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NOTES

Regulation of the *Escherichia coli secA* Gene by Protein Secretion Defects: Analysis of *secA*, *secB*, *secD*, and *secY* Mutants

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SecA protein synthesis levels were elevated 10- to 20-fold when protein secretion was blocked in *secA*, *secD*, and *secY* mutants or in a *malE-lacZ* fusion-containing strain but not in a *secB* null mutant. An active *secB* gene product was not required to derepress *secA*, since SecA levels were elevated during protein export blocks in *secB secY* and *secB malE-lacZ* double mutants.

A genetic approach has been used to study the molecular mechanisms responsible for protein localization in *Escherichia coli*. Genetic selections have identified a set of *sec* genes whose products are required to promote the secretion of cell envelope proteins. Conditional lethal *secA* (5, 6), *secY* (8, 9), and *secD* (1) mutants which when shifted to the nonpermissive temperature accumulate unsecreted protein precursors for most envelope proteins have been described previously. A nonconditional *secB* null mutant exhibiting substantial export defects for only certain envelope proteins also has been described (4). In addition, a strain carrying a *malE-lacZ* gene fusion which produces high levels of a MalE-LacZ hybrid protein that interferes with general protein export has been described elsewhere (3). It has been noted previously that *secA* gene expression is somehow coregulated with cellular protein secretion, since SecA synthesis levels increase dramatically when protein export is blocked in *secA* and *secD* mutants or by induction of a high level of synthesis of the MalE-LacZ hybrid protein by maltose addition (1, 7). Induction of SecA synthesis by production of the MalE-LacZ hybrid protein appears to be due to inhibition of protein export, since neither *secA* induction nor the protein export block occurs when the hybrid protein lacks a functional signal sequence (2). Furthermore, induction of SecA synthesis does not appear to be correlated with general conditions of cell stress. For example, *secA* induction is not seen under conditions that induce the heat shock response (either high temperature or production of abnormal proteins) and occurs normally in strains deficient in the heat shock response (*htpR* mutant [2]). SecA synthesis levels have not been reported in *secB* and *secY* mutants; therefore, we have measured them.

The following strains were used in this study: MC4100 (F^- *ΔlacU169 araD139 relA rpsL thi*) is the wild-type parent of MM52 [*secA51*(Ts)] (6), CK1953 (*secB*::Tn*5*) (4), IQ85 [*secY24*(Ts) *zhd*::Tn*10 rpsE*] (8), and MM18 [Φ(*malE-lacZ*)*72-47*(Hyb)] (3); MC1000 [F^- *araD139* Δ(*ara-leu*)*7697 ΔlacX74 galU galK rpsL*] is the wild-type parent of CG29 [*secD1*(Cs) *phoR recA1 srl*::Tn*10*] (1). All strains were grown initially in M63 minimal medium containing 20 μg of L-leucine per ml where required and 0.4% maltose or 0.4% glycerol (for MM18) at 30°C, except for strains CG29 and CK1953, which were grown at 37°C. When cultures reached a density of 1×10^8 to 2×10^8 cells per ml, export blocks were induced for a portion of each culture by a temperature shift to 41°C for MM52 and IQ85 or to 23°C for CG29 or by the addition of maltose to 0.4% for MM18. CK1953 has a constitutive export block. Samples of each culture were pulse-labeled 2 h later with [^{35}S]methionine (~1,000 Ci/mmol) at 20 μCi/ml for 1 min, and further radioisotope incorporation was halted by the addition of an equal volume of ice-cold 10% trichloroacetic acid followed by incubation on ice. Immunoprecipitation, polyacrylamide gel electrophoresis, and autoradiography techniques have been described elsewhere (5), except that autoradiography was carried out on Kodak X-Omat AR film that was preflashed according to the specifications of the manufacturer. Protein synthesis levels of SecA, MalE, MalE precursor (preMalE), OmpA, and OmpA precursor (preOmpA) were determined by densitometry of autoradiograms done with an LKB UltroScan XL laser densitometer and appropriate software.

The SecA synthesis levels and the protein export proficiencies of these strains are summarized in Table 1. Strains containing mutations in *secA* (MM52), *secD* (CG29), and *secY* (IQ85) and the *malE-lacZ*-containing strain (MM18) had only mild secretion defects, as indicated by low levels of preMalE and preOmpA, and only modest increases in SecA levels (1.5- to 5-fold) when these strains were grown under permissive conditions. When they were grown under nonpermissive conditions for 2 h, secretion defects were much more severe and SecA levels were elevated between 10- and 20-fold above the wild-type level. However, the strain with a *secB* null mutation (CK1953) had essentially wild-type SecA levels despite eliciting severe secretion defects for MalE and OmpA similar to those in the other export-defective strains examined under nonpermissive conditions. Results similar to those given in Table 1 were obtained in two additional experiments.

These results indicate that the *secA* gene is generally responsive to protein export blocks with the exception of secretion blocks due to a defective *secB* gene. In this light it is of interest that the *secB* gene is the only nonessential *sec* gene identified to date and that *secB* mutants are defective in the export of only a subset of envelope proteins (4). One possible explanation for the inability of SecA synthesis to increase in *secB* mutants is that SecB or a SecB-containing

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TABLE 1. SecA levels in *sec* mutant strains

Strain	Induction time (h)[a]	SecA level[b]	% preMalE[c]	% preOmpA[c]
MC4100	0	1.0	<5	<5
	2	1.1	<5	<5
MM52	0	5.2	13	40
	2	13.2	68	83
MM18	0	2.6	<5	14
	2	10.0	89	82
CG29	0	1.5	11	<5
	2	16.8	42	63
IQ85	0	5.6	9	17
	2	12.8	84	71
CK1953	0	1.2	87	51
IQ85.4	0	2.4	30	14
	2	6.5	67	32
MM18.4	0	1.3	>95	78
	2	12.7	>95	92

[a] Methods are as explained in the text.

[b] Ratio of SecA to total OmpA in a given strain divided by the comparable ratio for MC4100. Similar SecA levels were found when the SecA level was normalized to levels of RNA polymerase subunits RpoB and RpoC instead of OmpA.

[c] The percent precursor was determined by using the following formula: % precursor = [level of precursor/(level of precursor + level of mature form)] × 100%.

protein complex is required to activate *secA* expression in some manner. To test this possibility, we made two double-mutant strains containing either *secB* and *secY* mutations or *secB* and *malE-lacZ* mutations and tested their ability to elevate SecA levels when the strains were grown under nonpermissive conditions. The *secB*::Tn*5* mutation present in CK1953 was introduced into strains IQ85 and MM18 by P1 transduction to form IQ85.4 and MM18.4, respectively. It was clear that the *secB*::Tn*5* allele was present in these strains since they not only became kanamycin resistant but also grew only on minimal medium (4) and under permissive conditions showed severe export defects similar to defects in the original CK1953 donor strain (Table 1). When IQ85.4 and MM18.4 were shifted to the nonpermissive condition (42°C or maltose addition, respectively) for 2 h, they made, respectively, 6.5 and 12.7 times more SecA than did the wild type. Clearly, an active *secB* gene product is not required to derepress *secA* gene expression.

We conclude that SecA protein synthesis levels are elevated 10- to 20-fold when protein secretion is blocked in *secA*, *secD*, and *secY* mutants or in a strain overproducing the MalE-LacZ hybrid protein, but not in a *secB* null mutant. The fact that an active *secB* gene product is not required for *secA* derepression rules out models in which SecB is an activator of *secA* expression during a secretion block. We have considered two alternative models for the coregulation of *secA* gene expression with the protein export proficiency of the cell. In the first model, a secretory protein precursor would serve both as a monitor of export proficiency and as an activator of *secA* expression. The level of this precursor would rise during most export blocks, thus activating expression of *secA*. This hypothetical regulator would fall into the category of envelope proteins unaffected by the *secB* mutant block (4). In the second model, a particular Sec protein or protein complex would serve a dual role in catalyzing protein secretion and in regulating *secA* expression. Modulating the amount or activity of this regulator by protein export blocks could control SecA levels in either a positive or a negative fashion. In this scenario, *secB* mutants would not affect the level or activity of this hypothetical regulator. Discrimination between these or other models will require the additional genetic and biochemical experiments currently in progress.

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