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rpoB Mutation in *Escherichia coli* Alters Control of Ribosome Synthesis by Guanosine Tetraphosphate

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An isogenic pair of *relA*$^+$ and *relA* strains of *Escherichia coli* B/r with a mutation in the RNA polymerase subunit gene *rpoB* (Rif^r) was isolated in which the relationship between guanosine tetraphosphate (ppGpp) concentration and stable RNA (rRNA, tRNA) gene activity was altered. The RNA polymerase in the *rpoB* strains was found to be about 20-fold more sensitive to ppGpp with respect to its stable RNA promoter activity than was the wild-type enzyme. The existence of such mutants is consistent with the idea that ppGpp interacts with the RNA polymerase enzyme and thereby alters its promoter selectivity, i.e., reduces its affinity for the stable RNA promoters. Under most conditions, the *rpoB* mutants had a reduced rate of growth and about a 10-fold-reduced intracellular concentration of ppGpp compared with the *rpoB* wild-type strains. The reduction of the level of ppGpp in the *rpoB* mutants during exponential growth was presumably a reflection of an indirect effect of the *rpoB* mutation on the control of *relA*-independent ppGpp metabolism.

For *Escherichia coli*, the stable RNA gene activity, measured as the rate of rRNA and tRNA synthesis relative to the total instantaneous rate of RNA synthesis, r_s/r_t, has been shown to be related to the intracellular concentration of guanosine tetraphosphate (ppGpp) by a unique function (25). This relationship was shown to be independent of the growth medium, the status of the *relA* gene, or the imposition of amino acid starvation, and it appears to reflect only the selectivity of RNA polymerase with respect to rRNA and tRNA promoters in response to a particular ppGpp concentration (25). It was therefore proposed that the stable RNA gene activity in *E. coli* is metabolically regulated via a ppGpp-dependent partitioning of the cellular complement of RNA polymerase into two forms, one with a high and the other with a low affinity for stable RNA promoters (25). Irrespective of whether ppGpp interacts directly or indirectly via some other factor with the RNA polymerase, it would therefore be expected on the basis of this model that strains having mutations in RNA polymerase genes could be isolated in which this relationship is altered. One such strain with a mutation in *rpoD* (alt-1) appears to be of this type (30). However, since the effect of this mutation was studied with respect to the in vitro transcriptional specificity of RNA polymerase purified from the strain in response to ppGpp, an in vivo correlation of the stable RNA gene activity as a function of ppGpp would be required to properly classify this mutant.

Here we report the isolation of a *relA*$^+$/*relA* isogenic pair of strains having an *rpoB* (Rif^r) mutation which drastically alters the function describing the in vivo stable RNA gene activity versus ppGpp concentration. The results indicate that the RNA polymerase in these strains has about a 20-fold-increased sensitivity to ppGpp.

MATERIALS AND METHODS

The *rpoB* (Rif^r) mutation was derived from a spontaneous rifampin-resistant mutant of a *relA* derivative of *E. coli* strain NC51 (*relA*$^+$ *valS*(Ts); 18) obtained by intermittent near-UV irradiation (340-nm wavelength; 23). The mutation was transduced by bacteriophage P1 into *argH3* derivatives of RL331T and RL332T (25) by selecting for Arg$^+$ transductants and screening for rifampin resistance. The resulting isogenic strains RL81 and RL82 are identical, with the exception that RL81 is *relA*$^+$ and RL82 is *relA*.

Cultures were grown at 37°C in minimal medium C (10) supplemented with L-phenylalanine (50 μg/ml) and 0.2% glucose and 0.6% Casamino Acids (CAA), 0.2% glucose, or 0.2% L-alanine. Growth was monitored as the optical density at 460 nm (OD_{460}). Amino acid starvation was achieved by treating cultures with pseudomonic acid, a generous gift from Beecham Pharmaceuticals, at a concentration of 10 μg/ml, or 50 μg/ml for glucose-CAA medium (25).

The parameter r_s/r_t (the rate of stable RNA synthesis as a fraction of the instantaneous rate of total RNA synthesis) was measured by hybridization of [^{3}H]uridine pulse-labeled RNA to λ *dilv5* DNA as described previously (22, 24, 25). The concentration of ppGpp, in picomoles per OD_{460} unit, was determined from elu-

TABLE 1. Growth rates of the *rpoB* strains RL81 and RL82 and of their parental *rpoB*$^+$ strains in different growth media

Strain	Genotype	Growth rate (doublings per h)		
		L-Alanine	Glucose	Glucose-CAA
RL331T	*relA*$^+$ *rpoB*$^+$	0.45	1.40	2.31
RL81	*relA*$^+$ *rpoB*	0.47	1.13	1.71
RL332T	*relA rpoB*$^+$	0.60	1.40	2.31
RL82	*relA rpoB*	0.38	0.90	1.20

tion profiles of nucleotides in alkali extracts of cells after separation by ion-pair reverse-phase high-pressure liquid chromatography as described previously (16, 24).

RESULTS

Origin of the *rpoB* mutants. While we were isolating rifampin-resistant mutants from *relA* derivatives of *E. coli* B/r strain NC51, two Rif^r mutants among a total of 25 were fortuitously found that seemed to have an abnormal control of RNA synthesis. To facilitate the further characterization of the effect of these mutations, the *rpoB* alleles were introduced by phage P1 transduction into an isogenic pair of *relA*$^+$ and *relA* derivatives of *E. coli* B/r that had previously been used to analyze the relationship between ppGpp and ribosome synthesis (25). The isogenic pair of strains harboring one of the *rpoB* (Rif^r) mutations, RL81 (*relA*$^+$) and RL82 (*relA*), was used here to compare this relationship with that of the previously studied wild-type strains.

Control of rRNA synthesis and ppGpp accumulation at different growth rates in strains RL81 and RL82. The *rpoB* mutants had generally a lower growth rate for a given growth medium than did the parental *rpoB*$^+$ strains. The reduction in the growth rate was greater in RL82 (*relA*) than in RL81 (*relA*$^+$) and increased with the richness of the medium (Table 1). The maximum observed reduction was 50% for RL82 in glucose-CAA medium. The reason for the synergistic effect of the *relA* and *rpoB* mutations on the growth rate has been investigated and appears to be related to differences in the concentration and activity of the RNA polymerase in the *relA*$^+$ and *relA* derivatives having the *rpoB* mutation (R. Little, J. Ryals, and H. Bremer, manuscript in preparation). For a given growth rate, the mutant strains had about a 10-fold-lower ppGpp concentration, near the limit of detectability (Fig. 1b), and an up to 50% lower ribosomal gene activity, measured as the rate of rRNA and tRNA synthesis relative to the total instantaneous rate of RNA synthesis (r_s/r_t; Fig. 1a). In Fig. 2, the r_s/r_t values have been replotted as a function of ppGpp concentration for the *rpoB* mutants (squares) and, for comparison, the wild-type strains (circles; from reference 25); the open and closed symbols refer to *relA* and *relA*$^+$ strains, respectively. For a given concentration of ppGpp, the stable RNA gene activity in the *rpoB* mutant strains is in general much lower than in the wild-type parental strains; only at saturating concentrations of ppGpp (above 100 pmol/OD_{460} unit) was the same r_s/r_t limit value of 0.24 approached in both the mutant and wild-type strains. These results indicate that the *rpoB* mutation confers an altered control of ribosome synthesis by ppGpp.

DISCUSSION

Control of stable RNA synthesis by ppGpp. We have previously presented a model derived from in vivo data in which the control of stable RNA synthesis could be described by a ppGpp-medi-

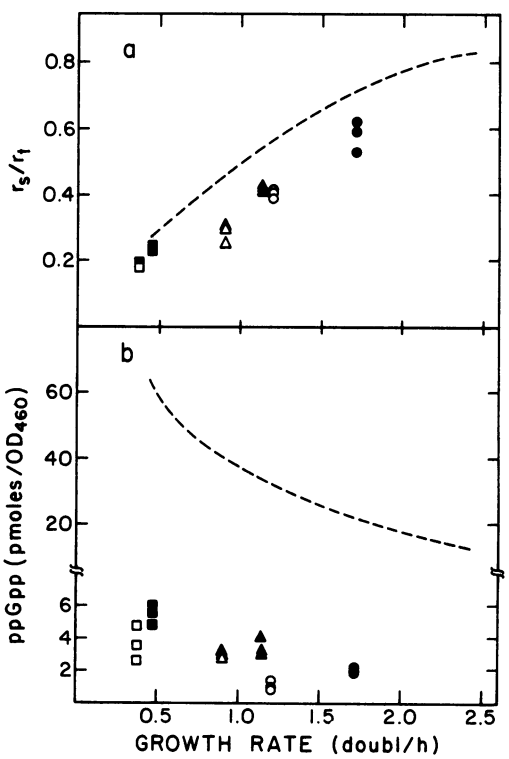

FIG. 1. Stable RNA gene activity, r_s/r_t (a), and ppGpp concentration (b) as a function of growth rate. Cultures were grown in glucose amino acids (●, ○), glucose minimal (▲, △), or L-alanine medium (■, □). Three measurements of r_s/r_t and two measurements of the ppGpp concentration were made for each of three cultures in the same medium. The values shown are average values for each culture. The dashed line indicates the average values for the *rpoB*$^+$ strains from reference 25. Closed symbols, RL81 (*relA*$^+$); open symbols, RL82 (*relA*).

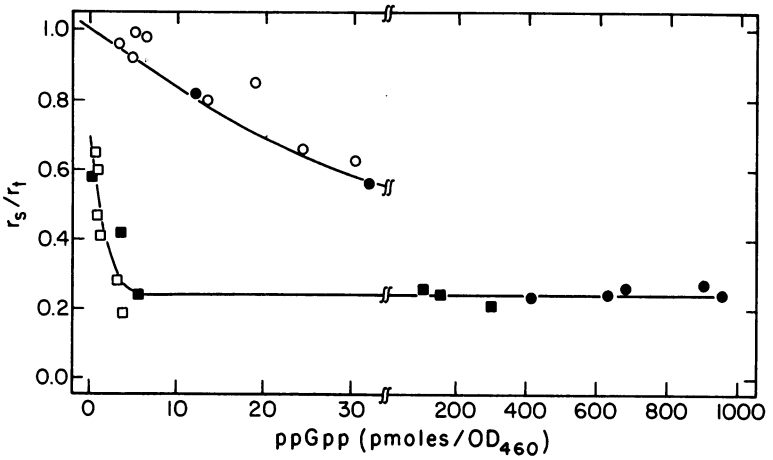

FIG. 2. Stable RNA gene activity, r_s/r_t, as a function of ppGpp concentration. The data of Fig. 1 were replotted and compared with the results obtained with the *rpoB*$^+$ strains (25). Symbols: (●) RL331T (*rpoB*$^+$ *relA*$^+$); (○) RL332T (*rpoB*$^+$ *relA*); (■) RL81 (*rpoB relA*$^+$); (□) RL82 (*rpoB relA*).

ated partitioning of the cellular RNA polymerase into two forms (25). In the absence of ppGpp, the RNA polymerase is in a form (I) which prefers exclusively stable RNA promoters over mRNA promoters. Thus, the probability that form I enzyme binds to stable RNA promoters, a_s(I), is 1.0. At saturating levels of ppGpp (>100 pmol/OD_{460} unit), the enzyme is in a form (II) which prefers mRNA promoters over stable RNA promoters at a ratio of 5:1. The probability that form II enzyme binds to stable RNA promoters, a_s(II), is therefore 1/6, i.e., 0.17. At intermediate ppGpp concentrations, the RNA polymerase exists as a mixture of form I and form II, the composition of which is given by the proportion of total (free) enzyme that has not reacted with ppGpp, i.e., in form I. This fraction, f_s, depends on the concentration of ppGpp and a parameter K, which is the concentration of ppGpp at which f_s = 0.5. K thus reflects the interaction of ppGpp with the RNA polymerase. The fraction f_s was found to decrease exponentially from 1.0–in the absence of ppGpp–to zero at saturating concentrations of ppGpp, and it can be described by the formula $f_s = 2^{-[\text{ppGpp}]/K}$. That this relationship is exponential rather than hyperbolic was proposed to arise from the RNA polymerase being accessible to ppGpp only during the interval between termination of an RNA chain and reinitiation of a new chain (25), a time which is probably very short (4) and insufficient to establish an equilibrium between the two forms of the enzyme.

The relationship between ppGpp concentration and r_s/r_t for wild-type *E. coli* B/r can accurately be described by a formula in which K = 20 pmol/OD_{460} unit, a_s(I) = 1.0, and a_s(II) = 0.17 (25; Fig. 2, upper curve). The same formula, but with K = 1 pmol/OD_{460} unit, can be used to describe the observed relationship for the *rpoB* mutant strains studied here (Fig. 2, lower curve). It is therefore concluded that in the *rpoB* mutants, at least K is affected. Apparently, a_s(II) is not affected since the curve for the mutants approaches the same final r_s/r_t plateau value of 0.24 as for the wild-type at saturating ppGpp levels (Fig. 2). It is not clear whether a_s(I) is altered by the mutation, since this would depend on whether the lower curve in Fig. 2 extrapolates to 1.0 for zero ppGpp concentration (as in the wild-type) or only to about 0.7. In the latter case, a_s(I) would also be altered by the mutation. This would in itself support the notion that RNA polymerase is heterogenous with respect to its promoter specificity, since it would imply that the affinity of one form of the enzyme for stable RNA promoters could be affected in the absence of a similar reduction in the affinity of the other form.

The difference between the K values for the wild-type and the *rpoB* mutant strains could mean either that the mutant RNA polymerase has a higher affinity for ppGpp or that the polymerase binds more slowly to all promoters, thereby allowing more time for the enzyme to interact with ppGpp. In either case, at a given ppGpp concentration, the fraction of the mutant RNA polymerase that has not reacted with ppGpp and is in form I would be decreased compared with the wild-type enzyme; i.e., in the mutants, more enzyme would be in form II at a particular level of ppGpp. Although the data do not favor one of the two possibilities, it seems more likely that the mutant RNA polymerase would bind more slowly to all promoters, since mutations generally result in reduced rather than

increased substrate affinities. This would not necessarily reduce the rate of RNA synthesis, since the slower binding would be compensated by the resulting increased concentration of free RNA polymerase, one of the factors that determine the rate of RNA synthesis (2).

Implications of the *rpoB* mutants for models of the control of stable RNA synthesis. It has been proposed on the basis of in vitro experiments that ppGpp acts by affecting the RNA chain elongation rate (11, 13). However, such a model is inconsistent with the observation that the stable RNA chain elongation rate in vivo is unaltered by ppGpp, even at high concentrations (22, 26). In the *rpoB* mutants used here, measurements of the RNA chain elongation rate indicated that both the rRNA and mRNA chain growth rates were the same as those in the *rpoB*$^+$ wild-type strains, indicating that the *rpoB* mutation had no effect on chain elongation (Little et al., manuscript in preparation). Thus, it is concluded that the abnormal control of ribosome synthesis in the *rpoB* mutants arises solely from the altered sensitivity of the RNA polymerase to ppGpp.

Implicit in the two-component RNA polymerase model of ppGpp action (see above and reference 25) are the assumptions that (i) the initiation frequency at a promoter depends on the concentration of free RNA polymerase; i.e., promoter binding rather than open initiation complex formation is the rate-limiting step in transcription initiation, (ii) stable RNA genes are transcribed constitutively, in the absence of any gene-specific controls, and (iii) the frequency at which stable RNA genes are transcribed is controlled only by the ppGpp-dependent partitioning of RNA polymerase between forms I and II, which would imply that all metabolic regulation (5, 21) is due to RNA polymerase heterogeneity. The first assumption, though at variance with in vitro measurements of the kinetic constants that determine "promoter strength" (27), has been shown to be essentially correct in that the concentration of free RNA polymerase rather than DNA has been shown to limit transcription in vivo (4). This observation is also at variance with the proposal (11) that a ppGpp-dependent pausing of the RNA polymerase at a site close to the promoter limits the rate of rRNA transcription (see above). Although not rigorously proven, it seems likely that the second assumption is also correct since, despite extensive study, no other factors have yet been implicated in the specific control of stable RNA gene activity (25). The third assumption of a ppGpp-dependent partitioning of RNA polymerase is supported by the observation that in the presence of ppGpp, the sedimentation velocity of purified RNA polymerase is altered, which is associated with a change in its promoter specificity (29, 31, 32). In addition, the binding of RNA polymerase to rRNA promoters has been shown to be decreased in vitro in the presence of ppGpp (9, 12, 33, 34). Moreover, the observation that ppGpp stimulates transcription from certain mRNA promoters both in vivo (8, 19) and in vitro (17, 35) is also consistent with the proposal that the RNA polymerase can exist in more than one form with respect to its promoter specificity. The observation that certain phage T7 or λ mRNA promoters exhibit little ppGpp dependence with respect to their ability to bind RNA polymerase in vitro (12, 13) does not disprove the idea of there being a functional heterogeneity of RNA polymerase, since the existence of such promoters would in fact be predicted from a generalization of the theory of the two-component model we have proposed (25). Thus, all known observations are consistent with there being a ppGpp-induced heterogeneity of RNA polymerase with respect to its promoter specificity, whereas contradictory evidence exists for a model in which ppGpp mediates its action by affecting the chain elongation rate.

On the basis of the two-component model of ppGpp action, it would be predicted that RNA polymerase mutants exist in which either one or a combination of the three parameters that describe the relationship between ppGpp and stable RNA gene activity, K, a_s(I), and a_s(II), is altered. In the *rpoB* mutant studied here, as discussed above, it is apparent that at least K is affected, whereas no change in the RNA chain elongation rate was observed. Thus, the data presented here are consistent with a two-component RNA polymerase model for the control of rRNA synthesis by ppGpp. In terms of this model, it would therefore be predicted that the transcription of genes that are under positive regulation of ppGpp, e.g., *lac* (17), would also be affected by the *rpoB* mutation.

Control of basal ppGpp levels in RL81 and RL82. During exponential growth in a given medium, essentially the same level of ppGpp is observed for *relA*$^+$ and *relA* strains (25; Fig. 1b), including those in which *relA* has been deleted (1). This suggests that a *relA*-independent pathway of ppGpp metabolism exists which is normally responsible for the adjustment of ribosome synthesis to the energy potential for protein synthesis afforded by a particular growth medium. Although evidence for such a pathway has been reported (6, 20), it is not known how the accumulation of ppGpp by this system is regulated.

In the *rpoB* mutants, the level of ppGpp is about 10 times lower than that for the wild-type strains growing in the same medium (Fig. 1b). These low levels of ppGpp most likely reflect an

indirect effect of the *rpoB* mutation on the control of *relA*-independent ppGpp metabolism. It has been observed that similarly low levels of ppGpp occur when protein synthesis is inhibited by chloramphenicol (14) or after amino acid starvation of *relA* strains (14, 15, 25), or when the protein synthesis potential is suddenly increased by a nutritional shift-up (7). These situations give rise to conditions in which the consumption of amino acids is less than what could be supplied by the energy potentially available from the medium. In the case of inhibition of protein synthesis, this imbalance results from decreased ribosome function, whereas for a nutritional shift-up, the initial ribosome concentration is less than that characteristic of the post-shift medium. Despite the differing causes for this imbalance, these conditions have in common that the charging of bulk tRNA would be increased since the amino acid supply to the tRNA aminoacylation reactions would exceed the drain of charged tRNA from the pool. It has therefore been proposed that charged or uncharged tRNA might be an effector in the control of ppGpp accumulation by the *relA*-independent pathway such that if the overall tRNA aminoacylation increases, the level of ppGpp would decrease (3, 28). With respect to such a model, it might be expected that uncharged tRNA would provide a wider range of responses, since normally most of the tRNA is charged (28). This would indirectly reflect the ATP level and hence would be a measure of the protein synthesis potential afforded by the particular growth condition. Thus, if the energy level were high but the protein synthesis rate were low, ppGpp accumulation by the *relA*-independent pathway would decrease and ribosome synthesis would increase to meet the protein synthesis potential. This idea could account for the low levels of ppGpp in the *rpoB* mutants in which the control of ribosome synthesis is defective. If ppGpp levels were normal in these mutants, ribosome synthesis would be repressed to the minimum level (i.e., $r_s/r_t = 0.24$) due to the greater sensitivity of the altered RNA polymerase to ppGpp. This would create a situation in which the amino acid supply would exceed the demand, resulting in low levels of uncharged tRNA, and ppGpp levels would be reduced. In response to these low ppGpp levels, ribosome synthesis could be increased, although apparently not to the wild-type level, since the *rpoB* strains were unable to achieve the same growth rates.

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