship implies that the conventional distinction between stringent control and growth rate control of ribosome synthesis (13, 21, 28) is unfounded. For the control of ppGpp itself, however, this distinction is still valid: during exponential growth ppGpp synthesis is catalyzed by a cytoplasmic protein for which no gene has yet been found (31, 40), whereas during amino acid starvation ppGpp is synthesized by a ribosome-associated factor, the product of the *relA* gene (15). If ppGpp is an effector in the control of stable RNA synthesis, as suggested by in vitro experiments (42, 43), then the rigid relationship between ppGpp concentration and stable RNA gene activity, despite the wide variety of conditions used, would indicate that ppGpp is the major, if not only, effector in the control of stable RNA gene activity (35).

The role of ppGpp in the control of ribosome synthesis is still controversial (13, 28). Recently, an alternate model of rRNA gene control which assumes that rRNA synthesis is regulated by free ribosomes acting as a repressor without the involvement of ppGpp has been proposed by Jinks-Robertson et al. (18) and Nomura et al. (28). These investigators did not explain the striking relationship between r_s/r_t and ppGpp concentration; they questioned the relevance of the parameter r_s/r_t (28) and did not consider the evidence (2, 36) demonstrating the superior utility and relevance of this parameter as a measure of stable RNA gene activity, as compared with the conventional RNA per DNA (19) or RNA per protein (18, 29) parameter. The ribosome feedback model was derived from a study of rRNA synthesis in bacteria with an increased copy number of rRNA genes and is based on the assumption that RNA polymerase in bacteria is in excess. At any given moment only 20 to 30% of all RNA polymerase in *E. coli* is actively engaged in transcription (39). However, despite an apparent abundance of idling RNA polymerase, DNA rather than RNA polymerase is in excess for transcription in vivo. This follows from a study of mutant bacteria in which an altered control of chromosome replication leads to a reduction in the concentration of every promoter, in comparison with wild-type bacteria (3). At the reduced DNA concentration (but unaltered total RNA polymerase concentration), all genes were found to be transcribed more frequently so that total transcription (per cell mass) was unaltered. This result has important implications for both metabolic regulation and stable RNA gene control. (i) The rate of transcription is limited by the concentration of free (active) RNA polymerase rather than by DNA; therefore, the excess idling RNA polymerase is not free to bind to promoters. Experiments with minicells indicated that it is bound to DNA (N. Shepherd, Ph.D. thesis, University of Texas at Dallas, Dallas, 1979). (ii) Different promoters must compete for RNA polymerase binding, and this binding, rather than the formation of the open promoter complex (25, 38), is rate limiting for transcription. (iii) As the rate of rRNA synthesis is not limited by the rRNA gene concentration, this rate would not be affected by an increased copy number of rRNA genes. Therefore, the observed gene dosage independence of rRNA synthesis (18) does not imply a negative control, either by free ribosomes or by any other factor. For these reasons, the role of ppGpp as a controlling element cannot be dismissed. Moreover, the effect of ppGpp on in vitro rRNA synthesis has been amply demonstrated (e.g., references 42 and 43), whereas efforts to demonstrate an effect of free ribosomes on in vitro rRNA transcription have failed (18).

The observed relationship between ppGpp and stable RNA gene activity has been interpreted in terms of a model that assumes a ppGpp-dependent partitioning of the cellular complement of RNA polymerase into two forms, I and II

* Corresponding author.
† Present address: Department of Chemistry, California Institute of Technology, Pasadena, CA 91125.

(35). In the absence of ppGpp all active polymerase is in form I and prefers, almost exclusively, stable RNA promoters. At saturating levels of ppGpp the enzyme is in form II and prefers mRNA to stable RNA promoters at a ratio of 5:1. The stable RNA gene activity that persists in the presence of excess ppGpp may arise primarily from transcription initiation at promoter P2 of *rrn* operons (37). At intermediate ppGpp concentrations, such as those observed during exponential growth, the RNA polymerase exists as a mixture of forms I and II. The partitioning under these conditions can be described by the fraction of total enzyme that is in form I: $f_s = 2^{-(ppGpp/K)}$, where K, the concentration at which half the enzyme is in form I, is 20 pmol per unit of optical density at 460 nm for wild-type *E. coli* B/r (35). The fraction of enzyme in form I thus decreases exponentially from 1.0 in the absence of ppGpp to 0 at saturating concentrations of ppGpp. The isolation of RNA polymerase mutants with altered values of K (23, 24) is consistent with this two-component model for the ppGpp-dependent partitioning of the RNA polymerase.

If correct, the above model predicts that the activity of any gene in a cell is modulated by ppGpp. For constitutive genes this would be the only control and would provide an explanation for the phenomenon previously described as metabolic regulation (5, 32, 44). In the case of regulated genes the ppGpp control would be obscured by the specific regulatory mechanisms and would only become apparent under conditions of physiological constitutivity, e.g., at saturating concentrations of an inducer or in the absence of a repressor. Depending on the relative affinities of the two forms of RNA polymerase for the particular promoter (41), the gene expression might increase with increasing concentration of ppGpp (as observed for an average mRNA gene), decrease (as observed for rRNA and tRNA genes), or remain constant if both forms of RNA polymerase have an equal probability of initiating transcription at the promoter.

To assess the effect of ppGpp on the expression of constitutive genes, we measured the transcriptional activity of ribosomal protein genes in the *spc-str* region of the chromosome and of the maximally induced *lac* operon for a *relA*$^+$/*relA* isogenic pair of strains at different rates of exponential growth, achieved by changing the composition of the growth medium, and after amino acid starvation in each of these media. Such systematic measurements of the constitutive *lac* gene activities have not been reported previously. Although *spc* gene activities have been measured during exponential growth in a *relA*$^+$ strain (6, 14) and after amino acid starvation in a single growth medium in a *relA*$^+$/*relA* strain (8, 10), the growth rate dependence of ribosomal protein gene expression has not been determined for a *relA* strain. Although some of the results obtained here are consistent with ppGpp-dependent metabolic control of ribosomal protein genes and of the *lac* operon, others appear to be at variance with this idea. The apparent deviant behavior is probably due to superimposed effects, unrelated to metabolic control, such as premature transcription termination during amino acid starvation, that affect transcription after initiation of RNA chains (see below). It appears that the hybridization methods and conditions that have been used here and elsewhere are inadequate for quantitative analysis of constitutive mRNA gene expression, as compared with the previous analysis of stable RNA gene expression. Hence, until more sophisticated methods are available, the transcriptional control of ribosomal proteins and the phenomenon of metabolic regulation may not be fully understood.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* B/r strains RL331T and RL332T are isogenic with the exception that RL331T is *relA*$^+$ and RL332T is *relA* (34). Cultures were grown at 37°C in medium C (16) supplemented with 50 μg of L-phenylalanine per ml, 10 mM isopropyl-β-D-thiogalactopyranoside (IPTG), 10 mM adenosine 3′,5′-phosphate (cAMP), 0.6% Casamino Acids (CAA) when used, and 0.2% glucose, glycerol, succinate, or L-alanine as carbon and energy sources. Amino acid starvation was achieved by the addition of pseudomonic acid, a generous gift of Beecham Pharmaceuticals, at concentrations of 10 μg/ml in the absence of CAA and 50 μg/ml in the presence of CAA (35). The mass doubling time of cultures was determined from the increase in optical density at 460 nm.

RNA labeling and hybridizations. Samples (10 ml) of cultures were removed and labeled for 1 min with [5-^{3}H]uridine (10 μCi/ml; 26 Ci/mmol). The pulse labeling was stopped by rapidly cooling the samples to 0°C in the presence of 1 mM NaN_3, and total cellular RNA was extracted as previously described (22, 26). The RNA concentrations were adjusted to 50 μg/ml in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

For examination of ribosomal protein gene activity, measured as the rate of ribosomal protein mRNA synthesis relative to the rate of total RNA synthesis (r_{spc}/r_t), and *lac* gene activity, measured as the rate of *lac* mRNA synthesis relative to the rate of total RNA synthesis (r_{lac}/r_t), 50-, 100-, 150-, and 200-μl volumes of the RNA preparations were hybridized at 67°C for 18 h to excess amounts of λ d*spc*1 and λ p*lac* DNAs that had been denatured and immobilized on nitrocellulose paper (22). The λ d*spc*1 DNA contains the genes for 15 ribosomal proteins and the α subunit of RNA polymerase (20, 21), and the λ p*lac* DNA contains the *lacZ* gene. Nonspecific hybridization was determined from filters containing λ DNA for r_{lac}/r_t or λ *trk* DNA for λ d*spc*1 DNA (22) and was subtracted from the counts obtained from the specific probes. The radioactivity in specific hybrids was plotted as a function of the RNA concentration in the hybridization mixture. The slope of this plot corresponds to the relative hybridization as a fraction of total radioactivity (r_x/r_t), which was used without further correlation for hybridization efficiency.

For measuring r_s/r_t, the RNA preparations were diluted 100-fold, and 100-μl portions were hybridized in triplicate to excess λ d*ilv*5 DNA, which contains an entire *rrn* gene (4). Nonspecific hybridization was determined from λ DNA filters. The hybridization efficiency (ranging from 65 to 85%) was monitored by including purified ^{14}C-labeled rRNA (12) in the hybridization reactions.

RESULTS

Ribosomal protein gene and *lac* gene activities during exponential growth at different rates. Strains RL331T (*relA*$^+$) and RL332T (*relA*) have been used previously to study stable RNA gene activities and intracellular ppGpp concentrations (35). To obtain growth rates of between 0.5 and 2.5 doublings per h, we used different carbon sources with or without CAA. All media included excess IPTG and cAMP to render the *lac* operon functionally constitutive (5). The presence of cAMP reduced the growth rate in some media, e.g., the bacteria grew extremely poorly in succinate minimal medium under these conditions. These effects were not expected to influence ppGpp-dependent metabolic regulation, as the

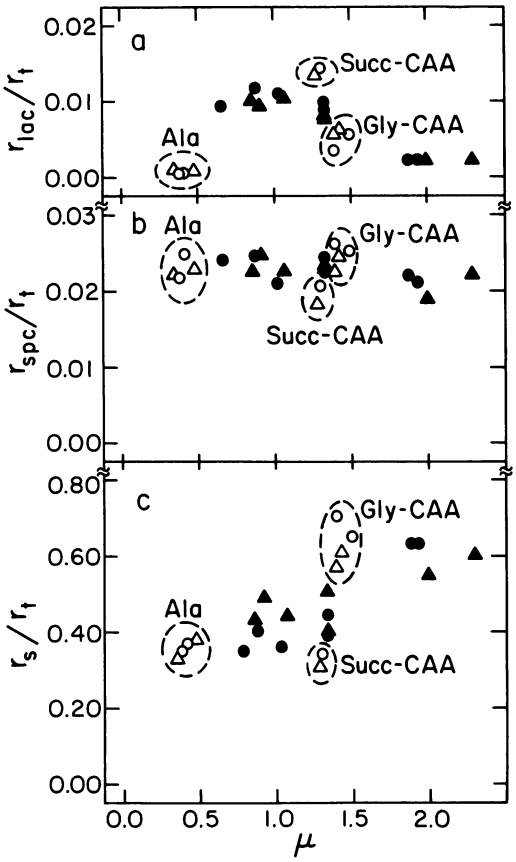

FIG. 1. Activities of stable RNA genes (r_s/r_t), ribosomal protein genes (r_{spc}/r_t), and *lac* genes (r_{lac}/r_t) as a function of the bacterial growth rate. Symbols: ● and ○, RL331T (*relA*$^+$); ▲ and △, RL332T (*relA*). Open symbols (surrounded by a broken circle) represent values obtained with succinate-CAA (Succ-CAA), glycerol-CAA (Gly-CAA), and L-alanine (Ala) media which seem to deviate from the general trend obtained with other growth media (solid symbols) (see text). All media contained 1 mM IPTG and 10 mM cAMP.

relationship between ppGpp and stable RNA gene activity is independent of particular growth conditions (35).

In Fig. 1 the activities of ribosomal protein genes (r_{spc}/r_t), the *lac* operon (r_{lac}/r_t), and stable RNA genes (r_s/r_t) are plotted as functions of growth rate. Whereas r_{spc}/r_t was rather independent of the growth rate (Fig. 1b), r_{lac}/r_t generally decreased (Fig. 1a) and r_s/r_t increased (Fig. 1c) with increasing growth rate, in agreement with reported data (14, 30, 35). However, deviations from the general relationships are apparent, most notably the low r_{lac}/r_t values in L-alanine medium. The gene activities did not depend on the function of the *relA* gene, as expected, as ppGpp accumulation during exponential growth is due to a *relA*-independent system (31, 40). When the data of Fig. 1a and b were replotted as a function of r_s/r_t (from Fig. 1c), similar relationships were obtained, i.e., r_{spc}/r_t was independent of r_s/r_t, and r_{lac}/r_t generally decreased with r_s/r_t (Fig. 2a and 3a).

In Fig. 2b and 3b ribosomal protein gene and *lac* gene activities are expressed as rates per total mRNA synthesis, r_{spc}/r_m and r_{lac}/r_m, respectively [$r_x/r_m = (r_x/r_t)/(r_m/r_t)$; $r_m/r_t = 1 - (r_s/r_t)$]. As different mRNA molecules compete for ribosomes, which are rate limiting for protein synthesis (11), it would be expected that r_x/r_m would be directly proportional to the rate of synthesis of protein X (per total protein

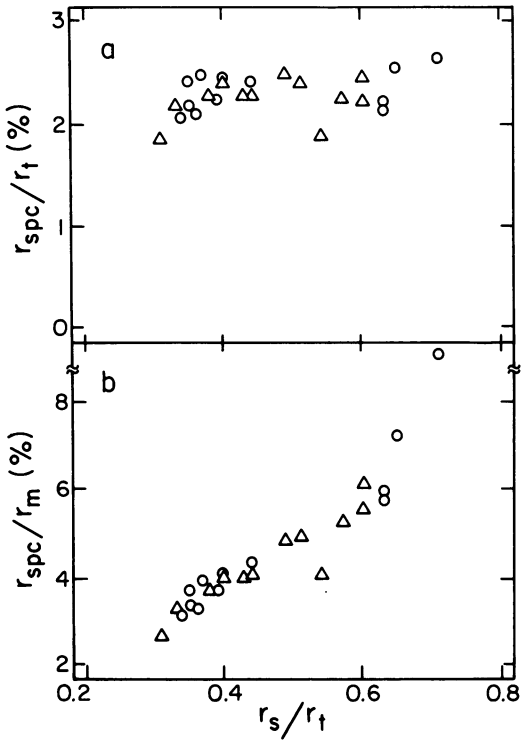

FIG. 2. Plots of ribosomal protein gene activities. (a) Replot of r_{spc}/r_t from Fig. 1b as a function of r_s/r_t (Fig. 1c). (b) r_{spc}/r_m, obtained as the ratio of r_{spc}/r_t to r_m/r_t. The symbols are the same as those described in the legend to Fig. 1.

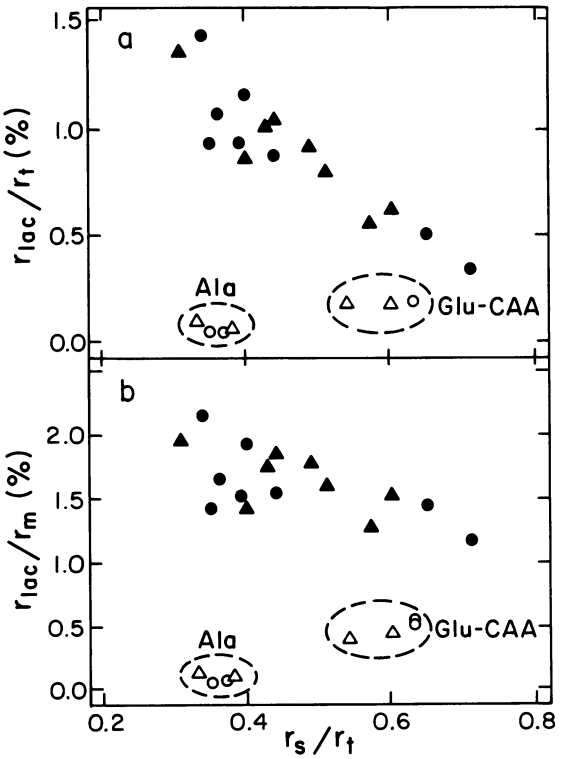

FIG. 3. Plots of *lac* gene activities. (a) Replot of r_{lac}/r_t from Fig. 1a as a function of r_s/r_t (Fig. 1c). (b) r_{lac}/r_m, obtained as the ratio of r_{lac}/r_t to r_m/r_t. The symbols are the same as those described in the legend to Fig. 1. Glu-CAA, glucose-CAA; Ala, L-alanine.

synthesis) in the absence of translational controls. In Fig. 2b r_{spc}/r_m is seen to increase in proportion to r_s/r_t. This suggests that rRNA and ribosomal protein synthesis are matched during exponential growth in the absence of extensive translational controls (21), as has been concluded previously (14). In Fig. 3b the observed decrease in r_{lac}/r_m with increasing r_s/r_t is consistent with previously observed decreases in the fractional rate of β-galactosidase synthesis, α_{lac}, with increasing growth rate (5, 44, 45).

Ribosomal protein gene and *lac* gene activities during amino acid starvation. During amino acid starvation r_{spc}/r_t decreased about 70% in the *relA*$^+$ strain and remained essentially unchanged in the *relA* strain (Table 1). Previous observations gave somewhat different results: during amino acid starvation ribosomal protein gene activity decreased in a *relA*$^+$ strain and increased in a *relA* strain (1, 8, 10), i.e., ribosomal proteins appeared to be clearly under stringent control, like rRNA and tRNA. In general, r_{lac}/r_t decreased to virtually undetectable levels during amino starvation in both the *relA*$^+$ and the *relA* strains.

DISCUSSION

Relationship between stable RNA and ribosomal protein mRNA gene expression. The stable RNA gene activity, r_s/r_t, was found to increase with growth rate (Fig. 1c), as expected (11, 14, 35). In some growth media, e.g., glycerol-CAA medium, stable RNA gene activity deviated from the expected value. This may have been because of the presence in the growth medium of cAMP, which also affected the growth of the bacteria. Although a rigid relationship between growth rate and r_s/r_t is not a theoretical necessity, a rigid relationship apparently exists between ppGpp concentration and r_s/r_t (35).

The ribosomal protein gene activity (r_{spc}/r_t) slightly decreased from 2.4 to 2.1% with increasing growth rate (Fig. 1b and 2a). These r_{spc}/r_t values are similar to, but somewhat higher than, those previously observed; whereas they decreased with growth rate from about 1.8 to 1.3% in one study (14), in another they decreased from 2.15 to 1.74% within the same range of growth rates (6). The differences in the absolute values of r_{spc}/r_t may reflect differences in hybridization efficiencies or in the corrections used to calculate hybridization efficiencies. Nonetheless, all the data are consistent with a slight decrease in r_{spc}/r_t with increasing growth rate.

As a fraction of total mRNA synthesis, the ribosomal protein gene activities were found to increase in proportion to rRNA gene activity (Fig. 2b). This indicates that during normal exponential growth ribosomal protein gene activities and rRNA gene activities are matched at the level of transcription initiation. To a first approximation, the fraction of total mRNA that is *spc* mRNA is equal to the fraction of total protein that is ribosomal protein derived from the cluster of *spc* genes (about 25% of total ribosomal protein). This is consistent with the suggestion that ribosomal protein mRNA is translated as efficiently as average mRNA (14). However, to quantitatively correlate ribosomal protein and ribosomal protein mRNA synthesis under different conditions, the functional life of the mRNA, which itself may be a function of growth rate, must also be taken into account. Using homogeneously labeled RNA, Dennis (6) and Dennis and Nomura (9) found that the amount of ribosomal protein mRNA as a fraction of total RNA increased with increasing growth rate. The meaning of this result is not clear, however, as the amounts measured by hybridization reflect the chemical, not the functional, life of mRNA, and the relationship between these two parameters is unknown. If the result reflects an increased functional life of ribosomal protein mRNA at higher growth rates, it would indicate that ribosomal protein mRNA is overproduced at higher growth rates. In this case, ribosomal protein and rRNA, although matched during exponential growth at the level of transcription initiation, would be unmatched at the level of the functional life of ribosomal protein mRNA, and a translational control would then have to be invoked to again match ribosomal protein to rRNA. Although evidence for translational control of ribosomal proteins has been obtained, it has only been shown under conditions when the relative transcription of those ribosomal proteins compared with that of others had been disproportionately increased (21, 22).

TABLE 1. Effect of amino acid starvation on r_{spc}/r_t and r_{lac}/r_t[a]

Medium	Strain	r_{spc}/r_t		r_{lac}/r_t	
		Before	After	Before	After
Glucose	RL331T	2.41	0.71	0.87	0.15
		2.23	0.67	0.93	0.13
	RL332T	2.36	1.98	0.85	0.15
		2.38	2.00	0.79	0.13
Glycerol	RL331T	2.44	0.87	1.16	0.32
	RL332T	2.45	2.38	0.91	0.16
L-Alanine	RL331T	2.46	0.69	0.36	0.51
	RL332T	2.26	3.09	0.06	0.03

[a] Calculated before and 15 min after the addition of pseudomonic acid.

Ribosomal protein gene control by ppGpp. After amino acid starvation r_{spc}/r_t decreased slightly or even increased in the *relA* strain and decreased about threefold in the *relA*$^+$ strain (Table 1). In a similar study partial amino acid starvation resulted in 20% stimulation of ribosomal protein mRNA synthesis in the *relA* strain and 50% repression in the *relA*$^+$ strain (10). The absolute differences between these and our results probably reflect differences in the amino acid starvation conditions. Whereas we produced complete amino acid starvation at constant temperature, the partial amino acid starvation was achieved by shifting the growth temperature of strains having a thermosensitive valyl-tRNA synthetase. Temperature shifts, however mild, have been shown to cause additional physiological and transcriptional effects which are difficult to analyze (34, 36). In another study in which homogenously labeled RNA was used the amount of ribosomal protein mRNA increased 3-fold in a partially amino acid-starved *relA* strain and decreased almost 10-fold in a partially amino acid-starved *relA*$^+$ strain (8), indicating that *relA* activity and presumably ppGpp have a much more pronounced effect on ribosomal protein mRNA accumulation. Again, the meaning of these results is not clear because of the effects of changes in temperature, long-term partial amino acid deprivation, and possible changes in the chemical lives of mRNA and total RNA.

The fact that the expression of a given gene depends on the function of the *relA* gene and, in particular, that it decreases after amino acid starvation in a *relA*$^+$ strain but not in a *relA* strain is insufficient to conclude that the gene is under stringent control. First, a nonspecific reduction of all gene activities due to an effect of ppGpp on the RNA polymerase activity was observed during amino acid starvation of a *relA*$^+$ strain but not of a *relA* strain (33). Second,

the proportion of total RNA synthesized that is mRNA increases or decreases when the proportion of stable RNA synthesis decreases or increases, respectively. Thus, even in ribosomal protein mRNA synthesis remained constant after amino acid starvation of a *relA*$^+$ strain, its fractional synthesis rate with respect to total mRNA would decrease as total mRNA synthesis increases (relative to total transcription). Correspondingly, ribosomal protein synthesis as a fraction of total protein synthesis would also decrease, thereby falsely suggesting stringent control of ribosomal proteins (7). The ratio r_{spc}/r_t, however, is independent of RNA polymerase activity and of changes in total mRNA synthesis.

Previous studies have indicated that no valid distinction exists between stringent control and growth rate control of stable RNA gene activity. Regardless of the conditions used, e.g., during exponential growth in a variety of media or after amino acid starvation of a *relA*$^+$ or a *relA* strain, the relationship between r_s/r_t and ppGpp is described by the same function (35). If a similar situation exists for ribosomal proteins, then the function r_{spc}/r_t versus ppGpp should also be the same for exponential growth at different rates and amino acid starvation, regardless of the *relA* allele used. For exponential growth conditions, the hybridization results indicate that r_{spc}/r_t is rather independent of r_s/r_t and thus also independent of ppGpp concentration. This observation would seem to be at variance with reports showing an in vitro ppGpp effect on ribosomal protein gene expression (20). However, as the in vitro data were obtained with a coupled transcription-translation system, the relevant parameter for comparison is the ratio r_{spc}/r_m rather than the ratio r_{spc}/r_t, which, as shown in Fig. 2a and b, readily explains the difference. If ribosomal proteins are metabolically regulated in the absence of other specific factors, then r_{spc}/r_t should be unaffected by amino acid starvation, regardless of the *relA* allele present. This was clearly not observed; a 70% decrease in r_{spc}/r_t was found during amino acid starvation of the *relA*$^+$ strain (Table 1).

Metabolic control of *lac* gene activity. To further study the effect of ppGpp on metabolic regulation, we chose the *lac* operon as a prototype for nonribosomal RNA genes, as the regulatory mechanisms involved in its expression have been well established. The concentrations of IPTG and cAMP used here have been shown to render the *lac* operon completely constitutive (5). We did not use the *lacUV5* promoter, which would have obviated the need to use high concentrations of cAMP, as its constitutive expression has been shown to be much less metabolically regulated than that of the wild-type promoter (45).

The data in Fig. 3 suggest that during exponential growth the *lac* promoter behaves in a manner opposite to the average stable RNA promoter; whereas transcription from stable RNA promoters decreases with increasing ppGpp concentration, transcription from the *lac* promoter decreases with increasing r_s/r_t and, therefore, by extrapolation, increases with increasing ppGpp concentration. This result agrees with in vitro data showing that *lac* gene activity is stimulated by ppGpp (30).

Again, exceptions that seem to be at variance with this interpretation were observed, in particular during amino acid starvation. During amino acid starvation one would predict a stimulation of *lac* gene activity caused by the accumulation of ppGpp in a *relA*$^+$ strain, but instead, a strong inhibition was observed (Table 1).

Differences in the constitutive expression of mRNA and stable RNA genes. In addition to the ppGpp-dependent partitioning of the RNA polymerase, two effects which are irrelevant for the metabolic regulation of stable RNA genes must be considered for the metabolic regulation of mRNA genes.

(i) During exponential growth the RNA polymerase on nascent mRNA chains is immediately followed by ribosomes which prevent premature transcription termination (polarity, references 17 and 27). During amino acid starvation, when ribosomes stall at codons for which no charged tRNA is available, this close coupling of transcription and translation is dissociated such that the value of r_x/r_t reflects not only changes in the gene activity but also effects of polarity on the transcriptional activity of gene *X*. Under similar conditions effects of polarity are not expected for stable RNA genes, as their transcripts are not translated. In addition, premature termination of stable RNA transcripts is prevented by strong transcription antiterminators at the beginning of *rrn* operons (M. Cashel, personal communication).

(ii) The transcriptional activity of any given gene necessarily depends on the activity of all other genes that are transcribed by the same form of RNA polymerase. If certain genes became very active during amino acid starvation or in a given growth medium, they would act as an RNA polymerase sink, and the effective concentration of that form of free RNA polymerase available for the transcription of other genes would decrease. Under these conditions r_x/r_t would not only measure the activity of gene *X* but also the different partitioning of RNA polymerase among mRNA genes. For stable RNA genes RNA polymerase sink effects are minimal because form I RNA polymerase transcribes, almost exclusively, stable RNA genes, i.e., other promoters do not significantly compete with stable RNA promoters.

With these considerations in mind, we have interpreted the results obtained here in terms of our model for the ppGpp-dependent partitioning of RNA polymerase (see above). During exponential growth r_{lac}/r_t generally decreased with increasing r_s/r_t, implying that form I has a lower affinity and form II has a higher affinity for *lac* promoters than for stable RNA promoters. During amino acid starvation or exponential growth in L-alanine medium, r_{lac}/r_t was severely depressed. We suggest that much of this depression arises from polarity or polymerase sink effects or both. The repression of *lac* gene activity in L-alanine medium suggests that growth in this medium is equivalent to growth during partial amino acid starvation, consistent with our previous measurements of r_s/r_t which also suggested mild amino acid starvation in L-alanine medium (26). The possibility cannot be excluded, however, that the cAMP-binding protein is less stable or that the cAMP concentration is not high enough under these conditions to completely eliminate catabolite repression.

During exponential growth r_{spc}/r_t was rather independent of r_s/r_t, suggesting that form I and form II RNA polymerases have about equal affinities for ribosomal protein promoters (Fig. 3). Amino acid starvation in *relA*$^+$ and *relA* strains differentially affected ribosomal protein gene activity but reduced it to a lesser extent than it did *lac* gene activity. This may be explained by assuming that, although effects of polarity were present, they were not as severe in ribosomal protein operons as in the *lac* operon. This result also indicates that the extent of polarity depends on the *rel* factor, as has been observed previously (27). The absence of a *rel* dependence of r_{lac}/r_t during amino acid starvation (Table 1) may be due to the *rel*-dependent effects of polarity in the *lac* operon being stronger than the ppGpp-dependent transcriptional control.

The results obtained here indicate that the analysis of

constitutively expressed mRNA genes is more complex than that of stable RNA genes. To demonstrate the general validity of the ppGpp-dependent metabolic regulation of all genes in vivo, a method that specifically measures initiation of mRNA transcripts is needed. Until such a method is available, the data observed here and reported elsewhere can neither prove nor disprove the model of ppGpp-dependent metabolic regulation.

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