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Guanosine 3′-diphosphate 5′-diphosphate is not required for growth rate-dependent control of rRNA synthesis in *Escherichia coli*

(transcription initiation/*relA*/*spoT*)

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ABSTRACT **rRNA synthesis in *Escherichia coli* is subject to at least two regulation systems, growth rate-dependent control and stringent control. The inverse correlation between rRNA synthesis rates and guanosine 3′-diphosphate 5′-diphosphate (ppGpp) levels under various physiological conditions has led to the supposition that ppGpp is the mediator of both control mechanisms by inhibiting transcription from *rrn P1* promoters. Recently, *relA*⁻ *spoT*⁻ strains have been constructed in which both ppGpp synthesis pathways most likely have been removed (M. Cashel, personal communication). We have confirmed that such strains produce no detectable ppGpp and therefore offer a direct means for testing the involvement of ppGpp in the regulation of rRNA synthesis *in vivo*. Stringent control was determined by measurement of rRNA synthesis after amino acid starvation, while growth rate control was determined by measurement of rRNA synthesis under different nutritional conditions. As expected, the *relA*⁻ *spoT*⁻ strain is relaxed for stringent control. However, growth rate-dependent regulation is unimpaired. These results indicate that growth rate regulation can occur in the absence of ppGpp and imply that ppGpp is not the mediator, or at least is not the sole mediator, of growth rate-dependent control. Therefore, growth rate-dependent control and stringent control may utilize different mechanisms for regulating stable RNA synthesis.**

rRNA synthesis constitutes a major fraction of the RNA synthesis in *Escherichia coli*. In cells growing under steady-state conditions, rRNA and tRNA synthesis rates are proportional to the square of the growth rate in order to meet the cell's requirements for protein synthesis, a phenomenon termed growth rate-dependent control (1–3). rRNA transcription is also subject to stringent control, a response to aminoacyl-tRNA limitation that results in severe inhibition of stable RNA (rRNA and tRNA) synthesis. rRNA synthesis is regulated primarily at the level of transcription initiation at the *P1* promoters of the seven rRNA operons (4–8). A negative feedback system responsive to the level of translationally competent ribosomes has been shown to control transcription from *rrn P1* and tRNA promoters (9–13) and has been proposed to be the mechanism for growth rate-dependent control (2, 7, 9).

The role of the *relA* locus in the control of ribosome synthesis is a longstanding question in bacterial physiology (14). A rapid decline in stable RNA synthesis after amino acid starvation in *relA*⁺ but not in *relA*⁻ cells coincides with a large increase in the concentration of guanosine 3′-diphosphate 5′-diphosphate ("guanosine tetraphosphate," ppGpp; see refs. 15 and 16). The *relA* locus encodes ppGpp synthetase I (17). The *relA* system monitors the ratio of charged and uncharged tRNA at the ribosome, and upon amino acid starvation is responsible for the synthesis of ≈1000 pmol of ppGpp per ml per OD_{600} unit of cells (17–20).

In the absence of a functional *relA* locus, basal ppGpp levels (<100 pmol·OD_{600}^{-1}·ml^{-1}) still vary inversely with the growth rate, implying that there is a second ppGpp-synthesizing system (ppGpp synthetase II; refs. 17 and 18). Growth rate-dependent regulation of stable RNA functions normally in *relA*⁻ cells (18).

The apparently perfect inverse correlation between ppGpp concentration and stable RNA synthesis rate under a wide range of steady-state and transient physiological conditions suggested a causal relationship between ppGpp concentration and rRNA transcription. The most common interpretation has been that ppGpp acts as an effector molecule negatively regulating stable RNA synthesis and that the two physiologically distinguishable regulatory phenomena, stringent and growth rate control, have a common mechanism (18, 19, 21). This hypothesis was supported further by the observation that mutations in a tRNA promoter region between the −10 consensus hexamer and the transcription start site destroy both stringent control and growth rate control of transcription from that promoter (22, 23). Nevertheless, the results of *in vitro* transcription experiments investigating the role of ppGpp have been inconclusive (reviewed in refs. 2 and 24), and no mechanism has been demonstrated for ppGpp as a negative effector specific to stable RNA promoters.

In a continuing effort to understand the role of ppGpp in cell physiology, Cashel, Glaser, and coworkers have determined the DNA sequences of *relA* and *spoT* and characterized in detail a number of existing and newly created *relA* and *spoT* alleles (refs. 21 and 25–28; M. Cashel, personal communication). Recently, the ppGpp synthetase II activity has been identified as a likely function of the *spoT* gene product (M. Cashel, personal communication), previously thought to be responsible only for the degradation of ppGpp. Cashel and coworkers constructed a strain in which both genes were deleted and replaced with antibiotic-resistance markers. The resulting *relA*⁻ *spoT*⁻ double mutant strain displayed multiple amino acid auxotrophies and other pleiotropic phenotypes, but surprisingly, when supplied with amino acids, the strain exhibited growth rates only slightly different from an isogenic wild-type strain under steady-state conditions (M. Cashel, personal communication, and Figs. 3 and 4).

Cashel and coworkers have generously provided us with the *relA*⁻ *spoT*⁻ strain so that we may directly test the requirement for ppGpp in growth rate-dependent control of rRNA synthesis. We confirm here that the double mutant lacks ppGpp. As expected, the strain is relaxed for stringent control. However, it is still able to regulate the synthesis of rRNA in a growth rate-dependent manner indistinguishable from that of the parental wild-type strain, suggesting that the two control systems utilize different mechanisms.

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MATERIALS AND METHODS

Bacterial Strains. Strains are listed in Table 1. The *relA251* allele (28) is a substitution of a kanamycin cassette for the *relA* locus. The DNA sequence of the *spoT* operon has been published (27). The *spoT207* allele is a substitution of a chloramphenicol cassette for 78% of the ORF 2 (*spoT*) open reading frame (positions 701–2354; ref. 27 and M. Cashel, personal communication). The *spoT209* allele is a substitution of a chloramphenicol cassette for all of ORF 1 (the ω gene), ORF 2 (*spoT*), and a portion of ORF 3 (*spoU*) (positions 6–2584; ref. 27 and M. Cashel, personal communication).

Growth Conditions. λ lysogens were grown at 30°C and all other strains at 37°C. Different growth rates were obtained by using LB or defined media [minimal A medium (33) plus 0.4% glucose, 10 μg of thiamine per ml, and supplements as indicated below]. Average growth rates (expressed as μ, doubling per hr) are given for the wild-type strain at 30°C: (A) Defined medium with each of the 20 amino acids at 40 μg/ml ($\mu \approx 0.66$); (B) A with serine at 500 μg/ml ($\mu \approx 0.71$); (C) A without amino acids but with 0.5% Casamino acids and tryptophan at 40 μg/ml ($\mu \approx 1.11$); (D) C with uracil at 50 μg/ml ($\mu \approx 1.15$); (E) C with 0.5% yeast extract ($\mu \approx 1.39$); (F) LB ($\mu \approx 1.40$). Cells were transferred from fresh plates to medium A (described above), grown overnight (12–16 hr), and then diluted to an OD_{600} <0.05 in the different media listed. Cells were grown exponentially for at least three to four generations to ensure balanced growth before sampling. The *relA*$^-$ *spoT*$^-$ strain grew about 12% more slowly than the wild type on the average, but uniformity of the culture with respect to growth, colony color (of *lacZ* fusions), and amino acid auxotrophy was reconfirmed at the conclusion of experiments. Uninduced β-galactosidase activity from the host allele in *lac*$^+$ strains was <0.5% of that from the *rrnB P1* fusion at the lowest growth rate. For evaluating the stringent response, MOPS medium (34) containing 4 mM K_2HPO_4, 50 μg of uracil per ml, and all 20 amino acids (each at 40 μg/ml) was used. Growth rates of strains in balanced growth (see above) were calculated by measuring OD_{600} of the cultures with a Beckman DU-40 spectrophotometer.

ppGpp Analysis. ppGpp was detected by HPLC as described (35). The final concentration of the elution buffer was set empirically by dilution with H_2O until a good separation of the ppGpp from the accompanying peaks was achieved.

Measurement of Growth Rate Dependence, Total Cellular Protein, and β-Galactosidase Activity. At OD_{600} 0.5–0.6, cells (10 ml) in balanced growth were chilled on ice, collected by centrifugation (5000 rpm, 10 min), washed, and resuspended in 15 mM Tris·HCl/4 mM EDTA/4 mM dithiothreitol. Cell extracts were prepared by sonication. Aliquots were used to measure RNA content, total protein, and β-galactosidase activity essentially as described (31). RNA was determined by the orcinol reaction (36), with yeast RNA as a standard. Protein content was measured by the Bradford method (37) with a kit (Bio-Rad), using bovine serum albumin as a standard. β-Galactosidase activity was assayed according to Miller (33). Linear regressions (Figs. 3 and 4) were performed using SIGMAPLOT (Jandel Scientific, Sausalito, CA). Slopes and standard errors were calculated using STATISTIX II (NH Analytical Software, Roseville, MN).

Stringent Response. After growth at 37°C to an OD_{600} of $\approx$0.2, two 0.5-ml aliquots were removed to prewarmed test tubes, and 5 μl of $^{32}P_i$ (H_3PO_4, 1 mCi/ml; DuPont; 1 mCi = 37 MBq) was added (time 0). After 10 min with continued shaking, amino acid starvation was induced by addition of lysine hydroxamate (ref. 38; Sigma) to 400 μg/ml. Samples (100 μl) were removed at 10, 20, 30, and 40 min into 5 ml of ice-cold 10% (wt/vol) trichloroacetic acid (TCA). After at least 30 min on ice, samples were passed through glass-fiber filters, the filters were washed with 5% TCA and dried, and

Table 1. Bacterial strains, phages, and plasmids

Name	Genotype	Source
Strains		
MG1655 (= CF1648)	Wild type	Ref. 29
CF1651 (= RLG850)	MG1655 *relA251*	Ref. 28
CF1693 (= RLG847)	MG1655 *relA251 spoT207*	M. Cashel
CF1678 (= RLG848)	MG1655 *relA251 spoT209*	M. Cashel
NK5031 (= RLG739)	Δ*lac*MS265 *supF* Nal^R	Ref. 30
RLG1231	CF1648 lysogen of λRLG1100	This work
RLG1229	CF1693 lysogen of λRLG1100	This work
RLG1235	CF1678 lysogen of λRLG1100	This work
RLG1242	CF1648 lysogen of λRLG1019	This work
RLG1248	CF1693 lysogen of λRLG1019	This work
RLG1252	CF1678 lysogen of λRLG1019	This work
RLG1262	CF1648 lysogen of λRLG479	This work
RLG1255	CF1693 lysogen of λRLG479	This work
RLG1259	CF1678 lysogen of λRLG478	This work
Strains carrying plasmids		
RLG1580	NK5031 lysogen of λRLG1100 carrying pBR322	This work
RLG1581	NK5031 lysogen of λRLG1100 carrying pNO1301	This work
RLG1582	NK5031 lysogen of λRLG1019 carrying pBR322	This work
RLG1583	NK5031 lysogen of λRLG1019 carrying pNO1301	This work
RLG1584	NK5031 *relA251 spoT207* lysogen of λRLG1100 carrying pBR322	This work
RLG1585	NK5031 *relA251 spoT207* lysogen of λRLG1100 carrying pNO1301	This work
RLG1586	NK5031 *relA251 spoT207* lysogen of λRLG1019 carrying pBR322	This work
RLG1587	NK5031 *relA251 spoT207* lysogen of λRLG1019 carrying pNO1301	This work
λ phages carrying promoter–*trpB'A lacZ* fusions		
λRLG1100	Carries *rrnB P1* promoter (−88 to +1)	Ref. 30
λRLG1019	Carries *rrnB P1* [C-1T,C-15G] promoter	Ref. 30
λRLG479	Carries *spc* promoter	Ref. 31
Plasmids		
pBR322	ColE1 derivative	Ref. 32
pNO1301	pBR322 carrying *rrnB* operon	Ref. 9

the retained radioactivity was determined by Cerenkov counting. Control cultures were followed by OD_{600}. The addition of lysine hydroxamate inhibited cell growth of wild-type, *relA*$^-$, and *relA*$^-$ *spoT*$^-$ strains to the same extent. ^{32}P counts incorporated into TCA-precipitable material were corrected for optical density at each time point.

RESULTS

Rationale. The *relA*$^-$ *spoT*$^-$ double mutant strain offers a direct means of testing the involvement of ppGpp in the regulation of stable RNA synthesis *in vivo*. If ppGpp is the only direct effector of growth rate control, then in the absence of ppGpp, neither stringent nor growth rate control should operate, and rRNA synthesis should continue at a high unregulated level independent of amino acid starvation or of changes in growth rate. We first confirmed the absence of ppGpp and then measured stringent control, growth rate dependence, and feedback regulation of the mutant strain.

Detection of ppGpp. Wild-type MG1655 and its *relA251 spoT209* derivative (CF1678) were grown at low and high growth rates. Nucleotides were extracted and analyzed by ion-pair reverse-phase HPLC (35) and compared to a ppGpp standard. Fig. 1 shows the appropriate portions of the chromatograms. The same amount of cell extract (250 μl) was loaded in each case. As shown previously (18), wild-type strains contain detectable amounts of ppGpp during steady-state growth, and this amount varies inversely with the growth rate. Our values of 73 pmol·OD_{600}^{-1}·ml^{-1} (± 10%) and 22 pmol·OD_{600}^{-1}·ml^{-1} (± 30%), respectively, are in good agreement with the values published previously (18). There was no detectable peak corresponding to ppGpp at either growth rate in the *relA*$^-$ *spoT*$^-$ strain. The same result was obtained with a second double mutant strain, *relA251 spoT207* (CF1693). Guanosine 3′-diphosphate 5′-triphosphate (pppGpp) was also undetectable in the double mutant strain (M. Cashel, personal communication). We note that there are a number of differences in the elution profiles of wild-type and double mutant strains growing at the same growth rate. We have not investigated these differences further. We would probably not have been able to detect ppGpp at much less than about 4 pmol·OD_{600}^{-1}·ml^{-1}, 5% of the low growth-rate level, or 0.4% of the amount present after amino acid starvation.

RNA Synthesis after Amino Acid Starvation. Since the double mutant strain requires several amino acids for growth (M. Cashel, personal communication), we induced amino acid starvation by the addition of an amino acid analog, lysine hydroxamate, a competitive inhibitor of the lysyl-tRNA synthetase (38). Since stable RNA is such a large fraction of total RNA synthesis (39), ^{32}P incorporation into total RNA was used as a measure of stable RNA synthesis. The wild-type strain showed the expected stringent response, whereas the *relA*$^-$ and *relA*$^-$ *spoT*$^-$ mutant strains exhibited typical relaxed responses (Fig. 2).

RNA Synthesis During Steady-State Growth. To compare the growth rate-dependent control of rRNA synthesis in the wild-type and double mutant strains, we measured their RNA/protein ratios. Since rRNA and tRNA are stable whereas mRNA under normal conditions is not, total RNA levels can be used as a measure of rRNA transcription (39). Since the double mutant strain is polyauxotrophic, all 20 amino acids were always supplied (either from individual amino acid mixes or from Casamino acids and tryptophan), and we achieved different growth rates by supplementing the medium with extra serine, uracil, yeast extract, or LB. The lag period from stationary phase before establishment of maximal growth rate was somewhat longer for the double mutant strain than for the wild type, but after establishment of steady-state growth, growth rates differed on the average by only about 12% (data not shown). Fig. 3 includes the

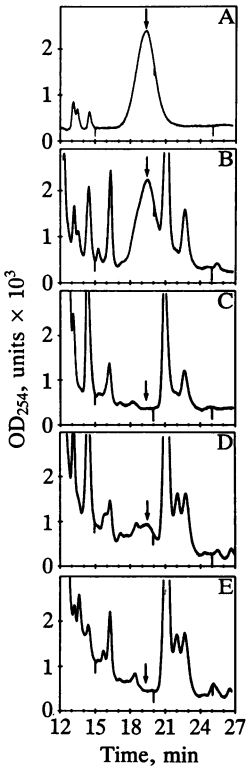

FIG. 1. HPLC elution profiles of extracts from strains CF1648 (wild type) and CF1678 (*relA*$^-$ *spoT*$^-$) at high and low growth rates. Extracts were obtained and chromatographed as described in *Materials and Methods*. Arrow indicates ppGpp. (*A*) Commercially prepared ppGpp (320 pmol; ICN). (*B*) Wild type, low growth rate. (*C*) *relA*$^-$ *spoT*$^-$, low growth rate. (*D*) Wild type, high growth rate. (*E*) *relA*$^-$ *spoT*$^-$, high growth rate.

results of three independent experiments. RNA/protein ratios in the double mutant strain displayed essentially the same growth rate dependence as in the wild type, implying that the regulatory mechanism affecting these ratios is unaffected by the absence of ppGpp.

rRNA Promoter Activity as a Function of Growth Rate. RNA/protein ratios reflect the cumulative effects of synthesis, processing, and decay of the RNA and protein products being measured. Therefore, it is formally possible that the two similar growth rate-dependence curves obtained above could reflect effects of compensating rRNA synthesis and decay rates in the double mutant strain. To control for possible interference from steps taking place after the initiation of transcription, we measured the growth rate regulation of an rRNA promoter by using single-copy *rrnB P1–lacZ*

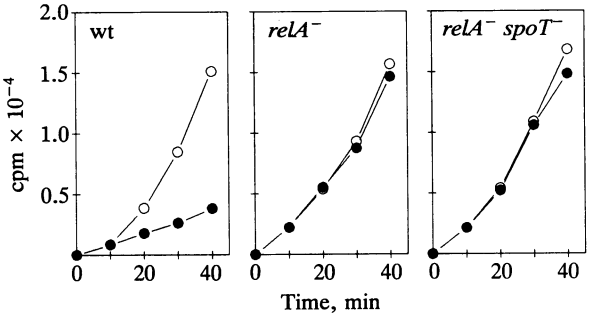

FIG. 2. Stringent control of wild-type (wt), *relA*$^-$, and *relA*$^-$ *spoT*$^-$ strains. Incorporation of ^{32}P into acid-precipitable material was measured as a function of time after addition of $^{32}P_i$. Amino acid starvation was induced by the addition of lysine hydroxamate at 10 min. ○, Unstarved cultures; ●, starved cultures.

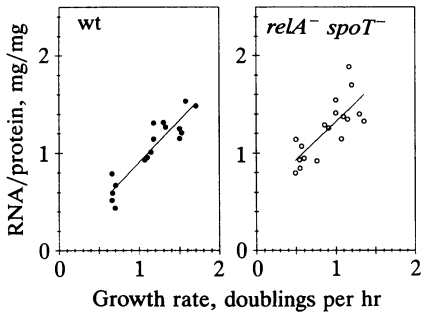

FIG. 3. RNA/protein ratios as a function of growth rate in the wild-type (wt) strain and the *relA*⁻ *spoT*⁻ mutant. Each point represents the average of two RNA and two protein measurements. The slope of the wild type is 1.02 ± 0.11, and the slope of the mutant is 0.92 ± 0.19.

operon fusions constructed previously (30) (see Table 1). In addition, this approach allowed the measurement of appropriate nonregulated promoters as negative controls.

λ lysogens were constructed in the wild-type (MG1655) and double mutant (CF1678) strains by using transducing phages from our collection of wild-type and mutant promoters (30). We used three well-characterized fusions to generate the following strains: RLG1231 and RLG1235 contain a growth rate-dependent rRNA promoter–*lacZ* fusion consisting of wild-type *rrnB P1* promoter sequences between positions −88 and +1 with respect to the transcription start site (30); RLG1242 and RLG1252 contain an *rrnB P1* promoter (C-1T,C-15G) with two mutations that render it growth rate-independent (8); and RLG1262 and RLG1259 contain a promoter from a ribosomal protein operon (*spc*) also shown previously to be growth rate-independent (7, 31).

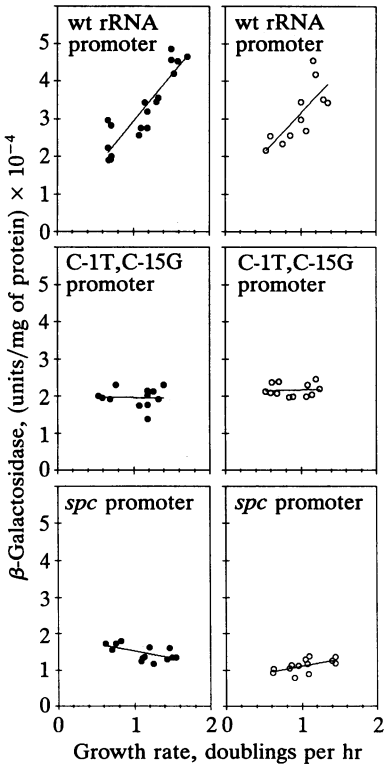

FIG. 4. Activities of promoter–*lacZ* fusions in wild-type (●) and *relA*⁻ *spoT*⁻ (○) strains as a function of growth rate. Each panel shows the results of three independent experiments. The identities of the strains and promoters used in each pair of fusions are indicated. For the wild-type (wt) rRNA promoter, the slope in the wild-type strain is 2.45 ± 0.29 and in the mutant strain is 2.15 ± 0.61. For the mutant (C-1T,C-15G) rRNA promoter, the slope in the wild-type strain is −0.02 ± 0.27 and in the mutant strain is 0.03 ± 0.21. For the *spc* promoter, the slope in the wild-type strain is −0.39 ± 0.16 and in the mutant strain is 0.39 ± 0.16.

Table 2. Feedback inhibition of *rrnB P1–lacZ* fusions by plasmids carrying extra rRNA genes

Host strain	Promoter	Ratio of β-galactosidase activities
Wild type	*rrnB P1*	0.65 ± 0.04
	C-1T,C-15G	0.92 ± 0.03
relA⁻ *spoT*⁻	*rrnB P1*	0.54 ± 0.02
	C-1T,C-15G	1.13 ± 0.01

Wild-type NK5031 or *relA*⁻ *spoT*⁻ NK5031 cells carrying a control plasmid (pBR322) or a plasmid with an intact *rrnB* operon (pNO1301) were inoculated directly from a fresh overnight plate into LB medium with ampicillin (100 μg/ml) and grown for three to four generations before β-galactosidase activities were measured. Values are the means of three experiments. The ratio of β-galactosidase activities (with standard deviation) refers to the activity of the strain carrying pNO1301/activity of the strain carrying pBR322.

Fig. 4 shows the growth rate dependence of the β-galactosidase activities obtained in three independent experiments for each fusion. In accord with the direct RNA measurements shown in Fig. 3, the wild-type *rrnB P1* promoter is growth rate-dependent both in the wild-type and in the double mutant strain. The control promoters are not growth rate-dependent in either strain, showing that our assay can distinguish between regulated and nonregulated promoters. Since the *spoT209* allele used above deletes all of the ω gene, all of *spoT*, and about half of the *spoU* open reading frame (ref. 27 and M. Cashel, personal communication), our results imply that growth rate-dependent control can occur in the absence of the ω factor as well as without ppGpp. In addition, experiments carried out in a second strain background (NK5031; ref. 30) or with double mutant strains carrying a different *spoT* deletion, *spoT207*, gave similar results to those observed in the *relA251 spoT209* MG1655 strain (data not shown). Finally, fusions containing the *rrnB P2* promoter or the *rac145* promoter (a mutant *rrnB P1* promoter with a 4-base-pair change in the spacer between the −10 and −35 consensus hexamers; refs. 7 and 8) were unregulated and regulated, respectively, in both the wild-type and double mutant strains, in accord with previous results and consistent with the conclusion that the lack of ppGpp does not affect growth rate control of rRNA transcription (data not shown).

Feedback Control. Cells provided with an increased rRNA gene dose reduce transcription from individual operons in order to keep the total rRNA synthesis rate constant (7, 9–11). The inhibitory effect of increased rRNA gene dose on the rate of transcription per gene was shown to be specific to stable RNA promoters (7). In order to ask whether the feedback mechanism is dependent on ppGpp, we tested the ability of the double mutant strain to inhibit β-galactosidase synthesis from an *rrnB P1–lacZ* fusion in the presence of a multicopy plasmid carrying an intact *rrnB* operon. An increase in rRNA gene dose decreased β-galactosidase activity by about 40% in both wild-type and double mutant strains (Table 2), comparable to the effect seen previously (7), and considerably greater than the effect on a control promoter–*lacZ* fusion not subject to growth rate control (C-1T,C-15G; Fig. 4 and ref. 8). Thus, ppGpp is not required for feedback regulation. Since we have shown that ppGpp is not required for growth rate-dependent control, the inhibition of the *rrnB P1* promoter–*lacZ* fusion and the lack of inhibition of the C-1T,C-15G promoter–*lacZ* fusion in the presence of increased rRNA gene dose are consistent with the hypothesis that the feedback system is the mechanism of growth rate control.

DISCUSSION

We have confirmed that *E. coli* deleted for the *relA* and *spoT* loci has no detectable ppGpp. Nevertheless, under steady-state growth conditions this double mutant strain regulates rRNA synthesis in a manner indistinguishable from that observed with the parental wild-type strain. Our conclusion that ppGpp is not required for growth rate control rests on the validity of two experimental approaches: measurement of RNA/protein ratios and promoter–*lacZ* fusion activities at different growth rates. While our data are internally consistent, our studies await further confirmation.

The above finding implies that if growth rate control and stringent control are achieved by a single mechanism, then this system does not require ppGpp; i.e., changes in ppGpp levels that parallel rRNA synthesis rates are coincidental but have no direct causal relationship. Alternatively, the two control systems utilize different mechanisms. In the latter case, ppGpp apparently is not required for growth rate control, but presumably it is required for the stringent response. The molecular mechanisms responsible for the control systems will have to be uncovered and the target sites and effector molecules identified before it will be possible to distinguish between these formal alternatives.

If ppGpp is not required for growth rate control, then why is there an inverse correlation between growth rate and ppGpp concentration? One possibility is that cells have evolved more than one way of controlling rRNA synthesis during steady-state growth, and ppGpp participates either directly or indirectly in one system but not in the other. In this situation, elimination of the ppGpp-synthesizing systems would not eliminate regulation of rRNA transcription initiation, even if ppGpp were normally used for this purpose in wild-type cells. A second formal possibility is that suppressor mutations were selected for during strain construction that allowed the cell to bypass the requirement for ppGpp. Transduction frequencies obtained during construction of double mutant strains do not suggest that this is likely (data not shown and M. Cashel, personal communication). Should our findings result from either redundancy of regulatory mechanisms (the first model) or a bypass by mutation (the second model), the double mutant still shows that growth rate regulation can be achieved without ppGpp.

Alternatively, the concentrations of ppGpp found in steady-state growth directly or indirectly regulate other macromolecular process(es), but not stable RNA transcription initiation. Such regulatory effects could occur at the level of transcription: numerous reports have implicated ppGpp as a negative effector of transcription elongation (40) or as a positive effector of nonstable RNA transcription initiation (41). In addition there have been reports of effects of ppGpp on translation initiation (42) or elongation (43). In this model, the *relA*-independent ppGpp synthesis mechanism responds to changes in growth rate, but the "basal" concentrations of ppGpp produced play no role in stable RNA transcription initiation. For example, one could imagine that ppGpp synthetase II, like ppGpp synthetase I, is responsive to the charged/uncharged tRNA ratio (20), or the variation in ppGpp with growth rate could be of use as a metabolic signal of growth to multiple cellular processes. Whether there are redundant systems for growth rate control or whether the variation in basal ppGpp levels is unrelated to the control of rRNA transcription initiation, our studies justify the continued search for molecular effectors of growth rate control of rRNA synthesis.

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