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mRNA Composition and Control of Bacterial Gene Expression

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The expression of any given bacterial protein is predicted to depend on (i) the transcriptional regulation of the promoter and the translational regulation of its mRNA and (ii) the synthesis and translation of total (bulk) mRNA. This is because total mRNA acts as a competitor to the specific mRNA for the binding of initiation-ready free ribosomes. To characterize the effects of mRNA competition on gene expression, the specific activity of β-galactosidase expressed from three different promoter-*lacZ* fusions (P_{spc}-*lacZ*, P_{RNAI}-*lacZ*, and P_{RNAII}-*lacZ*) was measured (i) in a *relA*$^+$ background during exponential growth at different rates and (ii) in *relA*$^+$ and Δ*relA* derivatives of *Escherichia coli* B/r after induction of a mild stringent or a relaxed response to raise or lower, respectively, the level of ppGpp. Expression from all three promoters was stimulated during slow exponential growth or at elevated levels of ppGpp and was reduced during fast exponential growth or at lower levels of ppGpp. From these observations and from other considerations, we propose (i) that the concentration of free, initiation-ready ribosomes is approximately constant and independent of the growth rate and (ii) that bulk mRNA made during slow growth and at elevated levels of ppGpp is less efficiently translated than bulk mRNA made during fast growth and at reduced levels of ppGpp. These features lead to an indirect enhancement in the expression of LacZ (or of any other protein) during growth in media of poor nutritional quality and at increased levels of ppGpp.

The expression of a bacterial gene can be studied by measuring the relative abundance of either its mRNA or its protein product. Intuitively these two methods might seem to be equivalent, but in fact they are not. Using artificially constructed promoter-*lacZ* fusions integrated into the *Escherichia coli* chromosome, we have previously determined the activities of a number of constitutive mRNA promoters expressed as *lacZ* transcripts per minute per promoter and as units of β-galactosidase activity (19). The transcriptional activities of these promoters increased with increasing growth rate, whereas the specific activity of β-galactosidase decreased. The rate of translation initiation of *lacZ* mRNA was found to be rather constant, with no indication of growth rate-dependent translational control (17). Therefore, the discrepancy was not caused by any control on the translation of the *lacZ* reporter mRNA.

What causes gene expression at the transcriptional and translational levels to respond in opposite directions to changes in the growth rate? The answer to this question is rather simple: the abundance in the cytoplasm of any given protein, or the specific activity of an enzyme (activity per amount of protein), reflects the distribution of translating ribosomes between the encoding mRNA and bulk mRNA. This distribution depends on two factors: (i) the relative amounts of the encoding mRNA and bulk mRNA and (ii) the translation frequencies of the encoding mRNA and bulk mRNA (see, e.g., reference 26).

In this report, we have considered the effects of bulk mRNA and free ribosomes on the synthesis of β-galactosidase expressed from three artificial promoter-*lacZ* fusions carrying the promoters for the *spc* ribosomal protein operon (P_{spc}), the pBR322 plasmid replication inhibitor RNAI (P_{RNAI}), and the pBR322 replication primer RNAII (P_{RNAII}). In previous studies involving measurements of transcripts by hybridization, P_{spc} and P_{RNAI} were found to be constitutive, without specific control; P_{RNAII} was positively regulated by ppGpp but was constitutive in the absence of ppGpp (19). The experiments presented here suggest to us that many poorly translated mRNAs (e.g., those with weak ribosome binding sites) accumulate during slow growth in poor media and, conversely, many frequently translated mRNAs (e.g., those with strong ribosome binding sites) accumulate during fast growth in rich media. This keeps the concentration of free ribosomes approximately constant as the growth rate increases, in spite of an increasing concentration of total ribosomes. Moreover, it produces an apparent stimulation in the expression of any given protein under conditions of slow growth or at increased intracellular levels of ppGpp. These results have implications for the expression of any bacterial gene, including the control of the synthesis of ribosomal RNA and ribosomal proteins, and for the interpretation of data obtained with reporter systems.

MATERIALS AND METHODS

Bacterial strains used. The *Escherichia coli* strains used in this work and their origins or constructions are described in Table 1. Fusions of *lacZ* with P_{spc} and the plasmid pBR322 promoters P_{RNAI} and P_{RNAII} were constructed as previously described (17, 19). The promoters were originally cloned in the plasmid vector pSL03 and then recombined into the *mal* locus of the chromosome of *lac* deletion derivatives of *E. coli* B/r (see Table 1 for details). pSL03 was derived from the W205 *trp-lac* operon fusion (24) from which the *trp* transcription terminator upstream of *lacZ* (25) has been deleted (17). The operon fusions carried the following promoter fragments: P_{spc}, from nucleotide (nt) −51 relative to the transcription start through *rplN* (the first gene of the *spc* operon, encoding ribosomal protein L14), ending at nt +453; P_{RNAI}, from nt −77 to +32; and P_{RNAII}, from nt −63 to +63. All promoter fragments carried *Eco*RI and *Bam*HI sites at their 5′ or 3′ ends, respectively, for insertion into the multiple cloning site of pSL03. For the experiment for which results are shown in Fig. 2, a *spc-lac*

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TABLE 1. Bacterial strains

E. coli strain	Genotype	Reference or construction
HB181	B/r A *malK*$^+$ Δ(*argF-lacIOZYA*) *phe*(*Am*) *thr*(*Am*) *hsdR*/M (K-12)	35
VH2732	MC4100 Δ*relA251*::*kan argA*::Tn*10 malB*::*malE'-rrnB* UAR-P1-ϕX174*E'-'lacZ*-T1T2-*kan*-T1T2-*'malK*[a]	12
XZ241	HB181 Δ*relA251*::*kan argA*::Tn*10 spoT*$^+$ *malB*::*malE'-rrnB* UAR-P1-box *BAC*-λ*'-lacZ*-T1T2-*kan*-T1T2-*'malK*	Zhang and Bremer, unpublished data; the Δ*relA251*::*kan argA*::Tn*10* alleles of VH2732 were transduced with phage P1 into XZ213 (reference 35), selecting for Tc^r and screening for the Rel$^-$ phenotype
SL104	HB181 *malB*::*malE'*-P_{spc}-*lacZ*-T1T2-*kan*-T1T2-*'malK*	17
SL106	HB181 *malB*::*malE'*-P_{spc}-*rplN-lacZ*-T1T2-*kan*-T1T2-*'malK*	17
SL111	SL104 Δ*relA251*::*kan*	The Δ*relA251*::*kan argA*::Tn*10* alleles from XZ241 were transduced with phage P1 into SL104, selecting for Tc^r and screening for the RelA phenotype; the strain was cured of Tn*10* and checked for Arg$^+$
YX101	HB181 *malB*::*malE'*-P_{RNAI}-*lacZ*-T1T2-*kan*-T1T2-*'malK*	Construction as described for SL104, except that a P_{RNAI} promoter fragment was cloned
YX102	HB181 *malB*::*malE'*-P_{RNAII}-*lacZ*-T1T2-*kan*-T1T2-*'malK*	Construction as described for SL104, except that a P_{RNAII} promoter fragment was cloned
YX103	YX101 Δ*relA251*::*kan*	The Δ*relA251*::*kan argA*::Tn*10* alleles from XZ241 were transduced with phage P1 into YX101, selecting for Tc^r and screening for the RelA phenotype; the strain was then cured of Tn*10* and checked for the Arg$^+$ phenotype
YX104	YX102 Δ*relA251*::*kan*	Construction as described for YX103, except that strain XZ102 was used as a recipient

[a] T1T2, transcription terminators of the *rrnB* operon.

operon fusion was used in which P_{spc} was directly linked to *lacZ* (from nt −51 to +59) without *rplN*. Previous studies have shown that strains in which *lacZ* is directly linked to P_{spc} may show an anomalous growth rate-dependent reduction in the accumulation of *lacZ* mRNA (17). The reason for this effect is not known; possibly, sequences close to the 5′ end of the *spc* operon transcript interact with sequences in the *trp-lac* mRNA leader to produce a fortuitous signal which either causes transcription termination or shortens the mRNA lifetime. Inclusion of *rplN* in the construct ensures that such effects are absent (17). Inclusion of additional sequences upstream of P_{spc} (up to 105 bp upstream of position −51) had no measurable effect on β-galactosidase expression; this suggests that the region upstream of P_{spc} is devoid of regulatory elements.

Conditions of growth. Cultures were grown at 37°C in medium C (11) supplemented with either 0.2% (vol/vol) glycerol or 0.2% (wt/vol) glucose, with or without 0.8% Difco Casamino Acids plus 50 μg of tryptophan/ml, or in Luria-Bertani (LB) medium (23) with 0.2% glucose. Minimal media were supplemented with phenylalanine and threonine as required, at 50 μg/ml. Experimental cultures were inoculated from overnight cultures in glycerol minimal medium by diluting at least 250-fold into minimal media or 2,000-fold into amino acid-supplemented media.

Growth was measured as the increase in turbidity at 600 nm with a 1-cm light path (optical density at 600 nm [OD_{600}]). Since the turbidity is not exactly proportional to the culture density, the observed values, after subtraction of the medium blank, were corrected for nonlinearity (2). The corrected OD values deviated by less than 1% from the average exponential curve, so that the inaccuracy of the average OD used for determination of the specific enzyme activity was about 1%.

β-Galactosidase assays. Assays for β-galactosidase activity were performed with four to five 10-μl samples of culture taken at different times during exponential growth as described previously (18). The specific activity of β-galactosidase was expressed as the increase in A_{420} per hour of incubation of the assay at 30°C per OD_{600} unit of culture in the assay. The specific activity values obtained from different samples of a given culture generally deviated from the average by less than 2%. Greater deviations, up to 10%, were sometimes observed in repeat experiments carried out with cultures grown on different days. The reproducibility of the assays can also be seen from Fig. 2c and d: the fact that, for growth without pseudomonic acid, all measured points lie on a straight line with a slope of 1.0 implies that the specific enzyme activity was exactly the same for all assays during threefold exponential growth of the culture.

RESULTS

Enzyme expression during exponential growth at different rates. The specific activities of β-galactosidase expressed from P_{spc}, P_{RNAI}, and P_{RNAII} in the ppGpp-proficient *E. coli* B/r strains SL106, YX101, and YX102 (Table 1; the promoter-*lacZ* fusions in these strains are integrated into the *mal* locus of the bacterial chromosome [see Materials and Methods]), respectively, were measured during exponential growth in different media (Fig. 1). For all three promoters, the specific activity of β-galactosidase decreased with increasing growth rate in the range between 1.0 and 3.0 doublings/h. The specific activities for P_{spc} and P_{RNAI} decreased in parallel, about 2.5-fold for the threefold increase in growth rate, whereas the specific activity for P_{RNAII} decreased more than fivefold over this range of growth rates (Fig. 1). Previous transcription assays with *E. coli*

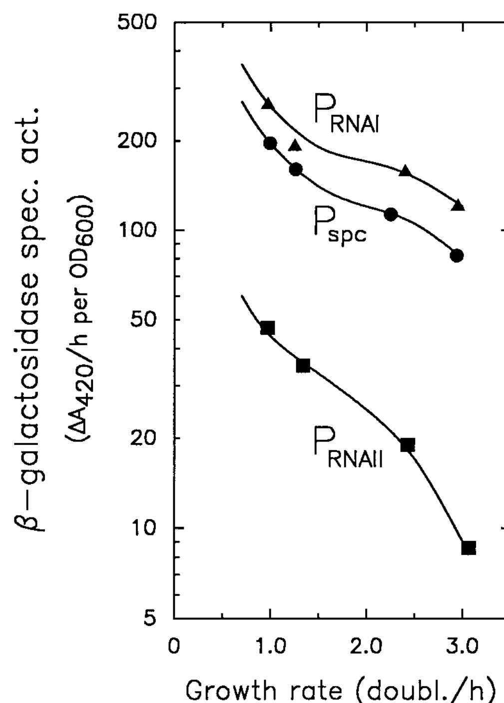

FIG. 1. Growth rate dependence of β-galactosidase expression from P_{spc}, P_{RNAI}, and P_{RNAII}. Strains SL106, YX101, and YX102, respectively, were used, and the growth media (given in order from the lowest to the highest growth rate) were glycerol minimal medium, glucose minimal medium, glucose-amino acids, and LB-glucose (see Materials and Methods).

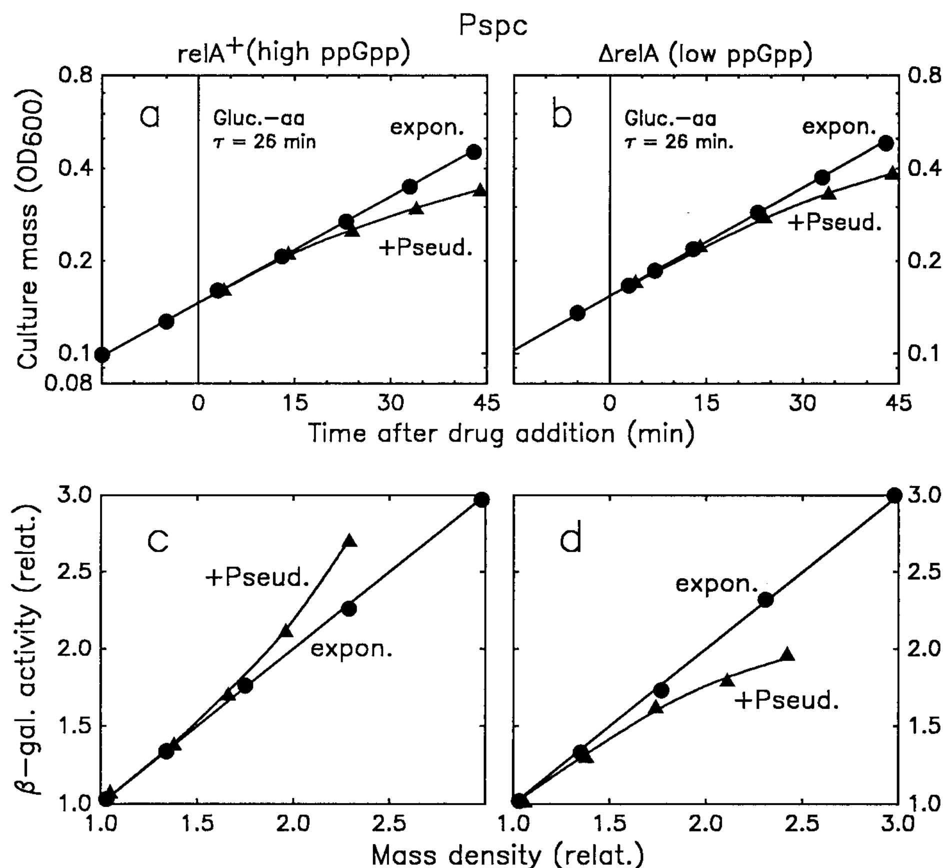

FIG. 2. β-Galactosidase expression from P_{spc} during the stringent and the relaxed response. The stringent (*relA*$^{+}$) strain SL104 (a and c) and the relaxed (Δ*relA*) strain SL111 (b and d) were grown exponentially (●) in glucose-amino acids medium. Mild amino acid deprivation was induced in part of the culture (▲) by treatment with 3 μg of pseudomonic acid/ml. (a and b) culture mass density (OD_{600}) as a function of time; (c and d) β-galactosidase activity plotted against cell mass density. Values are normalized to the β-galactosidase activity and culture mass (OD_{600}) observed at the time of drug addition. τ, doubling time.

K-12 strains showed that the activities of P_{spc} and P_{RNAI} are not significantly affected by the presence of ppGpp at the low ("basal") levels accumulating during slow exponential growth in ppGpp-proficient (*relA*$^{+}$ *spoT*$^{+}$) strains. In contrast, P_{RNAII} was stimulated by the low level of ppGpp under those conditions (19). Since cytoplasmic levels of ppGpp also decrease with increasing growth rate (31), the steeper decrease in the enzyme activity curve for P_{RNAII} in comparison to the curves for the other two promoters is consistent with positive control of P_{RNAII} by ppGpp. Results similar to those for P_{spc} in Fig. 1 have been reported previously (17); the data are shown here for the purposes of comparison and further analysis.

Enzyme expression during the stringent and relaxed response. The stimulation or reduction of the production of a protein under slow- or fast-growth conditions can be mimicked by artificially altering the intracellular level of ppGpp. Pseudomonic acid is a competitive inhibitor of isoleucyl tRNA synthetase and, at low concentrations, causes mild amino acid deprivation (14). A *relA*$^{+}$ and a Δ*relA* strain carrying P_{spc}-*lacZ* operon fusions (strains SL104 and SL111, respectively [Table 1]) were treated with pseudomonic acid. Culture mass accumulation was always reduced by the addition of pseudomonic acid (Fig. 2a and b). Under these conditions, expression of *lacZ* from P_{spc} was stimulated by a high ppGpp concentration (the stringent response [Fig. 2c]) and reduced by a low ppGpp concentration (the relaxed response [Fig. 2d]). As during exponential growth, the expression of *lacZ* from this promoter correlates with the ppGpp concentration.

The relaxed response can also be induced in *relA*$^{+}$ strains by treatment with chloramphenicol (5, 16). When a culture of the *relA*$^{+}$ strain SL104 was treated with chloramphenicol at a low concentration (1 μg/ml), the accumulation of β-galactosidase activity relative to the accumulation of total culture mass decreased in the same manner as that observed during mild amino acid starvation of a Δ*relA* strain (data not shown).

Amino acid deprivation experiments as shown in Fig. 2 for P_{spc} were also performed with *relA*$^{+}$ and Δ*relA* strains carrying a P_{RNAI}-*lacZ* or a P_{RNAII}-*lacZ* fusion (strains YX101 through YX104). Again, enzyme expression was stimulated when the level of ppGpp was raised and was inhibited when the ppGpp level was lowered (Fig. 3). However, the stimulation during the stringent response and the inhibition during the relaxed response were exaggerated with the P_{RNAII}-*lacZ* strains in comparison to those for the P_{RNAI} strains. Apparently, changes in the level of ppGpp during a mild stringent or a relaxed response affect enzyme expression from P_{RNAI} and P_{spc} (Fig. 2 and 3a and b) only indirectly, whereas enzyme expression from P_{RNAII} (Fig. 3c and d) is, in addition, stimulated by a direct effect of ppGpp on transcription. Together, the direct and indirect effects produce the stronger response of β-galactosidase expression from P_{RNAII} in comparison to those from the other two promoters, whose transcriptional activity is not affected by ppGpp during exponential growth (19).

Experiments similar to those for which results are shown in Fig. 2 and 3 were carried out with *lacZ* fusions carrying the phage λ promoter (P_L) and the β-lactamase promoter (P_{bla}),

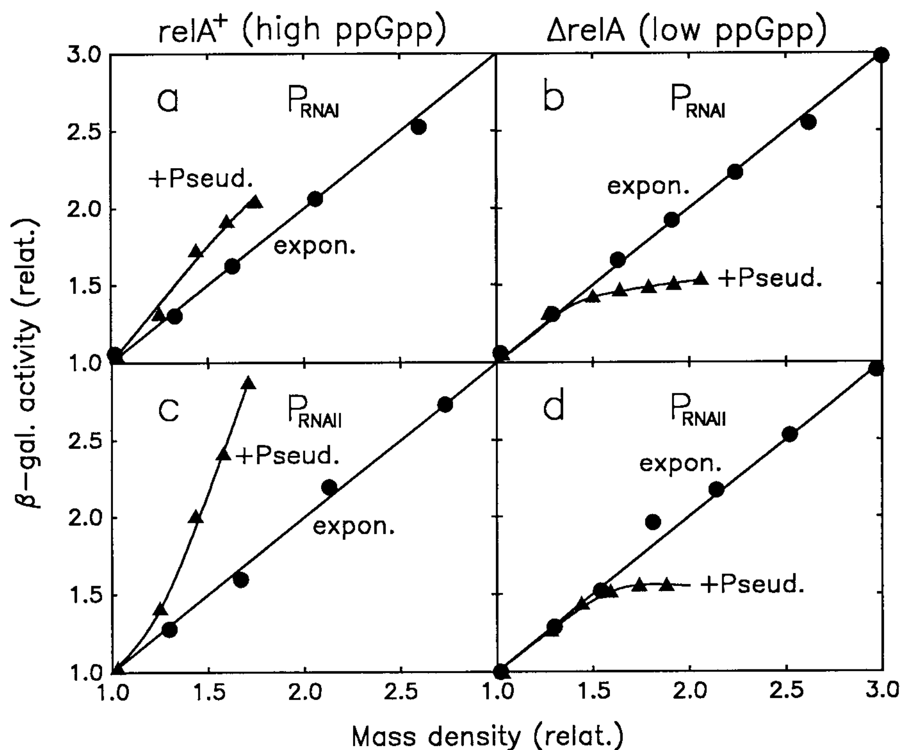

FIG. 3. β-Galactosidase expression from P_{RNAI} and P_{RNAII} during the stringent and the relaxed response. Stringent and relaxed strains carrying P_{RNAI}-*lacZ* and P_{RNAII}-*lacZ* operon fusions were grown exponentially in glucose minimal medium and treated with pseudomonic acid (3 μg/ml), as described in the legend for Fig. 2. (a and b) P_{RNAI}-*lacZ* fusion strains YX101 (*relA*$^+$) and YX103 (Δ*relA*); (c and d) P_{RNAII}-*lacZ* fusion strains YX102 (*relA*$^+$) and YX104 (Δ*relA*). The culture mass (OD_{600}) values and β-galactosidase activities were normalized to the values observed at time zero (addition of pseudomonic acid); only the differential plots, of β-galactosidase activity versus culture mass, are shown.

whose transcription is also not significantly affected by ppGpp during exponential growth (19). During the stringent and the relaxed response, the expression of *lacZ* from the promoters showed the same apparent positive correlation to the ppGpp concentration as that observed for P_{spc} and P_{RNAI} (data not shown). Since P_{spc}, P_{RNAI}, P_L, and P_{bla} are not known to have any common control elements, it is unlikely that the effect of ppGpp on enzyme expression is specific for these particular promoters. Rather, the effect is more likely to be related to general physiological aspects of bacterial growth that are affected by ppGpp (see Discussion).

DISCUSSION

Translational competition between bulk mRNA and specific mRNA. In the following, we consider the effect of total (bulk) mRNA and its translation on enzyme expression during exponential growth in different media. For this purpose, the expression of β-galactosidase from P_{spc} (Fig. 1) was chosen as an example. The same logic applies to the expression of any stable protein, whether it is measured by enzyme assays, radioactive labeling, protein staining, Western blotting, or some other means. This discussion requires additional information about *spc* promoter activity, bulk mRNA synthesis, total RNA synthesis, and protein synthesis. This information was obtained from previous measurements in the same *E. coli* B/r background and is summarized (with references) in Table 2.

During exponential growth, the relative amounts of stable components reflect their relative synthesis rates; e.g., the specific activity of β-galactosidase in the cytoplasm, expressed as the amount of enzyme as a proportion of total protein (β-Gal/P_t), equals the quotient of the rates of synthesis of β-galactosidase and total protein [(dβ-Gal/dt)/(dP_t/dt)]. If *lacZ* mRNA and average bulk mRNA were translated with equal efficiencies (equal translation initiations per minute per mRNA molecule), then the ratio of the synthesis rate of β-galactosidase to that of total protein (i.e., the specific activity) would reflect the proportion of *lacZ* mRNA in total (bulk) mRNA (R_{lac}/R_m). However, since *lacZ* mRNA and bulk mRNA may be translated differently, one has to consider the translation rate of *lacZ* mRNA [(dβ-Gal/dt)/R_{lac}] relative to the average translation rate of total mRNA [(dP_t/dt)/R_m]. Using these parameters and notations, the specific activity of β-galactosidase can be related to the transcription and translation of *lacZ* and bulk mRNA as follows:

$$\beta\text{-Gal}/P_t = d\beta\text{-Gal}/dP_t = (R_{lac}/R_m) \cdot \{[(d\beta\text{-Gal}/dt)/R_{lac}]/[(dP_t/dt)/R_m]\} \quad (1)$$

The first factor of the product on the right side of the equation (R_{lac}/R_m) reflects the relative abundance of *lacZ* mRNA, and the second factor, {[(dβ-Gal/dt)/R_{lac}]/[(dP_t/dt)/R_m]}, reflects the translation efficiency of *lacZ* mRNA relative to that of bulk mRNA.

The amount of *lacZ* mRNA (R_{lac}, in relative units) expressed from P_{spc} in *E. coli* B/r has been determined previously with hybridization assays applied to given amounts of total RNA (R_t) (17). As a consequence, R_{lac}/R_t, rather than R_{lac}/R_m, is the parameter actually observed. To obtain R_{lac}/R_m, the observed R_{lac}/R_t, is divided by the fraction of total RNA that is mRNA (R_m/R_t). Since no hybridization probe is available for

TABLE 2. Parameters related to *lacZ* expression from P_{spc} in strain SL106[a]

Parameter	Symbol	Unit[b]	Interpolated value at the indicated μ (τ)[c]					
			0.6 (100)	1.0 (60)	1.5 (40)	2.0 (30)	2.5 (24)	3.0 (20)
Total protein[d]	P_t/OD	10^{16} aa/OD_{460}	58	55	51	48	45	40
Total RNA[d]	R_t/OD	10^{16} nt/OD_{460}	3.3	3.8	4.4	5.3	6.3	6.7
β-Gal sp act[e]	β-Gal/OD	(ΔA_{420}/h)/OD_{600}	280	195	140	120	105	80
β-Gal sp act[f]	β-Gal/P_t	(ΔA_{420}/h)/10^{16} aa	3.0	2.2	1.7	1.6	1.5	1.3
lacZ mRNA/total RNA[g]	R_{lac}/R_t	Relative units	1.20	1.18	1.15	1.10	1.05	1.00
lacZ mRNA translation rate[h]	$(d\beta\text{-Gal}/dt)/R_{lac}$	Relative units	0.31	0.32	0.30	0.30	0.29	0.26
Stable RNA synthesis rate[i]	r_s/r_t	Fraction	0.41	0.52	0.68	0.78	0.85	0.90
Stable RNA synthesis rate[j]	r_s/OD	10^{14} nt/min/OD_{460}	2.7	5.1	8.8	14.4	21.2	27.3
mRNA synthesis rate[k]	r_m/r_t	Fraction	0.59	0.48	0.32	0.22	0.15	0.10
mRNA synthesis rate[l]	r_m/OD	10^{14} nt/min/OD_{460}	3.9	4.7	4.2	4.1	3.7	3.0
mRNA avg life[m]	τ_m	min	1.9	2.0	2.1	2.2	2.3	2.4
mRNA/OD[n]	R_m/OD	10^{14} nt/OD_{460}	7.4	9.4	8.8	9.0	8.6	7.3
mRNA/total RNA[o]	R_m/R_t	Fraction	0.022	0.025	0.020	0.017	0.014	0.0011
lacZ mRNA/total mRNA[p]	R_{lac}/R_m	Relative units	54	47	57	65	76	92
Protein synthesis rate/total RNA[q]	$(dP/dt)/R_t$	aa/min/nt	0.12	0.17	0.20	0.21	0.21	0.21
Peptide chain elongation rate[r]	c_p	aa polymerized/active ribosome	13	18	22	22	22	22
Distribution of ribosomes on mRNA[s]	d_r	nt/ribosome	143	160	129	108	88	70
Avg protein synthesis rate/mRNA[t]	$(dP/dt)/R_m$	aa/min/nt	5.5	6.8	10.0	12.4	15.1	19.0
mRNA translation rate[u]	$(di/dt)/R_m$	translations/min/mRNA	16	20	30	37	45	57

[a] Values are interpolated from observed data to match growth rate values in reference 4, Tables 2 and 3.

[b] aa, amino acids.

[c] μ, growth rate, expressed as doublings per hour; τ, doubling time in minutes.

[d] Per OD_{460} unit of culture mass (2).

[e] Per OD_{600} unit (Fig. 1) (17). The value at 0.6 doubling/h has been obtained by extrapolation and is consistent with similar data from *E. coli* K-12 strains, which grow more slowly in glycerol minimal medium than B/r strains (19).

[f] Per amount of protein, calculated as $(\beta\text{-Gal}/OD_{600})/(1.6\ P_t/OD_{460})$; the factor 1.6 converts OD_{460} units into OD_{600} units (2).

[g] Amount of *lacZ* mRNA per amount of total RNA in relative units, normalized to the hybridization value observed in LB medium at 3.0 doublings/h, which was set at 1.0 (17).

[h] Calculated as $(\ln 2/\tau) \cdot (\beta\text{-Gal}/P) \cdot (P_t/R_t)/(R_{lac}/R_t)$.

[i] Rate of stable RNA (rRNA plus tRNA) synthesis as a fraction of total RNA synthesis rate (30; Table 3 of reference 4). The value at 3.0 doublings/h was obtained by extrapolation.

[j] Per OD_{460} unit of culture mass; calculated as $(\ln 2/\tau) \cdot 0.98 \cdot 1.2 \cdot R_t$. The factors 0.98 and 1.2 reflect the facts that 98% of total RNA is stable RNA (about 2% is mRNA; see values for R_m/R_t below) and 20% of stable RNA precursors are rapidly degraded spacers.

[k] As a fraction of total RNA synthesis rate, calculated as $1 - r_s/r_t$.

[l] Per OD_{460} unit of culture mass; calculated as $r_s/\text{OD} \cdot (r_m/r_t)/(r_s/r_t)$.

[m] The average functional life of mRNA is assumed to be equal to the average functional life of *lacZ* mRNA (18) (see the text). For *E. coli* B/r in glucose minimal medium, the average life of total mRNA has previously been estimated from pulse-labeling data to be about 1 min (1).

[n] Amount of total mRNA/OD_{460} unit of culture mass; calculated as $(r_m/\text{OD}) \cdot \tau_m$.

[o] Calculated as $(R_m/\text{OD})/(R_t/\text{OD})$.

[p] Calculated as $(R_{lac}/R_t)/(R_m/R_t)$.

[q] Calculated as $(\ln 2/\tau) \cdot (P_t/R_t)$.

[r] Calculated as $(dP/dt)/R_t \cdot 4{,}566/(0.84 \cdot 0.85 \cdot 60)$. The factor 4,566 is the number of rRNA nucleotides per ribosome; 0.84 is the fraction of total RNA that is rRNA (14% is tRNA, and 2% is mRNA); 0.85 is the fraction of total ribosomes that is active at any given time; and 60 is the number of seconds per minute (see Table 3 in reference 4 for details).

[s] Average distance in mRNA nucleotides between translating ribosomes for average (bulk) mRNA; calculated as $60 \cdot c_p/[(dP/dt)/R_m]$.

[t] Calculated as $[(dP/dt)/R_t]/(R_m/R_t)$.

[u] Average rate of initiation (i) of translation (initiations per minute) per mRNA molecule; calculated as $3 \cdot (dP/dt)/R_m = 60 \cdot 3 \cdot c_p/d_r$, where the factor 3 represents the coding ratio (3 nt per amino acid) and the factor 60 is the number of seconds in a minute.

total mRNA, R_m/R_t was found indirectly. By using a hybridization probe for rRNA with pulse-labeled total RNA and correcting for the synthesis of tRNA, the rate of stable RNA synthesis (sum of rRNA plus tRNA) has previously been determined as a fraction of the rate of total RNA synthesis (r_s/r_t) (31). From r_s/r_t, the rate of mRNA synthesis was found, also as a fraction of the rate of total RNA synthesis, as the difference $r_m/r_t = (1 - r_s/r_t)$. By combining r_m/r_t with the average mRNA lifetime, τ_m, the ratio of the amounts, R_m/R_t, can be obtained. Using published data for R_{lac}/R_t, r_s/r_t, and τ_m, and additional data on the macromolecular composition of *E. coli* B/r (i.e., total RNA and protein), all parameters occurring in equation 1 above have been calculated (Table 2; for details and references, see table footnotes) and plotted as functions of the growth rate (Fig. 4). It can be seen that the specific activity of β-galactosidase decreases (Fig. 4a) even though the amount of *lacZ* mRNA as a proportion of total mRNA (R_{lac}/R_m) increases (Fig. 4b), and the rate of translation initiation per *lacZ* mRNA $[(d\beta\text{-Gal}/dt)/R_{lac}]$ is approximately constant (Fig. 4c). This apparent discrepancy is explained by the higher rate of translation of bulk mRNA at high growth rates (Fig. 4d). In other words, the specific activity of β-galactosidase decreases with increasing growth rate (Fig. 4a), despite an increasing abundance of its mRNA (Fig. 4b), mainly because of an increasing translation of bulk mRNA (Fig. 4d). The reason for this increased translation of bulk mRNA is discussed below.

The rate of translation of bulk mRNA $[(dP_t/dt)/R_m]$ was calculated both as the number of amino acid residues polymerized into polypeptides per minute per mRNA nucleotide (Table 2) and as the number of translation initiations per minute per average mRNA molecule $[(di/dt)/R_m]$ (Table 2; Fig. 4d). The latter was obtained from the former by multiplication by the coding ratio, 3 nt per amino acid residue. For example, according to Fig. 4d, an average mRNA molecule is translated almost once every second at a growth rate of 3 doublings/h.

Decay of bulk mRNA. In Table 2, the average life of total (bulk) mRNA (τ_m) has not been observed. The decay of total mRNA is expected to be complex, with higher-order kinetics, reflecting at first the fast decay of the least-stable mRNAs and later the slower decay of the more-stable mRNAs. Since no

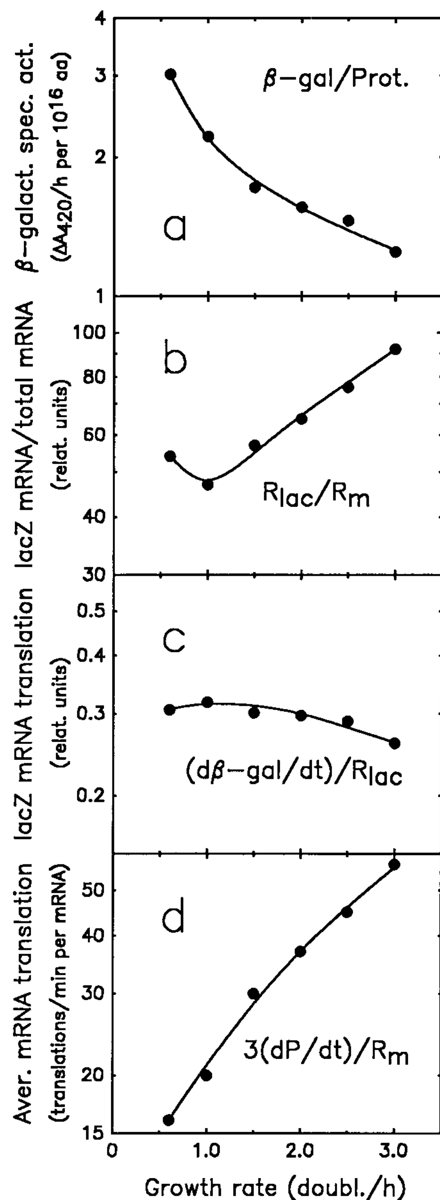

FIG. 4. Growth rate dependence of the β-galactosidase specific activity expressed from P_{spc} in strain SL106 and of transcription and translation parameters that determine this specific activity (see the text and equation 1). (a) β-Galactosidase specific activity; (b) relative abundance of *lacZ* mRNA as a proportion of total mRNA; (c) rate of translation initiation of *lacZ* mRNA in relative units; (d) average rate of translation initiation of total (bulk) mRNA in translations per minute per average mRNA molecule. This rate can be found either from the total rate of protein synthesis per amount of mRNA [$(dP_t/dt)/R_m$] or from the peptide chain elongation rate (c_p) and the average distance of ribosomes on the mRNA (d_r [see Table 2, footnote u]). Data represent interpolated values taken from Table 2 (see Table 2, footnotes f, p, h, and u, for the data in panels a, b, c, and d, respectively).

data about the growth rate dependence of the decay of total mRNA are available, we have used instead the average functional lifetimes of *lacZ* mRNA, which are 1.9 and 2.4 min in *E. coli* B/r growing at 1 and 3 doublings/h, respectively (18). These lifetimes were assumed to be representative for bulk mRNA. If the lifetimes of different mRNA species differ by constant factors from the *lacZ* mRNA lifetimes (i.e., a different factor for each mRNA species, but the same mRNA species-dependent factor for all growth rates), then the curves for R_{lac}/R_m (Fig. 4b) and $(dP_t/dt)/R_m$ (Fig. 4d) would shift in parallel either up or down, depending on whether the average τ_m values for bulk mRNA are greater or smaller than those of *lacZ* mRNA. However, if the τ_m of bulk mRNA increases with the growth rate more than the τ_m of *lacZ* mRNA, then the curves in Fig. 4b and d would increase less steeply. The lifetime of ribosomal protein mRNAs does indeed appear to increase with increasing growth rate more than the lifetime of *lacZ* mRNA (18); therefore, the relative abundance of *lacZ* mRNA (Fig. 4b) and the translation of bulk mRNA (Fig. 4d) might increase somewhat less steeply with the growth rate than is shown.

Translation frequency of *lacZ* mRNA at different growth rates. The approximately constant rate of translation of *lacZ* mRNA (Fig. 4c) could have several explanations: either (i) the *lacZ* ribosome binding site is saturated with ribosomes and initiation factors at all growth rates, (ii) the translation of *lacZ* mRNA is subject to a specific control that keeps it constant at all growth rates, or (iii) the concentrations of "initiation-ready" free ribosomes are nearly constant at all growth rates. Since the rate of translation of *lacZ* mRNA increases severalfold in the presence of the antibiotic rifampin when the amount of total mRNA decreases due to the inhibition of transcription (18), we conclude that, during normal exponential growth, the *lacZ* ribosome binding site is not saturated. Moreover, despite extensive study, there is no evidence to suggest that translation of *lacZ* mRNA is subject to any specific control. This leads us to suggest that the concentration of initiation-ready ribosomes (i.e., mature 30S ribosomal subunits with IF3, ready to bind to an mRNA ribosome binding site in the presence of saturating or constant concentrations of IF1, IF2, and initiator tRNA) is relatively constant and subsaturating at different growth rates. This causes the observed constant frequency of *lacZ* mRNA translation (Fig. 4c).

Translation frequency of bulk mRNA at different growth rates. Several features could contribute to the increasing average rate of translation initiation of bulk mRNA at increasing growth rates (Fig. 4d) despite a constant concentration of free, initiation-ready ribosomes and/or factors (see above). First, the average mRNA present during growth in rich media may have more-efficient ribosome binding sites than mRNA made during growth in poor media. Alternatively or in addition, the rate of translation of some mRNAs may be controlled by special regulatory sites such that translation is favored at high growth rates. The second possibility, i.e., control that favors translation at high growth rates, has been found to occur for ribosomal protein mRNAs (reviewed in reference 15), but it is not known to be the rule for most other mRNAs. Therefore, both more-efficient ribosome binding sites on constitutive mRNAs made during growth in rich media compared to more-repressible mRNAs made during growth in poor media and translational control of certain mRNAs are inferred to contribute to the increased translation of bulk mRNA at high growth rates. Whatever the exact cause, the increased translation makes bulk mRNA an increasingly better competitor for ribosome binding to *lacZ* mRNA as the population of mRNAs changes with increasing growth rate.

Effect of ppGpp on protein and enzyme expression. During a mild stringent or a mild relaxed response, the β-galactosidase specific activities expressed from both P_{spc}-*lacZ* and P_{RNAI}-*lacZ* operon fusions were increased and reduced, respectively (Fig. 2 and 3). Based on the analysis of the growth rate dependence of enzyme expression above, we suggest that ppGpp affects the accumulation and quality of bulk mRNAs and thereby causes the apparent positive control of enzyme expression by ppGpp. Indirect stimulation of *lacZ* expression by ppGpp (Fig. 2 and 3) could be produced in a number of ways. For

example, ppGpp might directly or indirectly stimulate the synthesis of mRNAs with weak ribosome binding sites, or it might inhibit the synthesis of mRNAs with strong ribosome binding sites. Indirect effects of ppGpp on transcription are expected for promoters controlled by DNA binding factors like Fis, H-NS, or Lrp (see, e.g., references 9, 29, 34 and 36), whose syntheses depend on ppGpp. LacZ expression may also be increased if ppGpp stimulates the decay of mRNAs with strong ribosome binding sites (e.g., ribosomal protein mRNAs [18]). Any one or all of these effects might contribute to the apparent stimulation of enzyme expression by ppGpp.

Combined effects of growth rate and ppGpp on bacterial gene expression. The effects of ppGpp and growth rate on bulk mRNA synthesis and translation are superimposed on the direct transcriptional control by ppGpp. Three cases are to be distinguished, as follows.

(i) If a promoter is under positive transcriptional control by ppGpp, as is P_{RNAII}, then the direct and indirect effects of ppGpp are additive, so that the specific activity of β-galactosidase decreases with increasing growth rate or at reduced levels of ppGpp more than the specific activity expressed from most other mRNA promoters that are not affected by ppGpp (e.g., P_{spc}, P_{RNAI}, λ P_L, and P_{bla} [19]), as observed (Fig. 1). Earlier reports suggest that the promoters of the histidine biosynthetic operon and the *lac* operon are also under positive control by ppGpp (27, 28, 32).

(ii) If a promoter is under negative transcriptional control by ppGpp, as are the P1 promoters of *rrn* operons (19), then both transcriptional activity and the expression of a reporter enzyme from P1 increase with increasing growth rate (10, 13, 19, 35). But in this instance, the transcriptional activity of *rrnB* P1 increases about 40-fold in the range of growth rates studied (between 0.7 and 3.0 doublings/h), whereas the specific activity of β-galactosidase expressed from *rrnB* P1 increases only about 15-fold (19, 35). Thus, the indirect effects of bulk mRNA subtract from the direct transcriptional effect of ppGpp on P1.

(iii) An intermediate situation is represented by the P2 promoters of *rrn* operons, whose transcriptional activity increases with growth rate less than transcription from *rrn* P1 but more than transcription from mRNA promoters (19). β-Galactosidase expression from *rrnB* P2 is approximately constant and independent of the bacterial growth rate (10, 19, 35). Thus, for *rrnB* P2, the effects of an increasing rate of transcription from the promoter and increasing competition of bulk mRNA for translation compensate for one another. The constancy of the specific activity of β-galactosidase expressed from *rrnB* P2 has previously been interpreted as an indication of a lack of "growth rate-dependent control" (see, e.g., reference 10). We suggest that it is more likely to be a coincidence of two opposing effects on gene expression.

Control of rRNA synthesis: role of free RNA polymerase concentration and ppGpp. Using a *lacZ* reporter system and *lacZ* mRNA hybridization assays, the absolute activities of the *rrnB* P1 and P2 promoters, expressed as number of transcripts per minute per promoter, were previously found to increase with increasing growth rate (19). Since no specific control factors or factor binding sites have ever been associated with *rrn* P2 promoters, we have assumed that *rrn* P2 promoters are constitutive, so that their activity is affected only by the concentration of free RNA polymerase and the promoter-specific parameters V_{max} and K_m, representing the maximum promoter activity at saturation with free RNA polymerase and the free RNA polymerase concentration at half-maximal activity. In the absence of ppGpp, the *rrn* P1 promoters are also constitutive; accordingly, their activity also depends only on the free RNA polymerase concentration and the associated V_{max} and K_m values. The K_m value for P1 promoters is affected by the DNA binding factors Fis and H-NS. In addition, in normal ppGpp-proficient strains, P1 activity was found to be inhibited by ppGpp. As a result, the P1 activity was lower than the P2 activity during slow bacterial growth, when basal (exponential-growth) levels of ppGpp are highest, and higher than the P2 activity during fast bacterial growth, when basal levels of ppGpp are lowest. Thus, in our model (19), the growth rate regulation of ribosome synthesis depends both on the concentration of free RNA polymerase, which determines the frequency of transcription initiation at both P1 and P2, and on the concentration of ppGpp, which selectively reduces expression from P1 under slow-growth conditions.

Role of ppGpp in the control of ribosomal protein synthesis. It has been reported that the rate of synthesis of *spc* mRNA from the normal *spc* operon relative to the rate of total mRNA synthesis increases with increasing growth rate (8, 20). This is similar to the growth rate-dependent increase in the mRNA amounts, R_{lac}/R_m, determined above for the P_{spc}-*rplN-lacZ* fusion (Fig. 4b). Since the rate of ribosomal protein synthesis as a proportion of total protein synthesis (denoted by α_r [3]) increases with increasing growth rate similarly to the rate of *spc* mRNA synthesis per total mRNA synthesis, it has been suggested that the control of ribosomal protein synthesis occurs mainly at the transcriptional level (4, 8, 20). This interpretation was based on the two implicit assumptions that (i) *spc* mRNA and bulk mRNA are translated approximately equally and (ii) *spc* mRNA and bulk mRNA have approximately equal lifetimes. Based on the considerations above (Fig. 4) and previous measurements of *spc* mRNA lifetimes (18), both assumptions appear to be unjustified. The increasing rate of synthesis of ribosomal proteins from the *spc* operon with increasing growth rate is mediated through control of the decay of *spc* mRNA; similar mechanisms presumably control the decay of other ribosomal protein mRNAs (18, 22). This control of the mRNA lifetime adjusts the synthesis of ribosomal proteins to the ppGpp-dependent synthesis of rRNA, overriding any transcriptional regulation of ribosomal protein operons or superimposed effects of bulk mRNA translation.

The synthesis rates of *spc* ribosomal proteins and of *spc* operon mRNA have been determined previously during mild amino acid starvation or chloramphenicol treatment of stringent and relaxed strains (3, 5, 6, 7, 21). Those studies suggested that the activity of P_{spc} is stringently controlled, i.e., reduced when ppGpp levels increase and enhanced when ppGpp levels decrease. Those observations contradict the apparent positive control by ppGpp of β-galactosidase synthesis expressed from P_{spc} observed here (Fig. 2). This discrepancy can be explained as follows. The wild-type *spc* operon mRNA carries a control site at which the regulatory ribosomal protein S8 binds under conditions of reduced rRNA synthesis (e.g., during the stringent response). The binding of S8 to its own mRNA initiates a regulatory pathway that accelerates the decay of *spc* operon mRNA, thereby adjusting ribosomal protein synthesis to the accumulation of rRNA (reviewed in reference 15). Therefore, we suggest that the changes in the synthesis of *spc* ribosomal proteins that have been observed previously during the stringent and the relaxed response or during chloramphenicol treatment are the result of a regulation of *spc* mRNA decay in response to ppGpp-dependent changes in rRNA synthesis (18). This regulation requires specific control sites on the normal *spc* mRNA which are not present in the *spc-lac* fusion mRNA used here.

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