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Transcription and Translation in a Pleiotropic Streptomycin-Resistant Mutant of *Escherichia coli*

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The role of the ribosomal protein S12 (streptomycin protein) in ribosome function and in other metabolic processes in the cell has been investigated. A spontaneous streptomycin-resistant strain of *Escherichia coli* (SM3) carrying a mutation in the *rpsL* gene is deficient in its ability to induce the synthesis of the enzyme β-galactosidase. It was demonstrated that the reduced rate of enzyme synthesis results from deficiencies in both the transcription of the lactose operon and translation of the lactose operon mRNA. The transcription deficiency was in part due to increased catabolite repression and could therefore be partially suppressed by the addition of cyclic AMP. Streptomycin also appeared to partially suppress catabolite repression. In the SM3 mutant strain, the translation of the lactose operon mRNA was only about 60% as efficient as in the parental control, and addition of streptomycin did not alter the translation efficiency. In contrast, both transcription and translation of ribosomal protein mRNA were equally efficient in the two strains. These observations imply that mutational alterations in the ribosomal protein S12 either directly or indirectly alter (i) the extent of catabolite repression, (ii) the efficiency of transcription of the lactose operon even in the absence of catabolite repression, and (iii) the efficiency of translation of some but not all mRNA species in the cell.

Certain mutations in the *rpsL* gene (*strA*) coding for the ribosomal protein S12 confer on the bacterial strain resistance to the antibiotic streptomycin (1, 10). One such mutant strain, SM3, exhibits, in addition to resistance to streptomycin, a variety of other pleiotropic effects (J. Zengel, Ph.D. thesis, University of Wisconsin, Madison, 1976). These include (i) a reduced growth rate, (ii) a reduced protein chain elongation rate, (iii) a reduced ability to synthesize the enzyme β-galactosidase in the presence of the gratuitous inducer isopropyl thiogalactosidase (IPTG), and (iv) a reduced ability to synthesize other inducible enzymes, including alkaline phosphatase and D-serine deaminase (12). Addition of streptomycin to a culture of SM3 bacteria results in suppression of many of the pleiotropic manifestations of the mutation. Furthermore, ribosomes isolated from the SM3 strain containing the mutant S12 protein exhibit altered specificity in initiation on natural mRNA's and altered translation termination properties in a DNA-dependent RNA-protein synthesis system primed with λ DNA (11; W. Gette, Ph.D. thesis, University of Wisconsin, Madison, 1976).

The fact that protein S12 is the target site of action of streptomycin implies that S12 plays an important and crucial role in ribosome function. For example, streptomycin-resistant mutations are known in general to decrease the in vivo level of suppression of nonsense and missense mutations and to reduce in vitro the error frequency caused by miscoding of mRNA (7). Protein S12, besides being an essential component of the ribosome, may have other activities in the cell. For example, certain *strA* mutants, when combined with a rifampin-resistant mutation in the *rpoB* gene (specifying the β subunit of RNA polymerase), are conditionally lethal, suggesting a direct or indirect relationship between RNA polymerase function and protein S12 (2). It has also been suggested that mutations resulting in streptomycin dependency and presumably in the *rpsL* gene specifically affect the expression of catabolite-sensitive enzyme systems (3).

In this paper an attempt has been made to further elucidate the role of protein S12 in both ribosome function and in other metabolic activities. This was accomplished by determining the nature of the inability of the SM3 mutant strain to synthesize the enzyme β-galactosidase at rates characteristic of the parental strain. The results indicate that the SM3 mutant (i) translates *lac* mRNA less efficiently than other types of mRNA and (ii) transcribes the lactose operon

less frequently, even in the presence of streptomycin and/or cyclic AMP (cAMP) than the parental control strain.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *Escherichia coli* parental strain C600 is a K-12 derivative requiring threonine, leucine, and thiamine and containing a mutation in the *lacY* (permease) gene. The SM3 strain is a spontaneous streptomycin-resistant derivative of C600 and contains the additional mutation in the *rpsL* gene specifying protein S12 (streptomycin protein) of the 30S ribosome subunit (12; Gette, Ph.D. thesis). Cultures were grown at 37°C in minimal glucose medium with required supplements as previously described (12). The SM3 strain was grown both in the presence (200 μg/ml) and in the absence of streptomycin. Growth was monitored by measuring the absorbance at 460 nm (A_{460}) in a Gilford spectrophotometer.

Induction of β-galactosidase. Cells were induced for synthesis of β-galactosidase by addition of IPTG to a final concentration of 10^{-3} M. In some experiments cAMP was added to a final concentration of 10^{-2} M 10 min before induction in order to overcome catabolite repression. The cAMP addition resulted in a slight reduction in the bacterial growth rate. The activity of β-galactosidase was assayed by using the procedure of Miller (9).

Measurements of protein accumulation. To determine the rate of protein synthesis, [^{14}C]leucine (1 μCi/ml; 2.6 μCi/μmol) was added to portions of the bacterial cultures at an A_{460} of 0.30 (time zero). In some instances cAMP was added to a final concentration of 10^{-2} M 2 min before [^{14}C]leucine addition. Samples were removed at 5-min intervals, precipitated with 5% trichloroacetic acid, collected on nitrocellulose filters, and counted. From the growth rate of the cultures (determined from A_{460} measurements before addition of radioactivity) and the initial rate of radioactive leucine incorporation in the absence of cAMP, the amount of total protein at time zero was calculated. Protein accumulation at times greater than zero was determined from the amount of incorporation of radioactivity. The mole fraction of leucine in *E. coli* protein was taken to be 8% (12). The rate of protein synthesis in the respective cultures was determined from the slopes of the protein accumulation curves illustrated in Fig. 2.

Cell labeling and RNA-DNA hybridization. To label cellular RNA to a constant specific radioactivity, bacteria were cultured in growth medium containing [5-^{3}H]uracil (10 μCi/ml; 3 μg/ml) for at least 10 cell doublings. To label newly synthesized RNA, cultures were exposed to [^{3}H]uracil (specific activity, 25 Ci/mmol; 10 μCi/ml) for 1 min before harvesting and preparation of RNA for hybridization (4, 5). The pulse labeling was performed between 9 and 10 min after addition of the inducer IPTG; in some cases cAMP was added 10 min before induction to reduce catabolite repression. Samples of the radioactive RNA preparations were hybridized to an excess of denatured nonspecific λ or λd*trk* DNA and specific λd*spc*1, λp*lac*5, and pcc720 DNA immobilized on nitrocellulose filters as previously described (4, 5).

RESULTS

Differential synthesis rate of β-galactosidase. The doubling time of strain C600 when cultured in minimal glucose medium was 55 min and corresponded to a growth rate, μ, of 1.15 doublings per h. The streptomycin-resistant strain SM3 under the same conditions had a doubling time of 118 min (μ = 0.52). When streptomycin was included in the growth medium at a concentration of 200 μg/ml, the doubling time of the mutant strain was reduced to 75 min (μ = 0.80). Portions of these three cultures were exposed to the gratuitous inducer, IPTG, to induce the synthesis of the enzyme β-galactosidase (Fig. 1). The rate of enzyme synthesis per A_{460} of bacterial mass in the SM3 culture was 10-fold lower than in the C600 parental culture. Growth of the SM3 mutant strain in the presence of streptomycin partially restored its ability to synthesize the enzyme after induction to a level only threefold below the parent C600 (Table 1).

The extent of induction of β-galactosidase is known to be subject to cAMP control mediated through the catabolite repression system (6). A less efficient utilization of the glucose carbon energy source in the SM3 mutant cultures, as evidenced by their slower rates of growth, could possibly contribute to accumulation of metabolic intermediates and result in a reduction in the intercellular levels of cAMP. Low cAMP levels

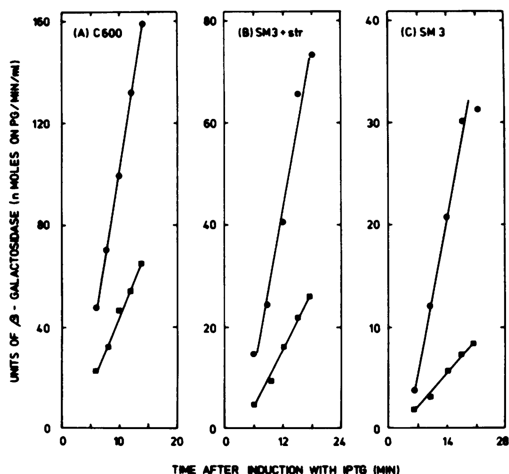

FIG. 1. *Induction of the synthesis of β-galactosidase. Exponential phase cultures of C600 (A), SM3 plus streptomycin (B), and SM3 without streptomycin (C) at an A_{460} of 0.25 to 0.30 were induced for the synthesis of β-galactosidase by the addition of IPTG (■). To a portion of each culture, cAMP was added 10 min before addition of inducer (●). The midpoints of the induction periods were 10, 12, and 14 min in the respective cultures.*

TABLE 1. *Differential rate of β-galactosidase synthesis*

Bacterial strain	Growth rate[a]	cAMP[b]	Enzyme synthesis rate[c]		Protein synthesis rate[d]		Differential synthesis rate of β-galactosidase[e] (U/nmol of aa)
			U/ml	U/A_{460}	nmol of aa/ml	nmol of aa/A_{460}	
C600	1.15	−	2.36	5.55	5.37	17.5	0.32 (1.00)
		+	5.78	14.64	4.05	13.2	1.11 (3.47)
SM3 + str	0.80	−	0.75	1.91	3.93	13.4	0.14 (0.44)
		+	2.08	5.43	3.34	11.4	0.48 (1.50)
SM3	0.52	−	0.17	0.46	2.09	7.0	0.07 (0.22)
		+	0.96	2.64	1.82	6.1	0.43 (1.34)

[a] Growth rate is in doublings per hour.

[b] cAMP was added to a final concentration of 10^{-2} M 10 min before induction or 2 min before addition of [^{14}C]leucine.

[c] Enzyme units are nanomoles of *o*-nitrophenol produced per minute per milliliter of culture. Enzyme units per A_{460} of bacterial mass were determined by using the mass of the culture at the midpoint of the induction interval. Data are taken from Fig. 1.

[d] The protein synthesis rates were determined from the slopes of the protein accumulation curves illustrated in Fig. 2. Values are further normalized per A_{460} unit of bacterial mass. aa, Amino acids.

[e] Differential synthesis rate of β-galactosidase is defined as the ratio of (synthesis rate of β-galactosidase/A_{460}) to (synthesis rate of total protein/A_{460}). Values in parentheses are normalized to the C600 control value set at 1.0.

cause catabolite repression and thus prevent maximum transcription of the lactose operon. Such a possibility was investigated by activating the positive acting catabolite repression protein in the three cultures by the addition of exogenous cAMP 10 min before induction. This resulted in a three- to fivefold increase in the induced enzyme synthesis rate compared to the induced rate in the absence of cAMP in the three cultures (Fig. 1 and Table 1). This observation suggested that catabolite repression could not account for the difference in the ability of the parental C600 and the mutant SM3 strains to induce the synthesis of β-galactosidase.

The rate of protein synthesis in the C600, SM3 (plus streptomycin), and SM3 (no streptomycin) cultures was determined by monitoring the incorporation of [^{14}C]leucine into acid-insoluble material in the presence and absence of cAMP. Figure 2 illustrates the accumulation of protein in the respective cultures during the 40-min labeling period as determined from the radioactive leucine incorporation kinetics. From the mass of the cultures at the time of addition of radioactive leucine and the slope of the protein accumulation curves, the rates of protein synthesis (in nanomoles of amino acids incorporated per minute per A_{460} of bacterial mass) was determined in the three cultures in the presence and absence of cAMP (Table 1). These results clearly indicate that the addition of cAMP to the respective cultures reduced the protein synthesis rate by about 15 to 30%.

The most accurate reflection of the ability of a culture to induce the synthesis of an enzyme is given by the magnitude of the differential synthesis rate of that enzyme (i.e., rate of synthesis of a specific enzyme/rate of total protein synthesis). The differential synthesis rates of β-galactosidase in the C600, SM3 (plus streptomycin), and SM3 (no streptomycin) cultures in the presence and absence of cAMP were calculated from the synthesis rate of enzyme per A_{460} of bacterial mass and the synthesis rate of total

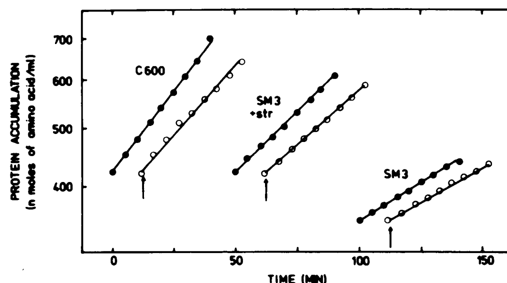

FIG. 2. *Protein synthesis in the presence and absence of cAMP. To exponential phase cultures of C600 (left), SM3 (plus streptomycin) (center), and SM3 (no streptomycin) (right) at an A_{460} of 0.30, [^{14}C]leucine was added and incorporation was monitored at 5-min intervals for 40 min (●). A portion of each culture received cAMP 2 min before addition of [^{14}C]leucine (○; curves displaced by 12.5 min on the time axis). From the initial rates of [^{14}C]leucine incorporation in the absence of cAMP and the growth rates of the culture before addition of radioactivity, the amount of total protein (nanomoles of amino acid per milliliter) at the time of [^{14}C]leucine addition was calculated. The amount of protein accumulating at later times was determined from the incorporation of [^{14}C]leucine in the presence (●) and absence (○) of cAMP. The arrows (↑) indicate that the amount of protein at the time of addition of [^{14}C]leucine to cAMP-containing cultures was the same as in the control (no cAMP) cultures.*

protein per A_{460} of bacterial mass (Table 1). These differential rate measurements demonstrate that the mutant SM3 strain can induce β-galactosidase only to about 1/4 or 1/5 the level in the parental C600 strain (i.e., a relative value of 0.22 compared to 1.00). Inclusion of streptomycin in the growth medium raised the differential synthesis rate in the mutant SM3 strain to slightly less than half the level in the parental strain (i.e., 0.44); streptomycin thus partially suppresses the phenotypic manifestation of the mutation in the *rpsL* gene. When cAMP is added immediately before induction in order to reduce catabolite repression, the SM3 mutant strain growing either in the presence or in the absence of streptomycin induced β-galactosidase to only about 40% of the parental C600 level (i.e., 1.50 and 1.34 compared to 3.47). This observation suggests that in the SM3 mutant strain streptomycin may either directly or indirectly enhance expression of the lactose operon by somehow altering the extent of catabolite repression.

Transcription of the lactose operon. The above results suggested that the SM3 mutant strain even in the presence of streptomycin and/or cAMP was either defective in the ability to initiate RNA transcripts at the *lac* promotor or that its mutant ribosomes were defective in recognition and/or translation of *lac* mRNA relative to the total mRNA population. To distinguish between these possibilities, the amounts and synthesis rates of various classes of mRNA, including lactose operon mRNA, were measured in the parental C600, SM3 (plus streptomycin), and SM3 (no streptomycin) cultures. To determine the amount of the various mRNA species, the strains were grown for 10 cell doublings in medium containing [^{3}H]uracil to label cellular nucleic acids to a constant specific radioactivity. The inducer IPTG was added one cell doubling before harvesting the cells to induce the synthesis of lactose operon mRNA. The radioactive RNA was extracted and hybridized to an excess of nonspecific λ DNA and λd*trk* DNA and specific λd*spc*1 DNA, pcc720 DNA, and λp*lac*5 DNA. Radioactive mRNA hybridized to λd*spc*1 DNA was mRNA specifying 15 ribosomal proteins and the α subunit of RNA polymerase; that hybridized to pcc720 DNA was mRNA specifying the β and β' subunits of RNA polymerase; and that hybridized to λp*lac*5 DNA was mRNA specifying β-galactosidase.

It was observed that the amount of mRNA hybridized to λd*spc*1 DNA represented 0.23 and 0.20% of the total cellular RNA in the SM3 (no streptomycin) and C600 parental cultures, respectively (Table 2). Consistent with this result is the previous observation that the differential synthesis rate of ribosomal protein in the SM3 strain was about 10 to 15% greater than in the C600 parental strain (12). Thus, the amount of mRNA available for translation, at least for ribosomal protein mRNA, correlates with the synthesis rate of the corresponding proteins. The amount of mRNA specifying the β and β' subunits of RNA polymerase was essentially constant in the respective cultures.

TABLE 2. *Quantitation of the amounts of ribosomal protein mRNA, RNA polymerase mRNA, and β-galactosidase mRNA*[a]

Bacterial strain	Input of RNA (cpm)	Radioactivity in specific RNA-DNA hybrids (%)		
		λd*spc*1	pcc720	λp*lac*5
C600	2.71×10^5	0.20	0.032	0.012 (1.00)
SM3 + str	3.28×10^5	0.21	0.035	0.010 (0.83)
SM3	3.18×10^5	0.23	0.032	0.007 (0.58)

[a] Hybridization assays were performed as described in references 4 and 5 with RNA inputs of 50, 100, 150, and 200 μl of RNA per assay. The specific activity of the RNA preparation was about 6×10^6 cpm per A_{260}. The input radioactivity is per 50 μl of RNA, and the percent input radioactivity hybridized specifically to λd*spc*1, pcc720, and λp*lac*5 is averaged from the four separate hybridizations. Each assay contained a blank filter, two λ DNA filters, two λd*trk* DNA filters, four λd*spc*1 filters, two pcc720 filters, and two λp*lac*5 filters. Each filter contained 167 fmol of denatured DNA (equivalent to 5 μg of λDNA). Values in parentheses are the λp*lac*5 hybridization values normalized to a value of 1.0 in the C600 control strain.

Precise quantitation of the amount of *lac* mRNA was difficult because of its very low level. However, the measurements clearly suggested that the amount was greater in the parental C600 culture than in the SM3 mutant cultures (Table 2); this is in qualitative agreement with the observed differential synthesis rates of β-galactosidase in the three cultures (Table 1), although the amount of *lac* mRNA appeared to be somewhat greater in the SM3 cultures than would have been predicted from the β-galactosidase synthesis rate measurements. (No attempt to add cAMP to these cultures was made because it would have affected the balanced growth of the cultures and almost certainly perturbed the specific activity of the pyrimidine nucleotide pools.)

The synthesis rates of specific mRNA species relative to total RNA synthesis were determined by pulse labeling the cultures and hybridizing the radioactive RNA to the specific DNAs. The IPTG-induced C600, SM3 (plus streptomycin), and SM3 (no streptomycin) cultures, both in the presence and in the absence of cAMP, were exposed to [^{3}H]uracil for 1 min to label with radioactivity the newly synthesized RNA tran-

scripts. The RNAs were again hybridized to nonspecific and specific DNAs (Fig. 3). The fraction of radioactivity from cultures labeled in the absence of cAMP that was ribosomal protein mRNA and hybridized to λ*dspc*1 DNA was between 1.4 and 1.7% (Table 3). This value was reduced to about 1.3% for cultures labeled in the presence of cAMP, suggesting that the cyclic nucleotide retards ribosome synthesis as well as total protein synthesis. The fraction of the radioactivity that was mRNA specifying the β and β' subunits of RNA polymerase and hybridized to pcc720 DNA was approximately constant and equal to about 0.2% in all of the cultures.

The fraction of radioactivity incorporated into *lac* mRNA, in contrast to RNA polymerases and ribosomal protein mRNA's, varied drastically among the various cultures. In the C600 cultures, cAMP enhanced the fraction of radioactive RNA hybridized to λp*lac*5 DNA by 3.75-fold (Table 3). This was virtually identical to the

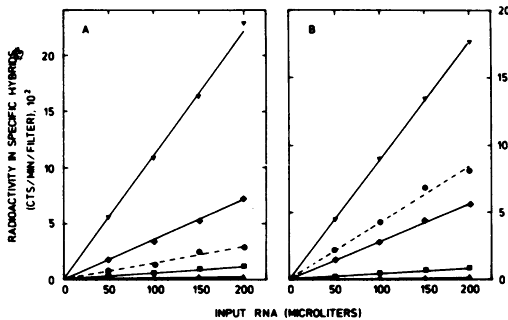

FIG. 3. *Hybridization of ribosomal protein, β and β' RNA polymerase, and lactose mRNA. Exponential-phase cultures of C600, SM3 (plus streptomycin), and SM3 (no streptomycin) were labeled for 1 min with [^{3}H]uracil in the presence of inducer and in the presence or absence of cAMP. The hybridization assays of the RNAs isolated for SM3 (plus streptomycin) labeled in the absence (A) or presence (B) of cAMP are illustrated. The RNAs were adjusted to a concentration of about 50 μg/ml, and 50, 100, 150, and 200 μl were hybridized to excess amounts of specific and nonspecific DNAs immobilized on nitrocellulose filters. Each assay contained one blank filter, two λDNA filters (▲), two λdtrk DNA filters (■), four λdspc1 DNA filters (▼), two pcc720 DNA filters (◆), and two λplac5 filters (●). The average radioactivity per filter corrected for the blank is illustrated. The difference in radioactivity associated with λdspc1 and λdtrk filters represents ribosomal protein mRNA. The difference between pcc720 or λplac5 and λ filters represents, respectively, β and β' RNA polymerase mRNA and lactose mRNA. The slopes of the respective curves represent the fraction of the input RNA which hybridizes to DNA. The results of these hybridization and similar hybridization using RNAs from the C600 and SM3 (no streptomycin) cultures are summarized in Table 3.*

cAMP-mediated 3.47-fold increase observed in the differential synthesis rate of β-galactosidase enzyme activity (Table 1). Similarly, the cAMP-mediated stimulations in lactose operon transcription in the SM3 mutant cultures were virtually identical to the stimulations in the differential synthesis rate of the enzyme.

These results clearly demonstrate that the synthesis rate of *lac* mRNA is proportional to the synthesis rate of β-galactosidase in the C600 and SM3 cultures. That is, addition of cAMP enhances transcription of the *lac* operon and synthesis of β-galactosidase to the same extent in the respective cultures (Table 4). Two further points are also apparent: (i) transcription of the *lac* operon and synthesis of β-galactosidase were greater in the parent C600 culture in the presence and absence of cAMP than in the corresponding SM3 mutant cultures; and (ii) in the SM3 cultures, both in the presence and absence of streptomycin and in the presence and absence of cAMP, *lac* mRNA was only about 60% as efficient in producing β-galactosidase as the parental C600 cultures (Table 4). In contrast to this latter point, ribosomal protein mRNA was equally efficient in producing ribosomal proteins in the two strains. In summary, it seems likely that the deficiency in the SM3 mutant in its ability to synthesize the inducible and catabolite-sensitive enzyme β-galactosidase results from both (i) a defect in the ability to initiate RNA transcripts from the lactose promotor and (ii) a defect in the SM3 mutant ribosomes to recognize and translate *lac* mRNA relative to other cellular mRNA's, such as ribosomal protein mRNA. The defect in the ability to initiate

TABLE 3. *Quantitation of the synthesis rates of ribosomal protein mRNA, RNA polymerase mRNA, and β-galactosidase mRNA*[a]

Bacterial strain	cAMP	Input of RNA (cpm)	Radioactivity in specific RNA-DNA hybrids		
			λ*dspc*1	pcc720	λp*lac*5
C600	−	1.48×10^5	1.52	0.24	0.12 (1.00)
	+	1.08×10^5	1.30	0.23	0.45 (3.75)
SM3	−	1.53×10^5	1.37	0.23	0.09 (0.75)
+ str	+	1.28×10^5	1.31	0.22	0.34 (2.83)
SM3	−	1.95×10^5	1.67	0.22	0.04 (0.33)
	+	2.03×10^5	1.38	0.15	0.30 (2.50)

[a] Cultures were induced with IPTG at time zero and labeled with [^{3}H]uracil from 9 to 10 min after induction; cAMP was added 10 min before induction. Hybridization assays were performed as previously described (4 and 5); the hybridization of the RNAs isolated from the SM3 (+ streptomycin) cultures labeled in the presence and absence of cAMP is illustrated in Fig. 3. Input radioactivity is per 50 μl of RNA. The RNA concentrations were about 50 μg/ml. Other details are given in the legend to Fig. 3 and footnote to Table 2. The values in parentheses are the hybridization to λp*lac*5 DNA normalized to a value of 1.0 for the C600 parent labeled in the absence of cAMP.

TABLE 4. *Efficiency of translation of lactose and ribosomal protein mRNA*

Bacterial strain	cAMP	β-Galactosidase[a]	*lac* mRNA[b]	Normalized[c] ratio	r-Protein[d]	r-Protein mRNA[e]	Ratio[f]
C600	−	1.00	1.00	1.00	0.165	1.52%	0.11
	+	3.47	3.75	0.93			
SM3 (+str)	−	0.44	0.75	0.59			
	+	1.50	2.83	0.53			
SM3	−	0.22	0.33	0.67	0.185	1.67%	0.11
	+	1.34	2.50	0.54			

[a] Differential rate of β-galactosidase activity normalized to a value of 1.0 for the C600 control (from Table 1).
[b] Relative synthesis rate of lactose mRNA normalized to a value of 1.0 for the C600 control culture (from Table 3).
[c] Normalized ratio is the enzyme synthesis rate divided by the lactose mRNA synthesis rate and reflects the relative efficiency of translation of lactose mRNA.
[d] Differential rate of ribosomal protein synthesis (from ref. 12).
[e] Relative synthesis rate of ribosomal protein mRNA hybridizing to λ*dspc*1 DNA (from Table 3).
[f] Ratio of the differential rate of ribosomal protein synthesis and the synthesis rate of ribosomal protein mRNA. The identical ratios indicate that ribosomal protein mRNA is translated with equal efficiency in the two cultures.

RNA transcription can be partially corrected by addition of cAMP; the addition of cAMP alone apparently corrects transcription about as well as cAMP and streptomycin together (Table 3). Neither streptomycin nor cAMP addition corrected the efficiency of translation of *lac* mRNA by the mutant SM3 ribosomes (Table 4).

DISCUSSION

Many mutations in the *rpsL* gene coding for the ribosomal protein S12 are pleiotropic in nature. In the past it has been generally assumed that such phenotypic manifestations were the result of defects in ribosome function. More recent evidence, however, suggests that the S12 protein may be involved in other cellular processes such as transcription and catabolite repression (2, 3). The results reported here indicate that the streptomycin-resistant strain SM3 is defective in both the synthesis and the translation of *lac* mRNA.

Transcription of the lactose operon. Growth on glucose partially inactivates the catabolite repression protein through a reduction in intracellular level of cAMP (6). The SM3 mutant strain exhibits a reduced growth rate compared to the wild-type parent in glucose minimal medium. The less efficient utilization of glucose could result in accumulation of metabolic intermediates, a further reduction in the level of cAMP, and, consequently, a stronger catabolite repression effect. Thus catabolite repression could easily contribute to the difference between the abilities of the mutant SM3 and the wild-type strain to transcribe the lactose operon. Consistent with this idea are the observations that (i) addition of streptomycin to the growth medium increases the growth rate of the mutant strain and at the same time increases its ability to transcribe *lac* mRNA and (ii) addition of exogenous cAMP to the mutant strain growing in either the presence or the absence of streptomycin results in the same higher level of induced lactose mRNA transcription. However, it is further apparent that the maximum level of transcription of *lac* mRNA in the mutant strain either in the presence or absence of cAMP is about 30 to 40% less than in the parental wild-type strain (Table 3). This means that the catabolite repression system is probably only partially responsible for the difference in the abilities of the strains to transcribe the lactose operon and implies that the mutant is partially defective either in initiation of transcription at the lactose promotor or in suppressing premature termination of the RNA transcripts. This transcriptional deficiency in the mutant may be related to the observation that certain streptomycin-resistant mutations are incompatible with rifampin-resistant mutations in the *rpoB* gene (2).

Translation of *lac* mRNA. The in vivo efficiency of ribosomes in polymerizing amino acids into protein in cultures of the mutant SM3 strain was observed to be reduced by 60% compared with the wild-type parent. Measurements of several distinct molecular-weight classes of protein indicated that the peptide chain growth rate in the wild-type parent and the SM3 mutant were 11.4 and 6.4 amino acids per s, respectively (12). In addition the induction lag for appearance of β-galactosidase after the addition of inducer was observed to be about 2.5 times as long in the mutant compared to the parent; in the presence of streptomycin, the induction lag was only 1.1 times as long. These results demonstrated that the ribosomes in the mutant SM3 strain are

defective in peptide chain elongation and that this effect is almost certainly general for all proteins being synthesized. Furthermore, addition of streptomycin to the growth medium is effective in partially suppressing this defect in general peptide chain elongation (12).

In the results reported here it was further observed that in the SM3 mutant strain, the efficiency of translation of β-galactosidase mRNA was specifically reduced relative to other classes of mRNA such as ribosomal protein mRNA. This specific effect could result from either (i) a defect in the ability of the mutant S12-containing ribosomes to recognize or initiate at the ribosome binding site on the lactose mRNA, (ii) premature termination of β-galactosidase peptides, or (iii) miscoding of β-galactosidase mRNA to produce inactive protein. Furthermore, this specific defect in *lac* mRNA translation could not be suppressed by the addition of streptomycin (Table 4).

In an extensive in vitro analysis, Gette (Ph.D. thesis) has shown that the SM3 mutant ribosomes exhibit an altered pattern of recognition of initiation sites on natural mRNA. This observation suggests that the failure of the mutant strain to efficiently translate *lac* mRNA in vivo could result at least in part from the reduced recognition of initiation sites on *lac* mRNA. The recent report that the antibiotic nitrofurantoin selectively inhibits translation of inducible mRNA supports the idea that some type of ribosome discrimination of initiation sites on mRNA's occurs (8). In addition, the rate of peptide chain elongation is known to be reduced in the SM3 strain (12). If this reduction contributes to premature termination, an enzyme such as β-galactosidase, because of its large molecular weight compared to the average *E. coli* protein, would be affected relative to proteins of average molecular weight. Finally, since streptomycin-resistant mutants in general show a decrease in the level of nonsense and missense suppression, it is unlikely that the reduced levels of β-galactosidase activity in the SM3 mutant strain result from miscoding of *lac* mRNA (7).

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