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Regulation of *Escherichia coli secA* mRNA Translation by a Secretion-Responsive Element

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The *Escherichia coli secA* gene, whose translation is responsive to the proficiency of protein export within the cell, is the second gene in a three-gene operon and is flanked by gene X and *mutT*. By using gene fusion and oligonucleotide-directed mutagenesis techniques, we have localized this translationally regulated site to a region at the end of gene X and the beginning of *secA*. This region has been shown to bind SecA protein in vitro. These studies open the way for a direct investigation of the mechanism of *secA* regulation and its coupling to the protein secretion capability of the cell.

Genetic approaches have been used in *Escherichia coli* to identify a set of genes whose products are required to promote the export of proteins to the cell envelope. Six *sec* genes are now known: *secA* (29), *secB* (18), *secD* (13), *secE* (34, 36), *secF* (14), and *secY/prlA* (11, 39). Biochemical analysis of these *sec* gene products in in vitro protein translocation systems is now being used to determine the function of this complex system. The cytoplasmic SecB protein possesses an antifolding activity that maintains the precursor protein in a conformation that allows functional binding to the membrane-associated SecA protein, which has been shown to possess essential protein translocation and ATPase activities (6, 8, 9, 19, 21, 44, 45). Of the four integral membrane proteins composing this system, no specific function has been assigned to SecD or SecF proteins (13, 14), while SecE and SecY/PrlA proteins in concert with SecA protein have been shown to be sufficient to catalyze protein translocation into reconstituted proteoliposomes (4, 42). Biochemical studies have revealed the presence of additional soluble components that facilitate in vitro protein export (46), as well as two distinct signal peptidases for processing secretory precursor proteins (43, 47).

Little is known about the regulation of this complex protein secretion machinery. It has been observed previously that *secA* expression is coordinated with the protein secretion capability of the cell, since the level of SecA protein synthesis increases approximately 10-fold when protein export is blocked genetically by using *sec* mutants or physiologically by treatment with sodium azide, a specific inhibitor of SecA ATPase activity (31, 32, 35). This form of regulation is exquisitely sensitive to the protein export status within the cell, since even mild protein export defects markedly derepress *secA* expression (30, 31, 34, 35). More recent studies have revealed that this regulation operates at the translational level and that SecA protein represses its own translation during conditions of normal protein export but not during a block in protein export (37). The secretion-responsive element (SRE), which is the site(s) of regulation of *secA* translation, has not been defined yet. To further elucidate the regulation of this operon and to determine the location of the SRE site, we used gene fusion and oligonucleotide-directed, deletion mutagenesis techniques. This approach indicated that the SRE site is located in the region at the end of gene X and the beginning of *secA*.

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MATERIALS AND METHODS

Media and reagents. M63 minimal medium and L broth, used for growth of bacteria, and TYE plates and H top agar, used for plating M13 bacteriophage, have been described previously (26). Restriction and modifying enzymes were obtained from New England Biolabs, Bethesda Research Laboratories, Inc., and International Biotechnologies Inc. T7 DNA polymerase (Sequenase) was obtained from United States Biochemical Corp. All enzymes were used as recommended by the manufacturer. Isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were obtained from Sigma Chemical Co. and Boehringer Mannheim Biochemicals, respectively. Radiochemicals, chemicals, and their manufacturers were as follows: [α-^{32}P]dATP (600 to 800 Ci/mmol) and [γ-^{32}P]ATP (6,000 Ci/mmol), ICN Radiochemicals or Dupont, NEN Research Products; [^{35}S]methionine (~1,000 Ci/mmol) and Tran-^{35}S-label (~1,100 Ci/mmol), Amersham Corp.; ATP, deoxynucleotide triphosphates, and dideoxynucleotide triphosphates, Pharmacia Fine Chemicals; IgSorb, the New England Enzyme Center; M13 *lac* universal primer no. 1211, New England Biolabs; Gene Clean, Bio 101, Inc.

Bacterial and bacteriophage strains. The parental *E. coli* strain MC4100 (F$^-$ Δ*lacU169 araD136 relA rpsL thi*) and its derivative MM18 containing the Φ(*malE-lacZ*)*72-47*(Hyb) fusion have been described previously (17). MGS68 (*recA1 srl*::Tn*10 secA22*) is an MM18 derivative containing a mutation inferred by genetic mapping methods to be in the *secA* gene. This alteration changes the electrophoretic mobility of the SecA protein but not its protein translocation activity. MC4100.2 (*recA1 srl*::Tn*10*) is a derivative of MC4100. MC1000 [F$^-$ Δ(*ara-leu*)*7697 araD139 lac*Δ*X74 galU galK*] (13) was used for plasmid constructions. MC1000.4 [*secA51* (Ts) *recA1 srl*::Tn*10*] is a derivative of MC1000 which was used to select for plasmid-encoded *secA* function. MM294 (F$^-$ *hsdR endA thi supE44*) is a Lac$^+$ strain obtained from Mark Ptashne. BW313 (*relA1 ung-1 dut-1 spoT1 thi-1*) (20) and JM101 [Δ(*lac-pro*) *supE thi* F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} *lacZ* Δ*M15*] were used as hosts for the oligonucleotide-directed mutagenesis studies. Bacteriophage M13-MS300 (M13mp18 containing a 460-bp *Pvu*II-*Hin*III fragment span-

ning the gene X-*secA* intergenic region) was used as a source of DNA for the SRE mutagenesis studies and has been described previously (38).

Plasmids. pMF8 (containing the gene X-*secA* operon) and pMF11 [a pMF8 derivative containing the Φ(*secA-lacZ*)*f181* (Hyb) fusion] have been described previously (37). Plasmid pMF9 is a derivative of pMF8 with the deletion of a 420-bp *Hpa*I fragment spanning the end of gene X and the beginning of *secA*. Plasmids pAX1 and pBA1 are pMF8 derivatives containing gene X-*lacZ*(Hyb) and *mutT-lacZ*(Hyb) fusions, respectively. Plasmid pAX1 was constructed by ligating the largest restriction fragment derived from an *Hpa*I and *Sal*I digest of pMF8 to the *lacZ* fragment derived from a *Sma*I and *Sal*I digest of pMC1871 (7). In order to construct pBA1, pMF8 was digested partially with *Mlu*I, and the incompletely digested, linear plasmid DNA was ligated with a *Mlu*I-*Bam*HI oligonucleotide adapter. One of the resulting plasmids, pAW13, contained the adapter located within the *mutT* gene. Plasmid pAW13 was digested with *Bam*HI, and the large restriction fragment was isolated and ligated to the *lacZ* fragment derived from a *Bam*HI digest of pMC1871 (7), resulting in plasmid pBA1. Both pAX1 and pBA1 were isolated as transformants of MC1000 that gave blue colonies on media containing X-Gal. Plasmid pBC1 is a derivative of pBA1 containing a 4-bp frameshift mutation in the plasmid-encoded *secA* gene due to repair of *Bgl*II-digested pBA1 DNA with Klenow fragment. Plasmid pBD1 is a *Sac*I-*Bgl*II deletion derivative of pBA1 missing the promoter region, gene X, and the proximal portion of the *secA* gene. It was constructed by digesting pBA1 completely with *Bgl*II and partially with *Sac*I and then treating the DNA ends with T4 DNA polymerase in the presence of 20 μM (each) deoxynucleoside triphosphates. The 7,615-bp DNA fragment was isolated, ligated, and used for transformation. Plasmid pCB9 is a derivative of pMF8 containing a Tn*phoA* insertion after nucleotide 719 of the published gene X-*secA* DNA sequence (38) and produces a hybrid protein containing the first 134 amino acid residues of gene X fused to alkaline phosphatase lacking its signal peptide. This Tn*phoA* fusion was made by the method of Manoil and Beckwith (23). The approximate position of the fusion joint was determined first by restriction enzyme analysis. A 3,732-bp *Hpa*I-*Bgl*II fragment containing the fusion joint was isolated from pCB9, cloned into M13mp19 cleaved with *Hin*cII and *Bam*HI, and subjected to DNA sequence analysis by using an oligonucleotide complementary to the early region of the *phoA* gene. All plasmid constructions were done from DNA fragments that were purified from agarose gels by using Gene Clean according to the manufacturer's instructions and were verified by restriction enzyme and DNA sequence analysis.

β-Galactosidase assays. Strains were subcultured in duplicate into L broth supplemented with 20 μg of ampicillin per ml and grown at 30°C to the mid-logarithmic phase of growth. Dilutions of the cultures were plated on TYE and TYE-ampicillin plates to score for plasmid loss, which was less than 5% in all cases. Cells were sedimented at 8,000 × *g* for 10 min and resuspended in an equal volume of Z buffer (26), and β-galactosidase assays were performed in triplicate by the method of Miller (26).

Oligonucleotide-directed mutagenesis of the gene X-*secA* intergenic region. M13-MS300 phage were used to infect BW313, and uracil-containing, single-stranded DNA was isolated as described by Schmidt et al. (38). In vitro mutagenesis was performed by annealing 10 to 20 ng of phosphorylated oligonucleotide containing the desired mutation to 100 ng of M13-MS300 template, and then second-strand synthesis in the presence of 25 μM (each) deoxynucleoside triphosphates and 13 U of Sequenase in a total reaction volume of 20 μl was carried out. The DNA was ligated overnight with T4 DNA ligase and used to transfect JM101, and this mixture was plated in H top agar overlaid on TYE plates. Plaques appearing after an overnight incubation at 37°C were picked onto master plates and subjected to plaque hybridization with the α-^{32}P-labeled oligonucleotide containing the desired mutation, as described by Maniatis et al. (22). M13 single-stranded DNA was prepared and subjected to DNA sequence analysis from plaques, giving a positive hybridization response. The SRE mutations were transferred to pMF8 by reconstruction techniques as follows. M13 MS300 replicative-form DNA containing a given mutation was digested with *Hpa*I, and the 420-bp DNA fragment was isolated and mixed with *Hpa*I-digested pMF9 DNA. This mixture was incubated overnight at 16°C with T4 DNA ligase and used to transform MC1000.4, and plasmid-encoded *secA* function was selected for by colony formation at 42°C on TYE-ampicillin plates. All plasmids were verified further by restriction enzyme analysis.

Protein analysis. Radiolabeling of proteins, immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography techniques have been described previously (30, 38). For the quantitation of radiolabeled protein, autoradiography was carried out on Kodak X-Omat AR film that was preflashed according to the manufacturer's specifications. Autoradiograms were quantitated with an LKB Ultroscan XL laser densitometer and LKB 2400 Gel Scan software.

RESULTS

Analysis of gene expression in the *secA* operon. We have noted previously that *secA* is the second gene in an operon; *secA* begins 64 nucleotides downstream of a gene predicted to encode a 147-amino-acid-residue polypeptide of unknown function termed gene X (38). We also noted that, 62 nucleotides past the end of the *secA* gene, there was the beginning of an open reading frame which continued beyond our sequence and which we now know corresponds to the proximal portion of the *mutT* gene (1). Previously, we have analyzed *secA* expression by *lacZ* gene fusion techniques using plasmid pMF8, which contains a 3,811-bp *E. coli* chromosomal fragment encoding gene X, *secA*, and the proximal portion of *mutT* (37). In order to continue our study of the regulation of this operon, we have made *lacZ* translational fusions to gene X, *secA*, and *mutT*, resulting in plasmids pAX1, pMF11, and pBA1, respectively. Figure 1 depicts these plasmids as well as two derivatives of pBA1 which were constructed to test whether *mutT* is part of the gene X-*secA* operon or is an independent transcriptional unit, as suggested by Akiyama et al. (1). Plasmid pBC1 contains a 4-bp frameshift mutation in the *secA* gene which should be polar during *mutT-lacZ* expression if these genes are in a single transcriptional unit. Plasmid pBD1 contains a 2,085-bp deletion removing a promoter, gene X, and the proximal portion of the *secA* gene. β-Galactosidase assays were performed on MC1000 carrying each of these plasmids, and the results are given in Table 1. First, the expression of these three genes appeared to differ dramatically, since the β-galactosidase activities found in the strains containing the gene X-*lacZ* and *mutT-lacZ* fusions were only approximately 1 and 10%, respectively, of that found in the strain containing the *secA-lacZ* fusion. These observations were consistent with the lack of a consensus ribosome-binding site for

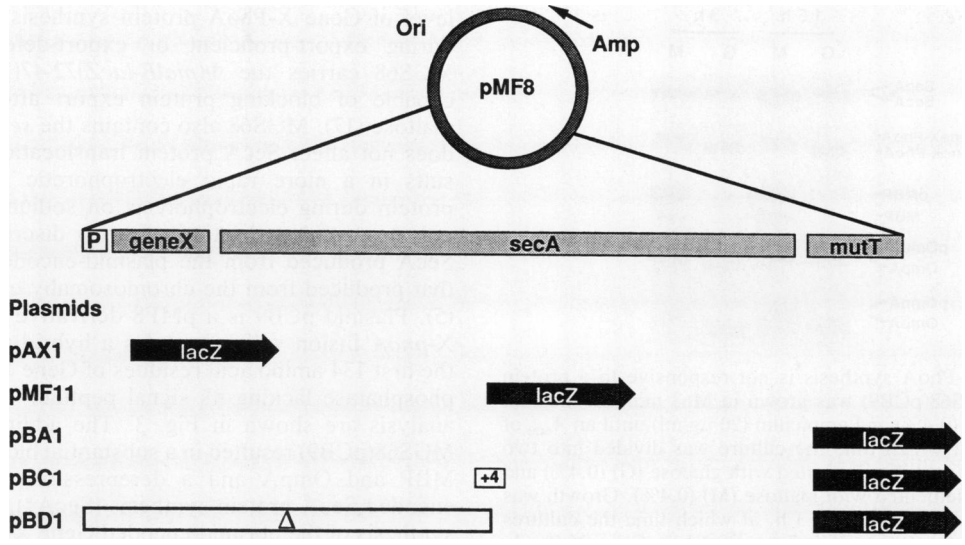

FIG. 1. Plasmids constructed for the study of gene X, *secA*, and *mutT* expression. Plasmid pMF8, containing a promoter (P), gene X, *secA*, and the proximal portion of *mutT*, is shown in the upper portion of the figure. The arrows containing *lacZ* indicate the sites of various *lacZ* gene fusions for the plasmids indicated on the left. +4 indicates the site of a 4-bp frameshift mutation. The bar containing the triangle indicates the extent of a large deletion mutation.

gene X and the presence of a predicted rho independent transcriptional terminator between *secA* and *mutT* (38). Furthermore, the level of β-galactosidase activity for the strain containing the gene X-*lacZ* fusion was artificially low, since gene X encodes an exported protein (see below) and *lacZ* fusions to such genes give rise to hybrid proteins with low β-galactosidase activity (3). Second, it appeared that *mutT* is part of the gene X-*secA* operon, since either a frameshift mutation in *secA* or a large deletion in the beginning of the operon reduced or largely eliminated *mutT-lacZ* expression.

Gene X expression is not responsive to a protein export defect. We have demonstrated previously that gene X-*secA* mRNA levels do not rise when SecA protein synthesis increases approximately 10-fold during a block in protein export (37). In order to determine whether Gene X protein synthesis increases similarly to SecA protein synthesis, we measured the level of Gene X-LacZ protein synthesis during export-proficient or export-defective conditions by using isogenic wild-type or *secA51*(Ts) mutant strains containing pAX1. These results are shown in Fig. 2. It can be seen that the level of Gene X-LacZ protein synthesis did not change appreciably under these two conditions, while SecA protein synthesis increased fivefold when protein export was blocked and proOmpA protein accumulated (Fig. 2, compare lanes 2 and 3; see right panel for quantitation). Significant derepression of SecA-LacZ protein synthesis was noted under export-defective conditions in a strain containing pMF11 (data not shown), as reported previously (37). Since in these and subsequent experiments we have compared the ratios of synthesis of a given protein (e.g., Gene X-LacZ and SecA-LacZ, etc.) under export-defective and export-profi-

TABLE 1. Expression of *lacZ* fusions to gene X, *secA*, and *mutT*

Plasmid	Genotype	β-Galactosidase activity[a]
pAX1	gene X-*lacZ*	36 ± 4.6
pMF11	*secA-lacZ*	3,096 ± 681
pBA1	*mutT-lacZ*	289 ± 61
pBC1	*secA*-fs[b] *mutT-lacZ*	148 ± 30
pBD1	Δ(gene X-*secA*)[c] *mutT-lacZ*	2.6 ± 0.9

[a] β-Galactosidase activity is given in Miller units (26).
[b] Indicates a 4-bp frameshift mutation in the *secA* gene.
[c] A deletion of gene X and the proximal portion of *secA*, as well as their upstream promoter.

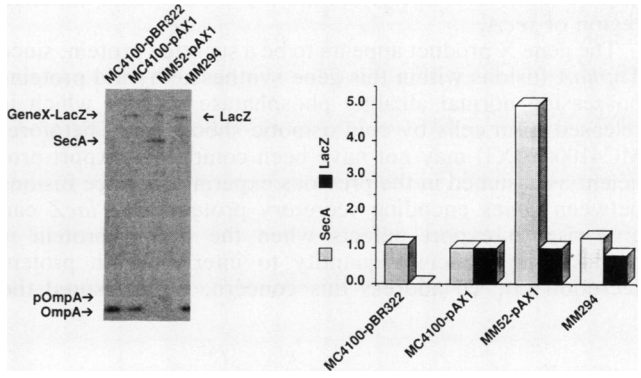

FIG. 2. Gene X-LacZ synthesis is not responsive to a protein export defect. Strains were grown in M63 minimal medium containing, when appropriate, glycerol (0.4%) and ampicillin (20 μg/ml) at 30°C until they reached an A_{600} of 0.3, at which time they were shifted to 42°C for 2 h. MM294 was grown at 37°C and induced with IPTG (1 mM). Aliquots (1 ml) of the cultures were pulse-labeled with [^{35}S]methionine for 1 min, and Gene X-LacZ, LacZ, SecA, and OmpA were analyzed by coimmunoprecipatation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography. The positions of Gene X-LacZ, LacZ, SecA, and OmpA precursor (pOmpA) and mature form (OmpA) proteins are indicated by arrows. Quantitation of the autoradiogram by densitometric analysis is given at the right. The level of SecA or LacZ synthesis found in each strain was defined as the ratio of SecA or LacZ to OmpA in a given strain divided by the comparable ratio for MC4100(pAX1). Similar values were obtained if the data were normalized to RNA polymerase subunits RpoB and RpoC instead of OmpA.

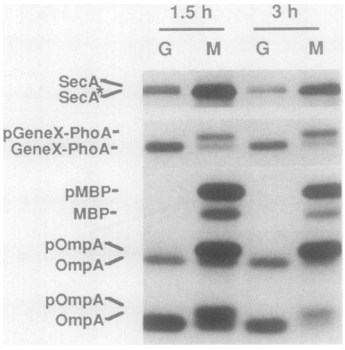

FIG. 3. Gene X-PhoA synthesis is not responsive to a protein export defect. MGS68(pCB9) was grown in M63 minimal medium containing glycerol (0.4%) and ampicillin (20 μg/ml) until an A_{600} of 0.3 was reached, at which time the culture was divided into two equal portions; one was supplemented with glucose (G) (0.4%) and the other was supplemented with maltose (M) (0.4%). Growth was continued for an additional 1.5 or 3 h, at which time the cultures were pulse-labeled for 1 min with Tran-^{35}S-label. Gene X-PhoA, SecA, MalE, and OmpA protein synthesis was analyzed as indicated in the Fig. 2 legend. The positions of the precursor and mature forms of the Gene X-PhoA fusion protein (pGeneX-PhoA and GeneX-PhoA, respectively), wild-type SecA (SecA), an electrophoretic variant form of SecA (SecA*), and precursor and mature forms of maltose-binding protein (pMalE and MalE, respectively) and OmpA (pOmpA and OmpA, respectively) are indicated. OmpA displays different electrophoretic forms here, as described previously (16).

cient conditions, our data should be normalized for any effects gene fusions or deletions (see below) have on the general stability of the resulting mRNA. Therefore, these results indicate that the SRE site is located somewhere between the gene fusion joints contained on pAX1 and pMF11 and, most likely, around the translational initiation region of *secA*.

The gene X product appears to be a secreted protein, since Tn*phoA* fusions within this gene synthesize hybrid proteins possessing normal alkaline phosphatase activity which is released from cells by cold osmotic shock (33). Therefore, MC4100(pAX1) may not have been completely export proficient as assumed in the previous experiment, since fusions between genes encoding secretory proteins and *lacZ* can give rise to export defects when the hybrid protein is produced in sufficient quantity to interfere with protein secretion (3). To address this concern, we measured the level of Gene X-PhoA protein synthesis in MGS68(pCB9) during export-proficient or export-defective conditions. MGS68 carries the Φ(*malE-lacZ*)*72-47*(Hyb) gene fusion capable of blocking protein export after induction with maltose (17). MGS68 also contains the *secA22* allele, which does not affect SecA protein translocation activity but results in a more rapid electrophoretic mobility of SecA protein during electrophoresis on sodium dodecyl sulfate-polyacrylamide gels, thus allowing discrimination between SecA produced from the plasmid-encoded *secA* gene and that produced from the chromosomally encoded *secA* gene (5). Plasmid pCB9 is a pMF8 derivative containing a gene X-*phoA* fusion which encodes a hybrid protein containing the first 134 amino acid residues of Gene X fused to alkaline phosphatase lacking its signal peptide. The results of this analysis are shown in Fig. 3. The addition of maltose to MGS68(pCB9) resulted in a substantial block in the export of MBP and OmpA and a derepression of chromosomally encoded SecA protein synthesis (SecA*), while the level of synthesis of the plasmid-encoded Gene X-PhoA protein was relatively unaffected by this treatment. In addition, during the export block a precursor form of this fusion protein accumulated, indicating that the gene X product contains a cleaved signal peptide. These results argue that sequences required for SRE function are distal to the gene X-*phoA* fusion joint, which is 261 nucleotides before the gene X termination codon.

Oligonucleotide-directed mutagenesis of the gene X-*secA* intergenic region. In order to determine whether the SRE site is located in the gene X-*secA* intergenic region, we created deletions in this region by oligonucleotide-directed mutagenesis techniques. The deletions generated and introduced into pMF8 are shown in Fig. 4. In order to determine the effect of these mutations on SRE function in a rapid and controlled fashion, we relied on our recent finding that sodium azide is a rapid and potent inhibitor of SecA ATPase function and protein export, derepressing *secA* expression similarly to other protein export defects that have been characterized previously (31, 35). Accordingly, the level of SecA protein synthesis was measured in MC4100.2 containing the relevant plasmid under export-proficient (without sodium azide addition) or export-defective (2 mM sodium azide treatment) conditions. These results are shown in Fig. 5. Under these conditions, the presence of high SecA levels in the strains containing the *secA* gene on a multicopy plasmid resulted in less severe protein export defects when either sodium azide or the *malE-lacZ* fusion (data not shown) was used to create

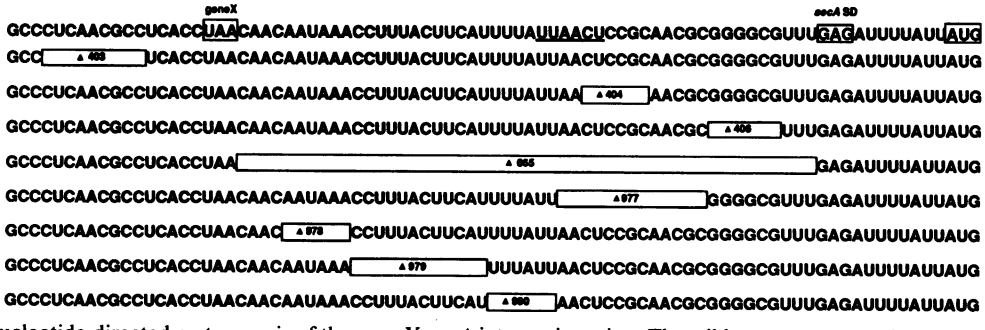

FIG. 4. Oligonucleotide-directed mutagenesis of the gene X-*secA* intergenic region. The wild-type sequence of the gene X-*secA* intergenic region is shown on the top line, where the gene X termination codon, *secA* SD sequence, and initiation codon are boxed. A consensus translational enhancer element, UUAACU, is underlined. The locations of the deletion mutations used in this study are indicated on subsequent lines by boxes containing the assigned number of the deletion interval.

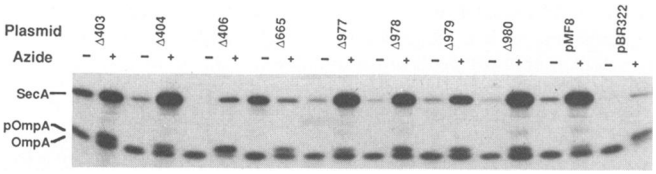

FIG. 5. Analysis of deletion mutants in the gene X-*secA* intergenic region. MC4100.2 containing the indicated plasmid was grown in M63 minimal medium containing glucose (0.4%) at 37°C until it reached an A_{600} of 0.6, at which time the culture was divided into two equal aliquots and sodium azide was added to one portion to a final concentration of 2 mM. Five minutes later, the cultures were pulse-labeled for 1 min with [^{35}S]methionine. SecA and OmpA proteins were analyzed as indicated in the Fig. 2 legend. The absence (−) or presence (+) of sodium azide is indicated. The positions of SecA protein and of OmpA precursor and mature form (pOmpA and OmpA, respectively) are also indicated.

a protein export block (e.g., compare the strains containing pMF8 or pBR322 in Fig. 5). However, since even mild protein export defects result in nearly maximal *secA* derepression (30, 31, 34, 35), the level of SecA protein synthesis during normal and export-defective conditions could be compared. Three of the deletions (Δ403, Δ406, and Δ665) resulted in altered patterns of *secA* regulation, while five others (Δ404, Δ977, Δ978, Δ979, and Δ980) resulted in a more wild-type pattern [similar to MC4100.2(pMF8)]. Both MC4100.2(pMF8Δ403) and MC4100.2(pMF8Δ665) displayed somewhat higher levels of SecA protein synthesis than MC4100.2(pMF8) under export-proficient conditions, but they differed in their responses to the protein export block. While SecA protein synthesis was derepressed in MC4100.2 (pMF8Δ403), SecA protein synthesis was reduced in MC4100.2(pMF8Δ665) by treatment with sodium azide. However, since both mutations affect the SecA induction ratio (the SecA synthetic rate during export-defective conditions divided by the rate during export-proficient conditions), both of these mutations somehow affect SRE function. In contrast, although the levels of SecA synthesis were reduced substantially in MC4100.2(pMF8Δ406) under both conditions of protein export, the SecA induction ratio was similar to that of the wild type, indicating that this mutation may affect *secA* translation but not SRE function. It was of interest that three deletions, Δ404, Δ977, and Δ980, which did not affect *secA* regulation removed portions of the sequence UUAACU, which has been shown to act as a general translational enhancer element for several systems tested (24, 25, 28). However, there is an additional UUAACU sequence beginning 13 nucleotides downstream of the *secA* initiation codon (38) which could have promoted a high level of SecA protein synthesis in these mutants. Previous observations indicate that such translational enhancer elements are equally effective when placed upstream or downstream of the translational initiation site (28).

DISCUSSION

Previous studies have indicated that the *secA* gene is in an operon with gene X, since the gene X promoter is required for normal *secA* expression (34) and gene X nonsense or insertion mutations are strongly polar during *secA* expression (12, 38). In this study, we have sought to define further the structure and regulation of the gene X-*secA* operon and to locate the site which is responsible for regulating SecA translation in response to the protein export proficiency of the *E. coli* cell. Our results indicate that this operon consists of three genes, gene X, *secA*, and *mutT*, whose products are synthesized in the ratio 0.01:1:0.1, respectively, as indicated by gene fusion techniques. This method, however, underestimates the amount of Gene X protein produced, since the Gene X-LacZ fusion protein had an artifically low β-galactosidase activity, analogous to other such chimeras that have been characterized (3). This reservation is consistent with the higher level of Gene X-LacZ protein synthesis observed in Fig. 2, even bearing in mind that such synthesis was derived from the gene fusion located on a multicopy plasmid. On the other hand, on the basis of our previous quantitation of the cellular level of SecA protein (30), our estimate of the relative amount of MutT protein in the cell agrees with that of Bhatnagar and Bessman (2). We also note that the *mutT* gene may be the last gene in this operon, since it is followed by several repetitive extragenic palindromic sequences, which have been found at the end of several other bacterial operons (1).

We have used gene fusion studies to help locate the SRE site, which somehow coordinates *secA* translation with the protein export-proficient state of the cell. Our present studies, combined with previous ones (37), showed that translational fusions to gene X were not subject to this regulation, whereas comparable fusions to *secA* were regulated correctly, indicating that the regulatory site is probably located somewhere distal in gene X, early in *secA*, or in the region between these two genes. Since our results indicate that Gene X is a secretory protein, it will be of interest to determine whether it plays any direct role in the protein export process or in *secA* regulation.

We have made a series of small deletion mutations in the gene X-*secA* intergenic region to specifically address the role of these sequences in *secA* regulation. Although certain of our deletion mutants (e.g., Δ403 and Δ665) clearly reveal the importance of this region in SRE function, the eight mutations that we made and analyzed using programs predictive of RNA secondary structure (48) have not allowed us to discern yet whether elements of mRNA secondary structure, a protein binding site, or both are required for the biological function of this region. For example, deletion mutations Δ403, Δ406, and Δ665 were designed to disrupt a favored, predicted RNA secondary structure which could have constituted the SRE element (see Fig. 5 of reference 37). However, the fact that one of these mutations (Δ665) was strongly defective in *secA* regulation, another (Δ403) was only moderately so, and the third (Δ406) displayed reduced SecA synthesis under both conditions but a normal induction ratio tended to rule out this particular structure. In addition, the fact that only the largest deletion displayed a marked defect in *secA* regulation may indicate the existence of multiple or redundant elements composing the SecA protein binding site. Additional mutagenesis was used to show that the UUAACU translational enhancer sequence upstream of the *secA* Shine-Dalgarno (SD) sequence was not needed for SRE function, but it left open the possibility that a second UUAACU sequence located 13 nucleotides downstream of the *secA* initiation codon contained the redundancy needed for SRE function. Previous studies by Olins and Rangwala (28) indicated that this hexanucleotide sequence can elevate translational initiation rates approximately 10-fold and that such elements can contribute to mRNA translational initiation when located either upstream of the SD sequence or downstream of the initiation codon, presumably by enhancing mRNA binding to a complementary region present on 16S rRNA. In this regard, it is somewhat curious that the

secA ribosome-binding site–initiation region has two such enhancer elements, since this region possesses good translational initiation signals which include a reasonably good SD sequence positioned optimally from the AUG initiation codon, as well as a U-rich sequence between these two elements with an A residue at the −3 position prior to the initiation codon (40, 41). Clearly, additional mutagenesis will be required to definitively locate the relevant SRE sequences and determine how they operate.

Recent studies indicate that purified SecA protein binds to gene X-*secA* RNA, as indicated by photo-cross-linking, filter-binding, or gel shift assays (10). In addition, a nested series of RNAs lacking either 5′ or 3′ portions of gene X-*secA* sequences were used to localize the SecA protein binding site to sequences around the gene X-*secA* intergenic region. The coincidence of the SRE region as determined here with the SecA protein binding site as determined by Dolan and Oliver (10) supports a simple model in which SecA protein serves as an autogenous translational repressor during normal protein export conditions by binding to the SRE site and, either directly or by stabilizing an mRNA secondary structure, blocking ribosomal binding and/or initiation. This model would be analogous to other well-studied examples of procaryotic translational regulation (for reviews, see references 15 and 27). When a protein export defect is present, SecA protein repressor activity must be somehow reduced, either directly by protein modification or indirectly by sequestering this activity through a protein-protein or protein-lipid interaction. In this regard, SecA protein's ATP-binding and ATPase activities, which are regulated by precursor protein and membrane binding (21), may provide a mechanism to modulate such an activity in keeping with the protein secretion status of the cell. Either of these mechanisms for inactivating SecA repressor activity would be rapid enough to explain the fact that SecA synthesis is derepressed within a couple of minutes after imposing a block in protein export (31). Further studies will be needed to verify this model, to define how the protein export proficient or defective state is sensed, to determine how this information is relayed to SecA protein, and to determine why this regulation is beneficial to the cell.

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