SecA Protein Autogenously Represses Its Own Translation during Normal Protein Secretion in *Escherichia coli*

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The *Escherichia coli* *secA* gene, whose expression is responsive to the protein secretion status of the cell, is the second gene in an operon. We found that both the basal and induced levels of SecA biosynthesis are dependent on prior translation of the upstream gene, gene X, and identified two large gene X-*secA* transcripts. The 10-fold derepression of *secA* expression by protein export defects was at the translational level since no further increases in gene X or *secA* mRNA levels were detected during this period, and a *secA-lacZ* protein fusion but not an operon fusion was appropriately derepressed. Furthermore, overexpression of the SecA protein severely reduced expression of only the *secA-lacZ* protein fusion, indicating that SecA autogenously represses its own translation.

A genetic approach was used to study the molecular mechanisms responsible for protein localization in *Escherichia coli*. Genetic selections identified a set of *sec* genes whose products are required to promote the secretion of cell envelope proteins. Conditional lethal *secA* (13, 18), *secY* (25), *secD* (9), and *secE* (21) mutants have been described which, when shifted to the nonpermissive temperature, accumulate unsecreted protein precursors for most envelope proteins. A nonconditional *secB* null mutant exhibiting severe export defects for only certain envelope proteins has been described also (11). It has been noted previously that expression of the *secA* gene is somehow coordinated with the protein secretion status of the cell, since SecA protein synthetic levels are elevated 10- to 20-fold when protein export is blocked in *secA*, *secD*, and *secY* mutants and in strains producing high levels of an export-defective hybrid protein between maltose-binding protein and β -galactosidase (MalE-LacZ), but not in a *secB* null mutant (9, 19, 22). The *secA* gene is the second gene in an operon in which it is downstream of a gene with an unknown function termed gene X (3, 24). To begin to understand how *secA* expression is coordinated with the secretion proficiency of the cell, we investigated the regulation of the gene X-*secA* operon. Our results indicate that *secA* expression is translationally coupled to gene X and that *secA* derepression by protein export defects occurs at the translational level. Furthermore, we found that SecA protein autogenously represses its translation. Since a second *sec* gene, *secY*, is a distal gene in a ribosomal protein operon and its expression is also translationally coupled (1, 8, 15), the coregulation of protein secretion with the translational machinery warrants further consideration.

MATERIALS AND METHODS

Media and reagents. Media used for growth of bacteria have been described previously (16). Restriction and modifying enzymes were obtained from New England Biolabs, Inc. (Beverly, Mass.), Bethesda Research Laboratories, Inc. (Gaithersburg, Md.), and International Biotechnologies, Inc. (New Haven, Conn.), and were used as recommended by the manufacturer. Isopropyl- β -D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside were ob-

tained from Sigma Chemical Co. (St. Louis, Mo.) and Boehringer Mannheim Biochemicals (Indianapolis, Ind.), respectively. The following radiochemicals were used. [32 P]dATP (600 to 800 Ci/mmol) was from ICN Radiochemicals (Irvine, Calif.) or Dupont, NEN Research Products (Boston, Mass.), and [35 S]methionine (~1,000 Ci/mmol) was from Amersham Corp. (Arlington Heights, Ill.). ATP, deoxynucleotide triphosphates, and dideoxynucleotide triphosphates were obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). The RNase inhibitors vanadyl ribonucleoside complex and RNaguard were from Bethesda Research Laboratories and Pharmacia, respectively. IgSorb was obtained from the New England Enzyme Center. The M13 *lac* universal primer 1211 was purchased from New England Biolabs.

Bacterial and bacteriophage strains. The parental strain *E. coli* MC4100 ($F^- \Delta lacU169 araD136 relA rpsL thi$) and its derivative MM18 containing the $\Phi(malE-lacZ)72-47$ (Hyb) fusion have been described previously (10). The gene X109(Am) *supF*(Ts) strain used was MM113 (12). The *secY*(Ts) strain used was IQ85 (25). Both of these strains were isogenic with MC4100. The *secY*(Ts) gene X109(Am) *supF*(Ts) strain was made by introducing the *secY24*(Ts) mutation into MM113 by P1 transduction with the linked *rpsE* marker. Strain MM171 [$\Phi(secA-lacZ)f181$ (Hyb) *leu::Tn5* (lambda PR9)] was kindly provided by Jon Beckwith (21). MM171.3 is a derivative of MM171 containing the following additional markers: *leu*⁺, *recA1*, and *srl::Tn10*. A *secA-lacZ* operon fusion was made by first integrating the plasmid pMF10 containing the *secA-lacZ* fusion into the *secA* locus of MC4100 [*leu::Tn5* (lambda PR9)] by transducing the strain to *polA1* by using a linked *Tn10*. A P1 lysate was made on this strain and was used to transduce MC4100 (lambda PR9) to kanamycin resistance, Lac⁺, and scoring for ampicillin sensitivity. An ampicillin-sensitive transductant was made into a *recA* mutant by conjugation with MS367 (a KL16 derivative containing *recA1 srl::Tn10*) selecting for tetracycline and kanamycin resistance and scoring for sensitivity to UV irradiation, resulting in strain DO308.1 [MC4100 $\Phi(secA-lacZ)mf10 leu::Tn5 recA1 srl::Tn10$ (lambda PR9)]. The correctness of the $\Phi(secA-lacZ)mf10$ fusion in DO308.1 was verified by Southern blot analysis with *secA* and *lacZ* oligonucleotide probes. Strains MM294 ($F^- hsdR endA thi supE44$) and MC1000 [$F^- \Delta(leu-$

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ara)7697 *araD*139 Δ *lacX*74 *rpsL galU galk*] were used to propagate plasmids. M13-MS300 is a derivative of M13mp18 carrying a 1,020-nucleotide *Pvu*II-*Hind*III DNA fragment encoding gene X and the proximal region of *secA* (24).

Plasmid constructions. Plasmid pE1 was made by cloning the 0.8- and 2.5-kilobase (kb) *Eco*RI fragments encoding the end of gene X and the entire *secA* gene (24) into the *Eco*RI site of a pBR325 derivative plasmid (lacking the *Hind*III site) in a clockwise orientation. Plasmid pW2 is a derivative of pE1 containing a *Bam*HI linker inserted into the *Bss*HII site within gene X. Plasmid pT7-*secA* was made by inserting a 4.8-kb *Bam*HI fragment from pE1 encoding the end of gene X, all of *secA*, and portions of the chloramphenicol acetyltransferase and tetracycline genes into the *Bam*HI site of pET-5 (23) in a counterclockwise direction. Plasmid pMF1 is a derivative of pT7-*secA* containing a *Sac*I linker inserted into the *Ava*I site of the pBR322 portion of this plasmid. A plasmid containing the gene X-*secA* operon, pMF8, was made by cloning a 620-base-pair *Sac*I-*Bss*HII fragment encoding the proximal region of gene X (isolated from M13-MS300 replicative form DNA cleaved with these enzymes [24]) into pMF1 that was partially cleaved with *Bss*HII (in the gene X region) and completely cleaved with *Sac*I. A plasmid containing a *secA* frameshift mutation, pAR1, was made by cleaving pMF8 with *Sal*I, filling in the 5' overhang with Klenow polymerase I, and religating the plasmid. A pMF8 derivative, pAS2, containing the *secA*51(Ts) mutation was made by integrating pMF8 into the *secA* locus of a *secA*51(Ts) strain by transducing the strain to *polA* by using a linked Tn10. Transductants containing the integrated plasmid were subsequently transduced to *polA*⁺ using a linked Tn5 to promote plasmid excision. Plasmid DNA was isolated from individual transductants and tested for *secA* function by complementation of a *secA*51(Ts) mutant. Negative complementing plasmids were subjected to restriction enzyme analysis and DNA sequencing to verify the presence of the *secA*51(Ts) mutation. The *secA-lacZ* operon fusion-containing plasmid pMF10 was made by inserting a 7.7-kb *Bam*HI fragment from pMC903 encoding the *lac* operon without its promoter (6) into the *Bgl*II site of pMF8 in a counterclockwise orientation. The *secA-lacZ* protein fusion containing plasmid pMF11 was made by inserting a 3.1-kb *Bam*HI fragment from pMC1871 encoding the *lacZ* gene lacking its translational initiation region (7) into the *Bgl*II site of pMF8 in a counterclockwise orientation. Both pMF10 and pMF11 were isolated by transforming MC1000 to ampicillin resistance and detecting blue colonies on medium containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside. All plasmids were verified by restriction enzyme analysis.

RNA isolation and quantitation. MM18 was grown in M63 minimal medium (made with sodium phosphate rather than potassium phosphate) with glycerol (0.4%) as the carbon source for 2 h at 37°C, when the culture was divided into two equal portions and either glucose or maltose was added to a final concentration of 0.4%. Total RNA was isolated 2 h later by the method of Sundaresan et al. (27). Total RNA concentrations were determined by UV adsorption at A_{260} and A_{280} . The gene X- and *secA*-specific RNA was quantitated by dot blot analysis (28) with a microfiltration apparatus (Bio-Dot; Bio-Rad Laboratories, Richmond, Calif.). Hybridization was performed as described by Maniatis et al. (14). RNA levels were determined by densitometric tracings of autoradiograms with a laser densitometer (Ultrosan XL; LKB Instruments, Inc., Rockville, Md.) and Gel Scan software (2400 Gel Scan; LKB).

S1 nuclease mapping. ³²P-labeled, single-stranded probes

for S1 nuclease mapping were made by primer extension by incubation of 10 μ Ci of [³²P]dATP; 30 μ M each of dTTP, dCTP, and dGTP; 2.5 ng of the *lac* universal primer; and 1 μ g of M13-MS300 single-stranded DNA in the presence of 2.5 U of the Klenow fragment of DNA polymerase at 37°C for 15 min. Different probes were generated by subsequent digestion of this mixture with the appropriate restriction enzymes followed by purification of the correct single-stranded fragment on a sequencing gel containing 6% acrylamide and 7 M urea. S1 nuclease analysis was performed as described by Barry et al. (2).

Protein analysis. The immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography techniques that we used have been described previously (19, 24). For quantitation of radiolabeled protein, autoradiography was carried out on film (X-Omat AR; Eastman Kodak Co., Rochester, N.Y.) that was preflashed according to the manufacturers specifications. Autoradiograms were quantitated with a laser densitometer (Ultrosan XL; LKB) and software (2400 Gel Scan; LKB).

RESULTS

***secA* expression is dependent on translation of gene X.** In order to begin to understand how *secA* expression is coordinated with the cellular secretion proficiency, we characterized the regulation of the gene X-*secA* operon. We have previously described the nucleotide sequence of this region and have presented evidence that these two genes constitute an operon based on their structure and the observation that a distal gene X amber mutation (*am*109) is strongly polar on *secA* expression (19, 24). The effect of the *am*109 mutation on *secA* expression is one of polarity rather than the fact that the gene X product is required for *secA* expression, since introduction of a plasmid that contained a copy of gene X into a strain containing this mutation had no effect on the reduction in SecA levels observed (unpublished data). In the present study we determined whether the *am*109 mutation is polar on both the basal and induced levels of *secA* expression. A positive result would indicate that *secA* induction occurs from a promoter upstream of gene X rather than a promoter located between these two genes. An *am*109 *supF*(Ts) strain was constructed so that we could vary the extent of gene X translation beyond the *am*109 position by growing it at different temperatures. Although this strain was already somewhat defective for protein export (see Fig. 1), it was unclear whether this partial defect alone was sufficient to completely induce *secA* expression. Therefore, in order to have a strain in which *secA* expression should be maximally induced by a strong secretion block, we introduced a *secY*(Ts) mutation into the *am*109 *supF*(Ts)-containing strain by P1 transduction. The *am*109 *supF*(Ts) single mutant and the *secY*(Ts) *am*109 *supF*(Ts) double mutant as well as the *secY*(Ts) mutant and wild-type strains were grown at 30°C or shifted to 42°C for 2 h and pulse-labeled with [³⁵S]methionine for 1 min in order to measure SecA synthesis levels and the cellular export proficiency of the two secretory proteins maltose-binding protein (MalE) and the outer membrane protein OmpA. Even at 30°C the *am*109 *supF*(Ts) and the *secY*(Ts) *am*109 *supF*(Ts) strains showed reduced SecA levels compared with those of the *secY*(Ts) mutant and wild-type strains (Fig. 1, lanes 1 and 2 versus lanes 3 and 4), which is consistent with the presence of a partial polar effect caused by the *am*109 mutation. The *supF*(Ts) suppressor had only a 30% suppression efficiency at this temperature, as measured by the introduction of a *lacZ*⁺- or *lacZ*(Am)-

FIG. 1. Dependence of SecA synthesis on gene X translation. Cells were grown in M63 minimal medium containing maltose (0.4%) at 30°C or shifted to 42°C for 2 h. The cultures were then pulse-labeled with [35 S]methionine for 1 min; and SecA, MalE, and OmpA were analyzed by immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography. The positions of SecA, MalE, and OmpA and the precursors to MalE (pMalE) and OmpA (pOmpA) are indicated.

containing gene into this strain (D. Oliver, unpublished data). On a shift to 42°C, the SecA level produced by the *am109 supF(Ts)* and *secY(Ts) am109 supF(Ts)* strains was undetectable, as was the suppression efficiency of the *supF(Ts)* suppressor at this temperature (Fig. 1, lanes 5 and 6). In this case *secA* expression should have been maximally induced since a complete export block for both secretory proteins monitored was obtained in the *secY(Ts) am109 supF(Ts)* double mutant at 42°C, and the *secY(Ts)* parent strain displayed the usual derepression of SecA synthesis compared with the wild-type strain (Fig. 1, lanes 6 through 8). These findings indicate that the *am109* mutation is polar on both the basal and induced levels of SecA and imply that *secA* gene expression is always dependent on the continued translation of gene X beyond the *am109* position.

Analysis of gene X and *secA* mRNA. Since *secA* expression is coupled to gene X translation, it seemed possible that *secA* derepression by protein export defects may be due to a form of translational rather than transcriptional control, as has been seen for translational coupling in the ribosomal protein operons (15, 17). Therefore, we measured gene X and *secA* mRNA levels before and after we imposed a protein secretion block. Strain MM18 produces an export-defective MalE-LacZ hybrid protein which blocks secretion when it is produced at high levels during maltose induction (10), causing SecA synthesis levels to rise from approximately 2-fold higher than that of the wild type to 10-fold higher than that of the wild type within 1 h after maltose induction (19, 22). Total RNA was isolated from MM18 with or without maltose induction for 2 h, and the amount of gene X- and *secA*-specific RNA was quantitated by dot blot analysis with a single-stranded antisense DNA probe containing all of gene X and the first 201 nucleotides of the *secA* gene. It was clear that the maltose-induced and -uninduced cultures contained

FIG. 2. The gene X-*secA* mRNA levels remain unaltered during an export block. Increasing concentrations of total RNA from MM18 grown with (solid bars) or without (hatched bars) maltose induction were loaded onto nitrocellulose with a microfiltration apparatus (Bio-Dot; Bio-Rad). The nitrocellulose blot was hybridized to a single-stranded antisense gene X-*secA* DNA probe. The concentration of gene X- and *secA*-specific RNA was determined by densitometric tracings of the autoradiogram.

the same level of gene X- and *secA*-specific RNA, even though at the time that they were harvested they showed a fivefold difference in SecA protein synthesis for more than 1 h (Fig. 2; data not shown). A similar result was obtained if a *secA*-specific probe containing the middle and distal portions of this gene was used for dot blot analysis or if RNA was prepared in the presence of the RNase inhibitors vanadyl ribonucleotide complex or RNAGuard (data not shown). Although we cannot rule out the possibility that the *secA* mRNA level was elevated during a secretion block but was very labile in vivo, the results obtained support the notion that *secA* derepression by export defects occurs at a posttranscriptional level.

We used the S1 nuclease mapping technique (4) to identify the 5' ends of the gene X-*secA* transcripts produced in MM18 cells grown with or without maltose induction. In no case did we find a difference in the quantity of the different transcripts produced in the presence or absence of the export block, further supporting the notion that *secA* derepression by secretion defects occurs at the posttranscriptional level. The S1 nuclease mapping results shown in Fig. 3 revealed two major transcripts encoding gene X and *secA* sequences. The largest one arose by readthrough of the terminator located at the end of the *envA* gene (3), while the 5' end of the other one mapped just downstream of a predicted promoter for gene X (3, 24). That a significant level of this readthrough transcript exists is not surprising in this case, since immediately upstream of the terminator there are four genes, *ftsQ*, *ftsA*, *ftsZ*, and *envA*, all of which are oriented in the same direction with a separate and in some cases strong promoter for each gene and no transcriptional terminator within this gene cluster (3). Besides these two large transcripts, several additional S1 nuclease-protected transcripts present in a lower amount were detected which

FIG. 3. Analysis of gene X-*secA* Transcripts. (A) M13-MS300 single-stranded DNA containing a 1,020-nucleotide *PvuII-HinIII* gene X-*secA* DNA fragment was extended with a universal *lac* primer. It was then digested with *SacI* to create probe 1, *SacI* and *EcoRI* to create probe 2, or *SacI* and *NcoI* to create probe 3. The seven RNAs detected are indicated by bars along with the positions of the *envA*, gene X, and *secA* genes. (B) S1 nuclease analysis of MM18 RNA by using probe 1. Lanes 1 to 3, Probe 1 without RNA and S1 nuclease addition, without RNA but with S1 nuclease addition, and with RNA and S1 nuclease addition, respectively; lanes 4 to 7, reference DNA sequence ladder for lanes 1 to 3 with the *lac* primer and M13-MS300 template used for the reaction.

had 5' ends mapping distal in gene X and proximal in *secA* (Fig. 3A; data not shown). Several lines of evidence indicate that these shorter transcripts were probably caused by in vivo or in vitro processing and degradation of the two larger transcripts. First, no predicted promoter elements have been found in the DNA sequence immediately upstream of *secA*

(24). Second, the two shortest transcripts would lack the 5'-coding region of the *secA* gene and therefore would be nonfunctional (Fig. 3A). Third, the fact that *secA* expression is always dependent on gene X translation (Fig. 1) argues that none of these shorter transcripts give rise to SecA protein synthesis.

A *secA-lacZ* protein fusion but not an operon fusion responds to a protein export defect. In order to study further the regulation of the *secA* gene by protein export defects, we constructed a plasmid, pMF8, containing the entire gene X-*secA* operon. In order to verify that this plasmid-encoded copy of the *secA* gene is correctly regulated by an export defect, we transformed pMF8 or the vector plasmid pET-1, which lacks any gene X-*secA* sequences, into MM18. These two strains were grown with or without maltose induction for 1.5 h, when they were pulse-labeled with [³⁵S]methionine for 1 min, and the level of SecA synthesis was measured by quantitative immunoprecipitation. MM18(pMF8) grown without maltose made approximately nine times more SecA than the control strain did (Fig. 4A). This high basal level is presumably caused by a gene dosage effect, and it allowed us to attribute most of the SecA made in this strain to the plasmid-encoded *secA* gene copy. After growth in medium containing maltose, the level of SecA synthesized in MM18(pMF8) was induced 3.5-fold over this basal level, indicating that the plasmid-encoded copy of the *secA* gene is induced by this export defect.

In order to gain additional evidence that regulation of the *secA* gene by protein secretion defects was at the translational level, we constructed *secA-lacZ* operon and *secA-lacZ* protein fusion derivatives of pMF8, resulting in plasmids pMF10 and pMF11, respectively (see above). These two plasmids were transformed into MM18. These two strains were grown with or without maltose induction for 1.5 h, when they were pulse-labeled with [³⁵S]methionine for 1 min; and the levels of SecA, LacZ, or the SecA-LacZ hybrid protein were measured by quantitative immunoprecipitation. Although the chromosomal *secA* gene responded correctly to maltose induction in both strains (Fig. 4B), only MM18(pMF11) showed derepression of the SecA-LacZ hybrid protein by maltose, whereas the level of LacZ remained constant in MM18(pMF10) (Fig. 4C). The derepression of the MalE-LacZ hybrid protein and the export defects for two secretory proteins MalE and OmpA were similar for both strains (data not shown). Since the level of LacZ should be proportional to the *secA* mRNA level, this result implies that the *secA* mRNA level does not increase during an export block and that the correct regulation of the *secA* gene requires its cognate translational initiation region.

SecA protein mediates autogenous translational repression. In order to test whether the translational regulation of the *secA* gene was caused by some sort of autogenous control mechanism, we determined whether artificially increasing SecA levels can repress the expression of a *secA-lacZ* reporter cassette. To this end, we constructed strains that contained *secA-lacZ* operon or protein fusions integrated into the chromosome at the *secA* locus and that overproduced various wild-type or mutant forms of SecA protein encoded on multicopy plasmids. In order to have wild-type *secA* function, these strains also carried a copy of the gene X-*secA* operon on a lambda prophage and were *recA* mutants, to prevent recombination from occurring between the different *secA* gene copies. Since all of these strains possessed the wild-type *secA* function and did not display protein export defects, as assayed by MalE and OmpA processing (data not shown), we could separate the autoge-

FIG. 4. A *secA-lacZ* protein fusion but not an operon fusion responds to a protein export defect. Cells were grown in M63 minimal medium containing glycerol (0.4%) at 30°C until they reached an A_{600} of 0.3, when they were split into two equal portions and glucose (–maltose) or maltose (+maltose) was added to a final concentration of 0.4%. The cultures were then pulse-labeled 1.5 h later with [35 S]methionine for 1 min; and SecA, LacZ, and the SecA-LacZ hybrid proteins were analyzed by coimmunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography. Protein synthetic levels were determined by densitometric tracings of autoradiograms. (A) SecA levels in MM18 containing pET-1 (solid bars) or pMF8 (stippled bars). (B and C) SecA and LacZ or SecA-LacZ levels, respectively, in MM18 containing pMF10 (solid bars) or pMF11 (hatched bars).

nous regulation of *secA* from its derepression during protein export defects. β -Galactosidase assays were performed on these strains, and the results are given in Table 1. First, the *secA-lacZ* operon fusion strain DO308.1(pBR322) produced 3.7 times more β -galactosidase activity than the comparable *secA-lacZ* protein fusion strain MM171.3(pBR322) did, in keeping with the contention that *secA* expression is translationally repressed in the latter strain. Quantitation of LacZ and SecA-LacZ protein levels in these two strains by radiolabeling and immunoprecipitation analysis indicated that the level of synthesis of these two proteins was in complete agreement with the β -galactosidase activities that were recorded (taking into account that the β -galactosidase activity of the SecA-LacZ hybrid protein is temperature sensitive above 30°C) (data not shown). Second, introduction of the plasmid pMF8, which overproduces SecA protein approximately ninefold (Fig. 4A), into these two strains resulted in greater than a threefold reduction in β -galactosidase activity in MM171.3, while only a 1.5-fold decrease was observed for DO308.1. This suggests that either gene X or the SecA protein is responsible for the repression that was observed and that repression occurs mainly at the translational level. The more modest reduction in expression of the *secA-lacZ* operon fusion by SecA overproduction in DO308.1(pMF8) may be due to polarity, since translational repression of the

upstream *secA* gene would result in a lower ribosomal density in this region of the gene X-*secA-lacZ* mRNA, which could affect overall mRNA stability or the utilization the *lacZ* ribosome-binding site. Alternatively, there could be a modest but real transcriptional effect in this case. In order to specifically determine whether the translational repression observed with MM171.3(pMF8) is caused by SecA protein overproduction, we transformed MM171.3 with a plasmid, pMF1, that lacked gene X and that produced only low levels of SecA protein or a derivative of pMF8, pAR1, which contained a frameshift mutation within the *secA* gene. Both plasmids were unable to repress expression of the *secA-lacZ* protein fusion contained in MM171.3 (Table 1). We conclude that SecA overproduction is necessary for the translational repression observed and that it is therefore some form of autogenous control, the precise mechanism of which needs further exploration. Further support of the notion that *secA* expression is autogenous was obtained by analyzing *secA* and *secA-lacZ* expression in MM171.3 carrying a pMF8 derivative, pAS2, that contains the *secA51*(Ts) mutation. MM171.3(pAS2) showed only a 1.6- and 1.8-fold reduction in β -galactosidase activity at 37 and 30°C, respectively, compared with MM171.3(pBR322), suggesting that this altered SecA protein is partially defective in mediating translational repression at either temperature. This conclusion was sup-

TABLE 1. SecA mediates autogenous translational repression^a

Strain	Plasmid	Plasmid genotype	β -Galactosidase activity at ^b :	
			37°C	30°C
DO308.1	pBR322	Gene X ⁺ <i>secA</i> ⁺	3,012 $\pm$ 344	ND ^c
	pMF8		1,952 $\pm$ 154	ND
MM171.3	pBR322	Gene X ⁺ <i>secA</i> ⁺	269 $\pm$ 11.5	805 $\pm$ 38.8
	pMF8		87.7 $\pm$ 8.2	255 $\pm$ 8.7
	pMF1	<i>secA</i> ⁺	273 $\pm$ 7.3	ND
	pAR1	Gene X ⁺ <i>secA</i> ⁰	245 $\pm$ 4.7	ND
	pAS2	Gene X ⁺ <i>secA</i> (Ts)	171 $\pm$ 38.7	453 $\pm$ 23.4

^a Strains were subcultured into LB supplemented with 20 μ g of ampicillin per ml in duplicate and grown at the indicated temperature until the mid-logarithmic phase of growth; they were then placed on ice. Dilutions of the cultures were plated on TYE (10 g of Bacto-Tryptone, 5 g of yeast extract, 8 g of NaCl, and 15 g of Bacto-Agar per liter) and TYE-ampicillin plates to score for plasmid loss, which was less than 5% in all cases.

^b β -Galactosidase assays were performed in triplicate by the method of Miller (16). β -Galactosidase activity is indicated in Miller units (16).

^c ND, Not determined.

over the gene X termination codon. A similar RNA structure (Fig. 5B) may also account for the translational coupling observed for the *secY* gene, which is the eighth gene in a row to be translationally coupled to *rplE* in the *spc* operon (1, 8, 15, 26). A major difference between these two structures is that the *rplO-secY* intergenic spacing is only 7 nucleotides long, as opposed to 61 nucleotides for the comparable gene X-*secA* intergenic region. This difference may be of regulatory significance, since short intergenic spaces are typical of translationally coupled genes whose protein products are made in equimolar amounts, whereas larger intergenic spaces are often seen when translationally coupled genes are translated at different levels (17). The larger gene X-*secA* intergenic region may be the target for the translational regulation observed here by SecA or some other *sec* gene product that serves a dual role by controlling stem formation and promoting protein secretion, thus coordinating the two processes in some manner. Whether this or another type of mechanism in fact accounts for the type of translational regulation observed here remains to be determined.

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