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Translational Coupling of the Two Proximal Genes in the S10 Ribosomal Protein Operon of *Escherichia coli*

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We have examined the translational coupling between the first two genes in the S10 ribosomal protein operon. We isolated mutations blocking the translation of the first gene of the operon, coding for S10, and monitored their effects on translation of the downstream gene, coding for L3. All of the mutations inhibiting S10 synthesis also affected the synthesis of L3. However, these experiments were complicated by decreased mRNA synthesis resulting from transcription polarity, which we could only partially eliminate by using a *rho-100* strain. To completely eliminate the problem of transcription polarity and obtain a more accurate measurement of the coupling, we replaced the natural S10 promoter with a promoter used by the bacteriophage T7 RNA polymerase. As expected, the T7 RNA polymerase was not subject to transcription polarity. Using this system, we were able to show that a complete abolishment of S10 translation resulted in an 80% inhibition of L3 synthesis. Other experiments show that the synthesis of L3 goes up as a function of increasing S10 synthesis, but the translational coupling does not assure strictly proportional output from the two genes.

The majority of the ribosomal protein (r-protein) genes of *Escherichia coli* are organized in multicistronic transcription units (14). The translation of most of these r-protein operons is under autogenous control. That is, one of the r-proteins encoded by a given transcription unit functions as a repressor of the translation of most or all genes in the unit (14, 22).

The S10 operon codes for 11 r-proteins. Translation of this r-protein operon is regulated by L4, the product of the third gene of the operon (9, 31). Genetic analysis has shown that the target for translation control of the S10 operon is located in the leader (9; L. P. Freedman, Ph.D. thesis, University of Rochester, Rochester, N.Y., 1985). This suggests that, whereas the first gene of the operon is under direct translational inhibition by L4, the regulation of the downstream genes is due to some indirect mechanism, analogous to what has been shown for several other r-protein operons (17, 30, 32).

It has been proposed that the propagation of translational regulation down an r-protein operon depends on translational coupling (22, 32), a phenomenon first demonstrated in the tryptophan operon by Oppenheim and Yanofsky (23). When two genes are translationally coupled, efficient translation of a downstream gene depends on the translation of the adjacent upstream gene. Translationally coupled genes are often separated by intercistronic regions which are 3 bases or less (2–4, 23, 25); in several of these cases, the termination codon of the upstream gene even overlaps with the initiation codon of the downstream gene. It has been speculated that the short distance between the genes is essential for efficient translational coupling (23), although coupling has been reported in several r-protein operons which contain genes separated by longer intercistronic regions (17, 30).

We have investigated the extent of coupling between the first two genes of the S10 operon. The translational relationship between these genes is especially interesting for several reasons. First, the distance between the first two genes of the S10 operon, encoding r-proteins S10 and L3, is 32 bases. This spacing is much longer than the spacing between other gene pairs for which translational coupling has been analyzed quantitatively (2–4, 23, 25, 27). Second, since the S10 operon contains 11 genes, it would require very strong translational coupling to propagate translational regulation from gene to gene through the entire operon. For example, incomplete coupling between the S10 and L3 genes would result in breakthrough translation by ribosomes initiating at the L3 gene independently of translation of the upstream S10 sequence. Thus, during L4-mediated inhibition of translation, more ribosomes would translate L3 (and all downstream genes) than would translate the S10 gene. Incomplete coupling between other gene pairs in the operon would amplify this leakiness: as more ribosomes gained access to the transcript at each intercistronic region, the regulatory effect exerted by L4 at the proximal end of the operon would eventually be diluted. Finally, L4 also regulates transcription of the S10 operon (9, 12, 34). Thus, unlike other r-protein operons for which autogenous control affects only translation (17, 33), regulation of the S10 operon is not absolutely dependent on the L4-mediated inhibition of translation.

Our results show that the S10 and L3 genes are indeed translationally coupled: blocking translation of the upstream S10 gene resulted in approximately 80% inhibition of L3 synthesis. This coupling is as strong as the coupling observed for several previously analyzed gene pairs separated by only a few bases. However, since the coupling is not complete, additional regulatory processes (including transcriptional control) are used to ensure effective regulation of the entire S10 operon.

MATERIALS AND METHODS

Strains and media. LL306 [*E. coli* K-12 Δ(*pro-lac*) *recA*] is from our strain collection. LL308 was derived from LL306 by introducing F′ $lacI^qZ^{\Delta M15}Y^+$ pro^+ (13). *E. coli* K-12

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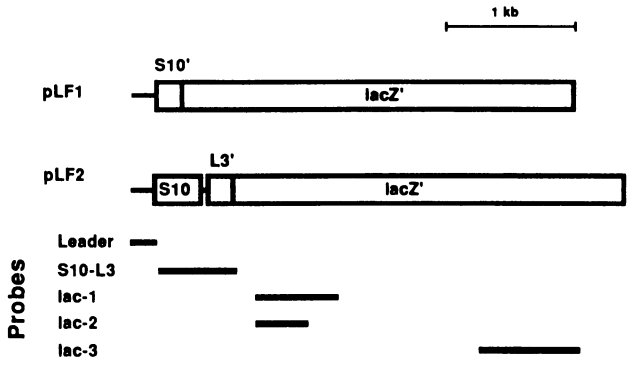

FIG. 1. Plasmids and hybridization probes. pLF1 and pLF2 were derived from pSC101 (10). The indicated DNA fragments were subcloned into M13 phage vectors in the appropriate orientation for hybridizing mRNA to single-stranded DNA from the recombinant phages.

strain W3110 (*trpE9851 leu*) and its *rho-100* derivative (20) were obtained from J. Richardson. Strain BL21(DE3), obtained from W. Studier, carries the lambda prophage DE3 with the gene for T7 RNA polymerase under control of the *lac* promoter (29). We confirmed that all strains were unable to suppress UGA termination codons by their inability to plate T4 bacteriophages carrying various UGA mutations (obtained from J. Wiberg and M. Yarus).

Cultures were grown in AB (7) or MOPS (morpholinepropanesulfonic acid) (21) minimal medium supplemented with thiamine, glucose, and required amino acids.

Plasmids and phages. Plasmids pLF1 and pLF2 (10) are derivatives of pSC101 containing gene fusions between *lacZ* and the first or second gene of the S10 operon, respectively (Fig. 1). Deletions within the S10 gene were generated at the unique *Sst*II site of pLF2 by BAL 31 exonuclease, using protocols recommended by vendors of the enzymes. Deletion plasmids were identified by restriction digestion and were mapped by subcloning and DNA sequencing. The mutant plasmid carrying the 15-base deletion removing the Shine-Dalgarno sequence of the S10 gene (ΔSD) was described previously (9, 12). The leader mutations used for comparing translation efficiencies of *lacZ* fusions in pLF1 and pLF2 derivatives included the Δ72 and 122,123 leader mutations described previously (9). Other mutations used for this analysis were derived from a mutant plasmid in which 142 bases of the 172-base leader were replaced with an 18-base linker (10). Although three of the four bases in the S10 Shine-Dalgarno sequence were left intact, this mutation (called leader 1 in this report) reduced translation of the S10 gene about 100-fold and rendered LL308 cells carrying this plasmid phenotypically Lac^-. Further leader mutants (including leaders 2 and 3 described below) were then generated by selecting Lac^+ phenotypes; all of the reverting mutations map within the S10 leader. These mutant plasmids will be described in detail in a separate publication (J. R. McCormick, J. M. Zengel, and L. Lindahl, manuscript in preparation).

Hybridization probes were constructed by cloning the indicated fragments (Fig. 1) into M13 vectors (18). The probe we call lac-1 is the SUM6 probe from the laboratory of C. Squires (2).

Protein synthesis. β-Galactosidase activity was measured in cells permeabilized with sodium dodecyl sulfate and chloroform (19). Rates of fusion protein synthesis were determined as described previously (9). In brief, samples of the culture were pulse-labeled with [^{35}S]methionine, and total cell lysates were fractionated on sodium dodecyl sulfate gels. The differential rate of S10'-*lacZ'* or L3'-*lacZ'* fusion protein synthesis was then determined as the ratio between the amount of radioactivity in the fusion protein band and the amount of total trichloroacetic acid-precipitable radioactivity applied to the lane of the gel.

RNA synthesis. Samples of the culture were pulse-labeled for 1 min with 75 μCi of [^{3}H]uridine per ml (40 to 50 Ci/mmol). Cells were lysed by being mixed with boiling buffer containing sodium dodecyl sulfate and were extracted with phenol and chloroform as previously described (26, 34). The amount of radioactivity in the indicated segments of the fusion gene transcript was then determined by hybridization to single-stranded M13 DNA with inserts from the fusion gene operons (Fig. 1). In some experiments, the single-stranded DNA was immobilized on filters (12). In other experiments, the hybrids were visualized by gel electrophoresis (15, 26) using a technique adopted from Hansen and Sharp (11). In this case, the hybrids were formed in a liquid reaction and were treated with RNase T1. The hybrids were collected on nitrocellulose filters, and the RNase-protected transcript fragments were eluted, fractionated on denaturing gels, and visualized by fluorography. Finally, the radioactivity in the various bands was eluted and quantitated by liquid scintillation counting.

RESULTS

Construction of mutants. To study translational coupling between the S10 and L3 genes, we used plasmid pLF2 carrying the beginning of the S10 operon including the promoter, the entire S10 gene, and the proximal part of the L3 gene (Fig. 1). The partial L3 gene was fused to *lacZ* so that L3 translation could be monitored by the synthesis of L3'-*lacZ'* fusion protein (10). From pLF2 we then isolated several mutations interfering with the translation of S10. First, we made short BAL 31 deletions at the *Sst*II site 68 bases into the 309-base S10 gene (Fig. 2). One mutant, Δ32, contained a 32-base frame-shifting deletion resulting in premature termination of translation about 90 bases from the end of the S10 gene, where there are two closely spaced UGA termination codons in the new reading frame (Fig. 2). In another mutant, Δ20-UGA, 20 bases were deleted and an 8-base *Bcl*I linker was inserted. Since the linker carries an in-phase UGA termination codon, translation in this mutant terminated at the *Sst*II site, but the reading frame distal to the mutation was not changed. As a control for these mutants, we used mutant Δ15 which contains an in-phase deletion of 15 bases.

We also reduced translation of the S10 gene by deleting 15 bases upstream of the gene, including the Shine-Dalgarno sequence (Fig. 2) (12). This mutation (ΔSD) results in a 10-fold reduction in protein synthesis from the S10 gene (Freedman, Ph.D. thesis). However, since loss of the Shine-Dalgarno region by itself did not completely abolish translation of the S10 gene, we also constructed a double mutant in which the Shine-Dalgarno deletion was combined with a frame-shifting 4-base BAL 31 deletion at the *Sst*II site (Fig. 2). In this mutant (ΔSDΔ4), not only is the translation initiation rate reduced about 10-fold, but ribosomes that do begin translation terminate prematurely in the middle of the S10 gene (Fig. 2).

Translation and transcription of the L3'-*lacZ'* fusion gene. To determine how interference with S10 translation affects translation of L3, we measured the rate of protein synthesis

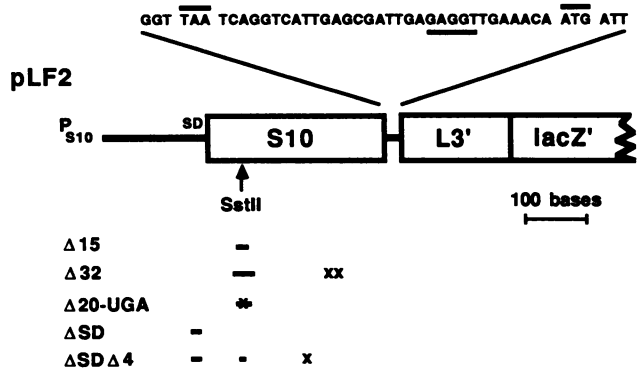

FIG. 2. Deletion mutations in the S10 gene of pLF2. The map shows the proximal portion of the fusion gene transcription unit of pLF2 with the sequence of the intercistronic region between the S10 and L3 genes. The termination codon of the S10 gene and the initiation codon of the L3 gene are overlined. The Shine-Dalgarno sequence of the L3 gene is underlined. The S10 promoter (P_{S10}) and the position of the Shine-Dalgarno region (SD) of the S10 gene are shown. The bars under the map show the positions and approximate lengths of the deletions used in this study. The name of each deletion created at the *Sst*II site indicates the number of bases deleted. UGA stop codons in the reading frame used downstream of the frame-shifting deletions (x) are indicated. In Δ20-UGA, an 8-base linker was inserted at the deletion, making a net loss of 12 bases. There is an in-frame UGA in the linker.

from the L3'-*lacZ'* fusion gene carried by the wild-type and various deletion plasmids. Several different *E. coli* strains were used, all unable to suppress UGA termination codons to any measurable extent. We anticipated that reduced translation of the S10 gene might affect expression of L3'-*lacZ'* in an indirect way, by causing decreased transcription of the fusion gene because of transcriptional polarity (1). We took two steps to address this complication. First, in all the experiments we monitored not only translation but also transcription of the fusion gene. Also, in several of the experiments we used a host strain containing the *rho-100* mutation, which we expected would at least partially suppress transcriptional polarity (20).

To measure the rate of fusion protein synthesis, we either assayed the level of accumulated β-galactosidase activity or measured the incorporation of radioactive methionine into fusion protein during a 1-min pulse. To measure the rate of mRNA synthesis, we labeled the cells for 1 min with [^{3}H]uridine and hybridized the radioactive RNA to DNA probes from the S10-L3 or *lacZ* region of the fusion operon.

These experiments showed that all of the mutations reducing translation of the S10 gene also reduced the synthesis of the L3'-*lacZ'* fusion protein (Table 1). The in-frame control, Δ15, showed no significant effect on fusion protein synthesis. As anticipated, in the *rho*$^+$ strains the mutations also reduced the rate of synthesis of fusion protein mRNA, particularly of the *lacZ* portion of the transcript (Table 1). The most severely affected mutant was the ΔSDΔ4 double mutant, in which the *lacZ* region was virtually untranscribed in the *rho*$^+$ strains. On the other hand, the *rho-100* mutation reduced the polar effect of the translation mutations on transcription of the *lacZ* region: even in the ΔSDΔ4 double mutant, a measurable amount of fusion protein mRNA was synthesized (Table 1).

The measurements of fusion protein synthesis (Table 1) reflect the cumulative effect on transcription and translation. Therefore, to calculate the efficiency of translation of the L3'-*lacZ'* gene (i.e., to correct for indirect effects on mRNA levels), we normalized the rates of protein synthesis (or accumulation) to the rates of mRNA synthesis. The resulting values are shown in Table 1. We found that the mutations reducing translation of the S10 gene did indeed inhibit the translation of the L3'-*lacZ'* gene. That is, the translation of the L3 gene appears to be coupled to the expression of the upstream S10 gene. Interestingly, the coupling does not appear to be absolute. Even when the S10 gene was totally inactivated (e.g., in the ΔSDΔ4 mutant), there was still residual expression of the downstream L3 gene. Unfortunately, the degree of coupling is difficult to quantitate accurately in these experiments, since, even in the *rho-100* mutant, we observed effects on transcription of the fusion gene. Consequently, there were differences in the translation efficiencies, depending on whether the transcription rates of the S10-L3 region or of the *lacZ'* segment were used for the calculations.

TABLE 1. Translation efficiency of L3'-*lacZ'* fusion gene when translation of upstream S10 gene is reduced

Host[a]	Plasmid	Relative synthesis rates of:			Translation efficiency normalized to RNA from[b]:	
		Fusion protein[c]	Fusion RNA[d]			
			S10-L3	*lac*	S10-L3	*lac*
LL306	pLF2	1	1	1	1	1
rho$^+$	Δ15	0.80	0.80	0.75	1.00	1.07
	Δ20-UAG	0.13	0.48	0.41	0.27	0.32
	Δ32	0.03	0.54	0.17	0.06	0.18
	ΔSD	0.10	0.44	0.24	0.23	0.42
W3110	pLF2	1	1	1	1	1
rho$^+$	Δ32	0.07[e]	0.68	0.31	0.10	0.23
		0.08	0.87	0.27	0.09	0.30
	ΔSD	0.12[e]	0.48	0.30	0.25	0.40
		0.12	0.51	0.29	0.24	0.41
	ΔSDΔ4	0.04	0.23	0.06	0.17	—
		0.04	0.32	0.03	0.13	—
W3110	pLF2	1	1	1	1	1
rho-100	Δ32	0.14[e]	1.37	0.60	0.10	0.23
		0.18	1.28	0.81	0.14	0.22
	ΔSD	0.35[e]	0.99	0.60	0.35	0.58
		0.35	1.42	0.74	0.25	0.47
	ΔSDΔ4	0.19	0.81	0.36	0.23	0.53
		0.19	1.25	0.48	0.15	0.40

[a] Host strains carrying the indicated plasmids were grown in AB glucose minimal medium.

[b] The translation efficiencies were calculated as the ratio between the protein synthesis value and the corresponding rate of fusion messenger synthesis, using the value for each of the two hybridization probes. All data were normalized to the values determined for pLF2 in the same host. No corrections for S10 mRNA derived from the chromosomal S10 operon were made because this RNA accounts for less than 15% of the total rate of S10 mRNA synthesis in strains carrying pLF1 and related plasmids (12; our unpublished experiments). Also, since the chromosomal *lac* operon was not induced, the lac-1 probe detected only plasmid-derived *lac* message. —, Below detection limit.

[c] The rate of fusion protein synthesis was determined either by assaying accumulation of β-galactosidase activity (superscript e) or by quantitating radioactivity in the pulse-labeled fusion protein purified by gel electrophoresis (no superscript).

[d] The rate of transcription of the fusion gene operon was determined by hybridizing pulse-labeled RNA to the S10-L3 or the lac-1 probes (Fig. 1) immobilized on nitrocellulose filters.

Translation of mRNA synthesized by the T7 RNA polymerase. To completely escape the problem of transcription

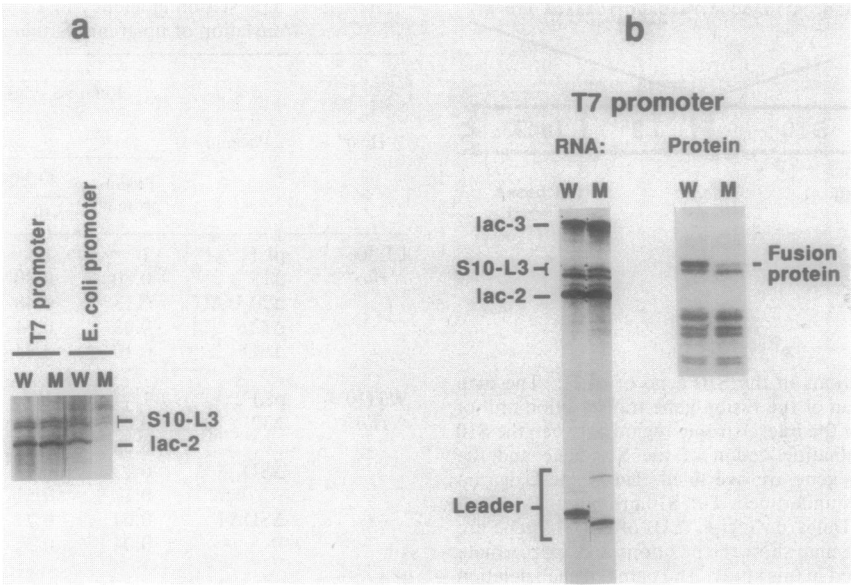

FIG. 3. Transcription and translation of the ΔSDΔ4 mutant expressed from the S10 and T7 promoters. Two versions of the pLF2 and pLF2-ΔSDΔ4 plasmids were used, one in which the fusion gene operon is transcribed from the natural promoter (harbored in strain LL308) and the other in which the T7 promoter has been substituted for the S10 promoter [harbored in strain BL21(DE3), in which T7 polymerase synthesis can be induced by the addition of isopropyl thiogalactopyranoside]. RNA and protein synthesized from the T7 promoter plasmids were labeled 10 min after induction of the T7 RNA polymerase gene. (a) Comparison of transcription by *E. coli* and T7 RNA polymerases. RNA from [^{3}H]uridine pulse-labeled cells was hybridized to a mixture of equimolar amounts of single-stranded DNA containing the S10-L3 and lac-2 inserts (Fig. 1). T1 nuclease-resistant hybridized RNA fragments were separated on a denaturing gel. The polymerase responsible for transcription of the labeled RNA is indicated above each lane. Lanes: W, plasmids without deletions in the fusion gene operon; M, plasmids containing the ΔSD and Δ4 deletions. (b) RNA and protein synthesis from the T7 promoter plasmids. Radioactive RNA was analyzed as described above, except that a pool of four probes was used. Protein from [^{35}S]methionine pulse-labeled cells was fractionated on a sodium dodecyl sulfate-polyacrylamide gel. Lanes are as explained above. The protected leader transcript from the ΔSDΔ4 deletion plasmid generated two bands because the trimming with RNase T1 was incomplete. The smaller of the two RNA fragments has the size expected from the position of the ΔSD deletion. For quantitation of the leader transcript, the two bands from the deletion mutant were combined. Even though strain BL21(DE3) carries the wild-type *lac* operon on the chromosome, the rate of synthesis of *lac* mRNA from the fully induced chromosomal operon is <5% of that of *lac* mRNA synthesized from the plasmid-borne P_{T7}-S10-L3′-*lacZ*′ fusion operon when the T7 RNA polymerase gene had been induced for 10 min (data not shown). Therefore, for the *lac* mRNA quantitation (Table 2) we made no correction for mRNA synthesized from the chromosomal *lac* operon. Since the *lac* operon in strain LL308 was not induced, it did not contribute to the synthesis of *lac* transcript.

polarity, we decided to transcribe the fusion gene operon with the T7 RNA polymerase. We expected this enzyme to be unaffected by the mutations reducing translation of the S10 gene, since nonsense mutations in phage T7 late genes have been reported to be nonpolar (28). To test this prediction, we constructed derivatives of pLF2 and the ΔSDΔ4 double mutant in which the S10 promoter was replaced with a T7 promoter positioned so that the T7 RNA polymerase would initiate transcription just one base upstream of the principal start site for the *E. coli* RNA polymerase at the S10 promoter (26). The T7 promoter plasmids were then introduced into a host carrying the T7 RNA polymerase gene under control of the *lac* promoter, such that synthesis of T7 RNA polymerase could be induced by the addition of isopropyl thiogalactoside (29). Following induction of T7 polymerase synthesis, we then monitored the synthesis of mRNA and protein from the fusion gene.

The rates of fusion protein and mRNA synthesis were measured 10 min after induction of T7 RNA polymerase synthesis. The synthesis rate of fusion protein was determined as described above. The rate of fusion protein mRNA synthesis was determined by hybridizing pulse-labeled RNA to a mixture of equimolar amounts of M13 phage DNA containing inserts from the S10 leader, the S10-L3 genes, the middle part of *lacZ* (lac-2), and the distal part of *lacZ* (lac-3; Fig. 1). After hybridization, nonhybridized RNA was degraded by RNase T1, and the various protected RNA fragments were electrophoresed through a denaturing polyacrylamide gel. Since the phages used in the hybridization mixture contain probes of different lengths, the RNA segments protected by each probe could be separated on the gel. This procedure allowed us to both visualize and quantitate the amount of message synthesized from various regions of the fusion protein operon. For comparison, we also analyzed transcripts made by the *E. coli* RNA polymerase from the S10 promoter.

As we had hoped, the synthesis of mRNA from the ΔSDΔ4 mutant by T7 polymerase was no longer subject to polarity effects (Fig. 3). Quantitation of the bands obtained with the T7 promoter derivatives of pLF2 and the ΔSDΔ4 mutant plasmid showed that all segments of the L3′-*lacZ*′ fusion operon message were transcribed at about the same rate from the ΔSDΔ4 mutant plasmid as from the wild-type plasmid (Fig. 3; Table 2). This is in contrast to the results obtained with the *E. coli* RNA polymerase, in which virtually no transcripts were seen for the L3′-*lacZ*′ fusion gene in the ΔSDΔ4 mutant (Table 1; Fig. 3a). Thus our results confirmed that the T7 RNA polymerase does not terminate transcription prematurely, even though the S10 mRNA is untranslated.

Although the mRNA synthesis rates were the same for both pLF2 and ΔSDΔ4, in the double mutant the L3′-*lacZ*′

TABLE 2. Expression of the L3′-*lacZ*′ fusion gene by using T7 RNA polymerase[a]

Plasmid	Relative synthesis rate				Translation efficiency normalized to RNA from:		
	Fusion protein	RNA from:			S10-L3	Mid-*lac* (lac-2)	Dist-*lac* (lac-3)
		S10-L3	Mid-*lac* (lac-2)	Dist-*lac* (lac-3)			
WT	1	1	1	1	1	1	1
ΔSDΔ4	0.23	0.74	1.17	0.93	0.31	0.19	0.25

[a] Differential rates of protein and mRNA synthesis were determined by quantitating the radioactivity in the pertinent bands of the gels shown in Fig. 3b. All data for the plasmid carrying ΔSDΔ4 mutations were normalized to the values obtained for the plasmid with the wild-type leader and S10 sequence. The table shows results from one of two experiments which gave essentially identical results. Mid-*lac*, Middle of *lacZ* gene; Dist-*lac*, distal portion of *lacZ*; WT, wild type.

fusion gene was translated at only 23% of the rate in the wild-type plasmid (Table 2). Since the use of the T7 RNA polymerase eliminated the influence of the ΔSDΔ4 double mutation on transcription, the protein synthesis measurements directly reflect the coupling of L3 translation to the translation of the S10 gene. These results confirm the conclusion from the experiments above, that the translation of L3 is indeed coupled to the translation of the S10 gene. However, it is also evident that the translational coupling is incomplete: even though translation of the S10 gene in the double mutant presumably is negligible, the downstream fusion gene is still translated at a significant rate.

Correlation of rates of translation of the S10 and L3 genes. The mutants used above were designed to essentially abolish expression of the S10 gene to confirm that translation of L3 is coupled to S10 synthesis. Once the coupling was established, we wanted to assess how less severe reductions in the rate of S10 synthesis affect the translation rate of the downstream L3 gene. For these studies we used a series of leader mutations which reduced S10 synthesis from about twofold to more than 99%. These mutations (see Materials and Methods), all mapping within the S10 leader, were constructed in pLF1, a plasmid containing the S10 promoter and leader followed by an S10′-*lacZ*′ fusion gene (Fig. 1). The mutated leaders were then transferred from pLF1 to pLF2 (Fig. 1), regenerating the intact S10 gene and creating an L3′-*lacZ*′ fusion gene. For each leader mutation, we then determined the translation efficiencies of the fusion genes on the pLF1 and pLF2 derivatives as the ratio between the radioactivity in the fusion protein band and the radioactivity in the band of lac-2 hybrid. Examples illustrating the data used for these measurements are shown in Fig. 4.

The translation efficiency of the S10′-*lacZ*′ fusion gene on the pLF1 derivatives measures the direct effect of a given leader mutation on translation of the S10 gene. The translation efficiency of the L3′-*lacZ*′ fusion gene on the pLF2 derivatives measures the indirect effect of the same leader mutation on the L3 gene through translational coupling. The translation efficiencies of the pLF1 mutants are plotted along the abscissa in the graph shown in Fig. 5; the corresponding pLF2 mutant measurements are plotted along the ordinate. Each point in the graph therefore illustrates the correlation for a given leader mutation between its direct effect on translation of S10 and its indirect effect, via coupling, on translation of the L3 gene. The graph confirms our earlier conclusion that, at very low rates of S10 translation, there is still 15 to 20% residual translation of the L3 gene. Furthermore, as the expression of the S10 gene improves, so does the rate of L3 synthesis. However, efficient expression of the L3 gene does not require optimal translation of S10: when translation of the S10 gene was decreased by 50 to 80%, translation of L3 was nearly maximal.

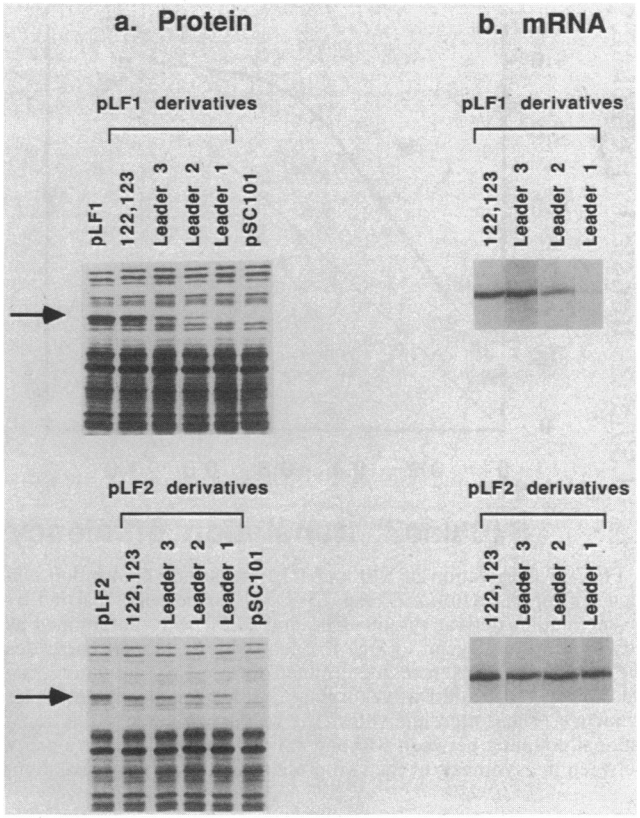

FIG. 4. Transcription and translation of leader mutant derivatives of pLF1 and pLF2. Cultures of strain LL308 carrying pLF1 or pLF2 or a mutant leader derivative of one of these plasmids (9; see Materials and Methods) were grown exponentially in glucose minimal medium. (a) Rates of fusion protein synthesis were determined for each culture by gel electrophoresis of extracts of [^{35}S]methionine-pulse-labeled cells. Arrows identify the fusion protein bands in autoradiograms of such gels. (b) Rates of fusion mRNA synthesis were determined on the same cultures by hybridizing RNA from [^{3}H]uridine-pulse-labeled cells to single-stranded DNA carrying the lac-2 insert (Fig. 1) and isolating RNase T1-resistant fragments on polyacrylamide gels. The mRNA bands for the pLF1 and pLF2 fusion genes are not shown in the fluorograms but had intensities similar to those observed for the 122,123 and leader 3 mutants. The mRNA bands from the leader 1 and 2 derivatives of pLF1 are relatively weak due to transcriptional polarity. Similar polarity was not observed for the corresponding pLF2 derivatives, even though polarity was observed for several other poorly translated pLF2 derivatives (Table 2; Fig. 3). We do not understand why some pLF2 derivatives exhibit transcriptional polarity and others do not. Translation efficiencies were determined as described in the text. By normalizing to the lac-2 probe, we corrected for the transcriptional polarity exhibited by the mutants showing very low fusion protein synthesis. The resulting values were then normalized to the translation efficiency for the relevant wild-type parent plasmid. The translation efficiencies obtained for the pLF1 derivatives were 0, 0.06, 0.03, and 0.13 for the leader 1, 2, and 3 and 122,123 mutants, respectively. The corresponding values for the pLF2 derivatives were 0.16, 0.23, 0.29, and 0.24. See also Fig. 5.

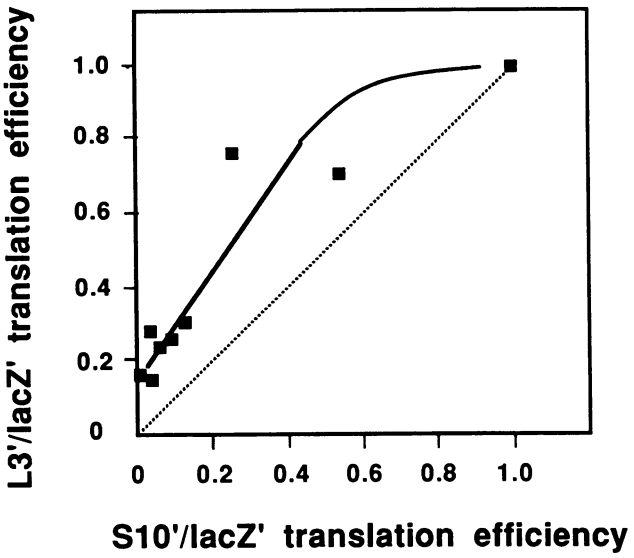

FIG. 5. Correlation of S10 and L3 translation. Translation efficiencies for the S10'-*lacZ'* and L3'-*lacZ'* fusion genes carried by leader mutant derivatives of pLF1 and pLF2 were determined as described in the legend to Fig. 4. Each point in the plot correlates the translation efficiencies determined for a pLF1 derivative (abscissa) and a pLF2 derivative (ordinate) with the same mutant leader sequence. The dotted line shows the results expected if the translational coupling between S10 and L3 had assured proportionality between the synthesis of the two proteins at all rates of translation.

DISCUSSION

Our experiments show that mutations decreasing translation of the S10 gene result in as much as an 80% inhibition of the translation of the downstream L3 gene. Thus, the translational coupling between the S10 and L3 genes is as efficient as the coupling between the *trpE* and *trpD* genes (23), the *trpB* and *trpA* genes (2, 8), and the *galT* and *galK* genes (25). Since these latter gene pairs are separated by 3 bases or less, and the S10-L3 intercistronic region is 32 bases long, our data show that a short intercistronic region is not a requirement for coupled translation of two genes.

The translational coupling between the S10 and L3 genes provides a mechanism for propagating L4-mediated inhibition of translation from the first to the second gene of the S10 operon. However, the coupling does not assure strict proportionality between the synthesis of S10 and L3, evidenced both by the 15 to 20% residual translation of the L3 gene during blocked S10 synthesis and by the near-normal rates of L3 translation observed when the translation efficiency of the S10 gene was still only about half the optimal level. Note that the coupling between S10 and L3 is considerably less efficient than the coupling in the two-gene L11-L1 r-protein operon (3, 27).

We have not analyzed the degree of translational coupling between the remaining genes of the S10 operon, but if the coupling between these other genes is also leaky, translational regulation by L4 would be further reduced (and eventually disappear) for the more distal genes of the operon. Interestingly, this prediction is consistent with the results of in vitro translation experiments by Yates and Nomura (31), which showed that only the proximal genes of the S10 operon are regulated by the addition of L4. Although the lack of translational regulation of downstream genes might result from artificial fragmentation of the DNA template or messenger RNA in the in vitro system (32), the experiments reported here suggest that the limited range of L4 regulation of translation may be a natural characteristic of the S10 operon. Weak translational coupling between the genes in the S10 operon would also explain the observation that a nonsense mutation in the L3 gene has a relatively strong polar effect on the adjacent downstream gene, weaker effects on the next two genes, and essentially no effect on the remaining genes of the operon (6).

We know from previous in vivo experiments that oversynthesis of L4 results in a strong inhibition of the synthesis of all the proteins encoded by the 11-gene S10 operon (34). Given the incomplete translational coupling of the first two genes of the operon (and possibly of other genes in the operon), we infer that other regulatory processes are necessary for efficient L4 control of the entire operon. In fact, we have shown that L4 also mediates premature termination of transcription upstream of the structural genes of the S10 operon (9, 12, 34). This form of autogenous control plays a major role in regulating the S10 operon; the results reported here suggest that the transcriptional regulation could be particularly important for the more distal genes which have escaped the translational control. We cannot exclude that expression of the operon is also modulated by indirect effects of the translation inhibition, such as *rho*-dependent transcription polarity and increased mRNA turnover.

A priori, there is no obvious reason why such a long operon needs both translation and transcription control. For example, coupled translation (and possibly the indirect effects of inhibition of translation just mentioned above) is apparently sufficient to regulate the 8 distal genes of the 10-gene *spc* operon. There is no evidence for a major contribution of transcription control in this r-protein operon (17) or, for that matter, for any other autogenously regulated r-protein operon (3, 16, 30, 33).

Two molecular models have been proposed for translational coupling (23). First, the secondary structure of the mRNA could sequester the ribosome-binding site of the distal gene, preventing access by initiating ribosomes unless ribosomes translating the upstream gene disrupted the structure. There are several examples in which mRNA secondary structure appears to play a role in coupling the translation of two genes (see, e.g., references 4 and 24). The short intercistronic region common to many pairs of coupled genes has suggested a second mechanism for coupling translation: ribosomes could be "handed over" from the proximal to the distal gene, perhaps because the efficiency of translation initiation of the distal gene is enhanced when ribosomes are delivered directly to the initiation region by the translation termination process at the upstream gene (23). A hand-over mechanism is likely to be responsible for the coupling of the V and VII genes of phage f1 (4; M. Ivey-Hoyle and D. A. Steege, J. Mol. Biol., in press) and may also be involved in the coupling of several other closely spaced genes (2, 8, 25, 32). Yates and Nomura (32) have suggested that such a mechanism could lead to sequential and equimolar translation of all genes in an r-protein operon; i.e., ribosomes translating a multicistronic r-protein message may bind to the mRNA only at the proximal gene and proceed through the operon without the discharge or binding of new ribosomes at the downstream translation initiation codons.

We do not know which molecular model applies to the translational coupling of the S10 and L3 genes. The genetic experiments reported here indicate that the L3 ribosome-binding site is capable of accepting ribosomes directly from the pool of nontranslating ribosomes without the ribosomes being routed through the S10 gene (the L3 gene is preceded

by a reasonable Shine-Dalgarno sequence). This conclusion is also supported by in vitro experiments showing that the first dipeptide of the L3 protein can be formed even when ribosomes initiating at the S10 gene are restricted to forming only one peptide bond (5). However, the in vivo translation of the L3 gene is clearly enhanced by translation of the upstream S10 gene. Although it is not clear what the molecular mechanism might be, it is conceivable that ribosomes terminating at the end of the S10 gene are more effective at initiating translation of L3 than are ribosomes from the free pool. Alternatively, ribosomes translating through the end of the S10 gene may melt out a secondary structure that prevents efficient binding of ribosomes at the beginning of the L3 gene. Computer analysis of the S10 operon sequence shows that the transcript can form a secondary structure involving the distal end of the S10 gene and the intercistronic region between the S10 and L3 genes. This hypothetical turnstile structure would sequester the Shine-Dalgarno sequence of the L3 gene, requiring translation of the distal part of the S10 gene for ribosomes to gain access to the L3 gene. Our preliminary analysis of mutations deleting the distal portion of the S10 gene is consistent with this model; such deletions, which we predict would disrupt the secondary structure, also destroy the translational coupling between the two genes. More experiments are clearly required for a critical test of this turnstile model.

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