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Regulation of *Escherichia coli secA* by Cellular Protein Secretion Proficiency Requires an Intact Gene X Signal Sequence and an Active Translocon

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***secA* is translationally regulated by the protein secretion proficiency state of the *Escherichia coli* cell. This regulation was explored by making signal sequence mutations in the gene upstream of *secA*, gene X, which promotes *secA* translational coupling. Gene X signal sequence mutants were constitutive for *secA* expression, while *prlA* alleles partially restored *secA* regulation. These results show that interaction of the pre-gene X protein with the translocon is required for proper *secA* regulation. Furthermore, gene X signal sequence mutations disrupted *secA* regulation only in the *cis* configuration. We propose that nascent pre-gene X protein interacts with the translocon during its secretion to constitute the secretion sensor.**

Eubacterial protein secretion is facilitated by a number of different soluble and membrane proteins that comprise the secretion machinery (21, 28). Central to this picture is the SecA protein, the translocation ATPase, which binds both preproteins and SecYEG protein, the putative preprotein channel and receptor for SecA (1, 12–14). Protein translocation requires insertion of SecA into the membrane, a step that is regulated by its amino-terminal ATP-binding domain as well as the SecG, SecY, and SecDFyajC proteins (6–8, 15, 18, 23). Protein translocation appears to require cycles of SecA membrane insertion and retraction to drive successive portions of the preprotein across the membrane (9, 27). However, it has been suggested that protein translocation utilizing SecA that is permanently imbedded within the inner membrane can also occur (4).

secA is the only *Escherichia coli sec* gene that has been shown to be regulated (19). This regulation involves repression of *secA* translation under conditions of excess protein secretion capacity and derepression when protein secretion becomes limiting (20). While the basis for this secretion-responsive regulation is not clear, it is known that (i) *secA* translation is normally coupled to translation of gene X, which lies immediately upstream of *secA* in the gene X-*secA-mutT* operon (26); (ii) *secA* repression occurs by an autogenous mechanism in which SecA binds to a translational operator site on the gene X-*secA* mRNA to block or dislodge ribosomes that initiate at the *secA* ribosome-binding site (24); (iii) at the end of gene X there exists a secretion-responsive element which appears to positively regulate the system (16); and (iv) gene X encodes a secretory protein that is nonessential for cell growth (22). Despite these advances, the exact role that gene X plays in *secA* regulation is unclear, as is how *secA* regulation is tied to the status of protein secretion proficiency.

The observation that gene X is crucial for proper *secA* regulation, and the fact that it is itself a secretory protein, struck us as being a potentially important linkage. In particular, we hypothesized that the secretion-responsive regulation of *secA* may originate from the secretability of the gene X protein by the translocon. To test this idea, we constructed two small deletions in the gene X signal sequence that were predicted to disrupt its function based on the length of the residual hydrophobic core region (2). Deletions of gene X codons 8 to 11 (ΔLPAL) or 6 to 10 (ΔLGLPA) were performed by oligonucleotide-directed mutagenesis methods on a plasmid-borne copy of the gene X-*secA* operon containing a *secA-lacZ* translational fusion, pPhIF, which has been shown previously to be regulated correctly (16). The mutations were verified by DNA sequence analysis of the entire gene X-*secA* region. These pPhIF derivatives were transformed into a wild-type strain (CG155) and a strain containing a *secD1*(Cs) mutation (CG29), which shows a strong protein secretion block when grown at reduced temperatures (11). Strains were grown at 39°C and shifted to 23°C, the temperature at which the effect of the gene X signal sequence mutations on *secA* regulation was determined. Wild-type gene X allowed nearly a fivefold repression of the expression of the *secA-lacZ* fusion in the secretion-competent strain CG155(pPhIF) compared to that in its isogenic secretion-defective counterpart, CG29(pPhIF) (Fig. 1). In contrast, little repression was observed for the gene X signal sequence mutants; the β-galactosidase levels were nearly as high in the CG155 host as they were in the fully derepressed host, CG29. The residual level of repression observed with the gene X signal sequence mutations in the CG155 host may have been due to residual targeting of gene X protein to the translocon, which is likely to occur inefficiently even in the absence of a good signal sequence (5). Similar results were obtained with a point mutation in the gene X signal sequence that resulted in the introduction of a positively charged amino acid residue within the hydrophobic core region (a Pro-to-Arg change in the ninth amino acid residue) (data not shown). These results indicate that an intact gene X signal sequence is necessary for proper *secA* repression.

To determine whether suppression of the presumed gene X protein secretion defect restored *secA* repression, we employed strains SE6004 and SE4014.1, containing the *secY prlA4* and *prlA3* alleles, respectively, which have been shown to suppress a variety of signal sequence defects, including complete signal sequence deletions (5, 10). Indeed, the *prlA* strains containing the gene X signal sequence mutations showed partial restora-

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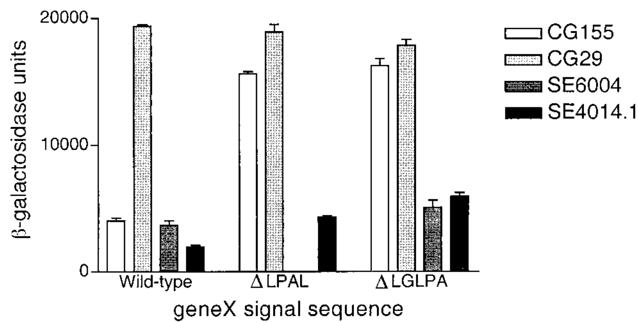

FIG. 1. CG155 (MC1000 *recA*), CG29 [MC1000 *recA1 secD1*(Cs) *phoR srl*::Tn*10*], SE6004 (MC4100 *prlA4 lamBS60*), or SE4014.1 [MC4100 *prlA3 lamBS60 rpsE trp*(Am) *supF*(Ts) *zch*::Tn*10*] (from left to right) containing pPhIF with the mutation indicated was grown in Luria broth containing 100 μg of ampicillin per ml at 39°C to mid-logarithmic phase, at which time the culture was shifted to 23°C for 4 h. β-Galactosidase assays were performed in duplicate for each of two duplicate cultures as described previously (17). The average results are given, with the error bars indicating the standard deviations.

tion of *secA* repression (Fig. 1). These results provide compelling evidence that interaction of the pre-gene X protein with the translocon is required for proper *secA* regulation. Interestingly, we found that the plasmid containing the ΔLPAL allele is synthetically lethal in SE6004, further suggesting that interaction of the pre-gene X protein with the translocon is an important element for control of this system.

Nearly constitutive *secA-lacZ* expression was observed in CG155 containing the gene X signal sequence defects, despite the fact that this strain contains an intact chromosomal copy of the gene X-*secA* operon. This implies that the gene X signal sequence mutations are dominant. However, it might be argued that the higher dosage of the plasmid-borne copy of gene X is the cause of the dominant phenotype observed in this case. To explore this point further, we used Western blotting to compare the regulation of the chromosomal copy of *secA* to that of the plasmid-borne copy of the *secA-lacZ* fusion in CG155 containing the different gene X signal sequence alleles. The results demonstrate clearly that while the gene X signal sequence mutations disrupted repression of the *cis*-linked *secA-lacZ* fusion, they did not affect repression of the *trans* copy of *secA* (Fig. 2). This result argues against these mutations being dominant, since correct regulation was observed for the chromosomal copy of *secA* despite the low dosage of wild-type gene X. This result is most readily understood in terms of

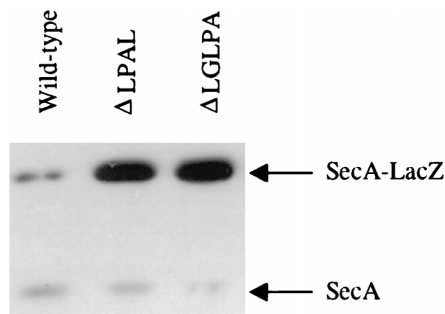

FIG. 2. Gene X signal sequence mutations are active only in *cis*. Wild-type CG155(pPhIF) or this strain containing the mutation indicated was grown as described in the legend to Fig. 1. The cells were then isolated, and SecA and SecA-LacZ proteins were analyzed by Western blotting as described previously (3). The positions of SecA and SecA-LacZ fusion proteins are indicated.

the obligate translational coupling of *secA* and gene X (26), which is required for proper *secA* regulation (16).

These data led us to propose a model in which an interaction between the translation and secretion machineries would promote proper *secA* regulation. In particular, the cotranslational secretion of gene X protein would be critical for this process. We suggest that there exists a translational pausing mechanism by which a pause in the translation of the distal portion of gene X provides an opportunity for ribosomes to initiate translation at the *secA* ribosome-binding site on the gene X-*secA* mRNA. Translation at this site is normally blocked by an RNA secondary structure or by SecA bound within this region (16, 24, 25). Presumably an active translocon, and perhaps SecA protein itself complexed with some other Sec protein(s), such as SecY, efficiently releases this translational pause under secretion-proficient conditions but not under secretion-defective conditions. Thus, the exportability of the gene X protein, along with the secretion activity of the translocon, provides the necessary cell sensor which determines the secretion-responsive regulation of *secA* that has been observed previously. This proposal is consistent with gene X signal sequence defects rendering *secA* expression constitutive, but only in *cis*, since the inability of nascent gene X protein to interact properly with the translocon would prevent efficient release of the pause in gene X translation and allow additional rounds of *secA* translation initiation to occur. It is also consistent with the observed restoration of *secA* regulation when gene X signal sequence mutations are suppressed by *prlA* alleles. While other proposals for this regulatory mechanism can be entertained as well, they need to postulate a central role for gene X translation and secretion in the process.

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