JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5928–5930
0021-9193/88/125928-03$02.00/0

Vol. 170, No. 12

Two Cellular Components, PrlA and SecB, That Recognize Different Sequence Determinants Are Required for Efficient Protein Export

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Received 1 February 1988/Accepted 19 September 1988

We exploited the conditional-lethal phenotype of *secB* null mutations to demonstrate that SecB function was required for PrlA-mediated suppression of signal sequence mutations. The results of these experiments provide information about the functions performed and the sequence determinants recognized by each of these components of the protein export machinery of *Escherichia coli*.

The *prlA* (*secY*) gene, the most distal gene in the *spc* ribosomal protein operon (6), encodes a protein of 443 amino acids (1) that performs an essential function(s) in protein localization (3, 4, 7). It was originally identified as the site of suppressors which alleviate the export defects imparted by many different signal sequence mutations (3). Because the existence of *prlA* suppressors implies an interaction with the signal sequence and because PrlA is localized to the cytoplasmic membrane, at least one of its functions may be to serve as the membrane receptor for precursor polypeptides which are destined for translocation from the cytoplasm. Indeed, in this regard, PrlA may be similar to the signal sequence receptor in the mammalian endoplasmic reticulum recently identified by Wiedmann et al. (9).

The *secB* gene specifies a small cytoplasmic protein (12,000 daltons) that functions in the export of a specific subset of noncytoplasmic proteins, including the outer membrane protein LamB and the periplasmic maltose-binding protein (5; G. Kumamoto, unpublished data). Other proteins, such as ribose-binding protein, require PrlA but are exported at normal rates without SecB (5). Although the export defect observed in the absence of SecB function is profound, *secB* null mutants can survive provided that they are maintained on minimal medium. On rich media, the *secB* null mutations are lethal (5).

The mutations *lamBS78* and *lamB13D* alter the signal sequence of the outer membrane protein LamB by removing residues 10 through 13 and by substituting Asp for Ala at position 13, respectively. Both of these mutations cause the precursor form of LamB to accumulate in the cytoplasm. This defect is seen clearly in pulse-chase experiments that follow LamB localization (Fig. 1). Using this pulse-chase assay, the suppression caused by *prlA4* can be measured by monitoring the appearance of mature LamB. Since the processing of LamB to its mature form is catalyzed by leader peptidase and occurs on the exterior face of the cytoplasmic membrane, this event can serve as an indicator for translocation of LamB across the inner membrane. As reported previously (8), *prlA4* causes strong suppression of both *lamBS78* and *lamB13D* (Fig. 1).

Figure 1 shows the defect in LamB export that is caused by the null mutation *secB*::Tn*5*. As evidenced by the accumulation of LamB precursor, this mutation blocks a step in protein export before proteolytic processing. In this respect, the *secB* mutation resembles the signal sequence mutations *lamBS78* and *lamB13D*, except that it is more leaky. Accordingly, we cannot determine, using the pulse-chase assay, if *secB* and the signal sequence mutations block the same step or if they act at different stages in the export process.

The *secB*::Tn*5* mutation was introduced into strains carrying each of the *lamB* signal sequence mutations and the *prlA4* suppressor by a transductional cross selecting for the drug resistance determinant of Tn*5*. The resulting strains, NT193 (*lamBS78 prlA4 secB*::Tn*5*) and NT197 (*lamB13D prlA4 secB*::Tn*5*), exhibit the phenotype of a *secB* null mutant; i.e., they do not grow on rich media. In addition, these strains are more resistant to bacteriophage lambda (a LamB$^-$ phenotype) than are either of the corresponding parents (NT191 *lamBS78 prlA4*, NT195 *lamB13D prlA4*, or NT187 *secB*::Tn*5*), suggesting that *prlA4* suppression is reduced significantly in the absence of SecB. To more carefully determine the effect of the *secB* mutation on LamB export in these strains, the pulse-chase assay was employed. The results established that SecB is required for PrlA-mediated suppression of the signal sequence mutations *lamBS78* and *lamB13D* (Fig. 1).

Quantitation of the pulse-chase data shown in Fig. 1 by densitometer scan showed that the effect of a *secB* mutation in a *lamB prlA* double mutant was larger than that predicted by the simple sum of the *secB prlA4* export defects. In the *secB* null strain NT299 (*lamB*$^+$ *prlA*$^+$ *secB*::Tn*5*), the $t_{1/2}$ of processing was 4 min. In strains containing the *lamBS78* signal sequence mutation and *prlA4*, the $t_{1/2}$ for processing was 1 min. Taking into consideration the degradation rate for cytoplasmic LamB precursor ($t_{1/2}$ = 2 min; J. Stader, unpublished data), we estimated that about 10 to 20% of the LamB present at 4 min would be in the mature form in NT193 (*lamBS78 prlA4 secB*::Tn*5*), provided that the effects of *secB* and *prlA* are additive; this value is well within the sensitivity of the assay. In fact, there was no measurable mature LamB present. This result indicates that *secB* and *prlA* acted in the same pathway, since their effects would be additive if they acted in independent pathways. In addition, the results show that SecB function is more critical for export of LamB with a defective signal sequence under PrlA4-suppressing conditions than for export of wild-type LamB.

Since SecB is required for PrlA-mediated suppression of

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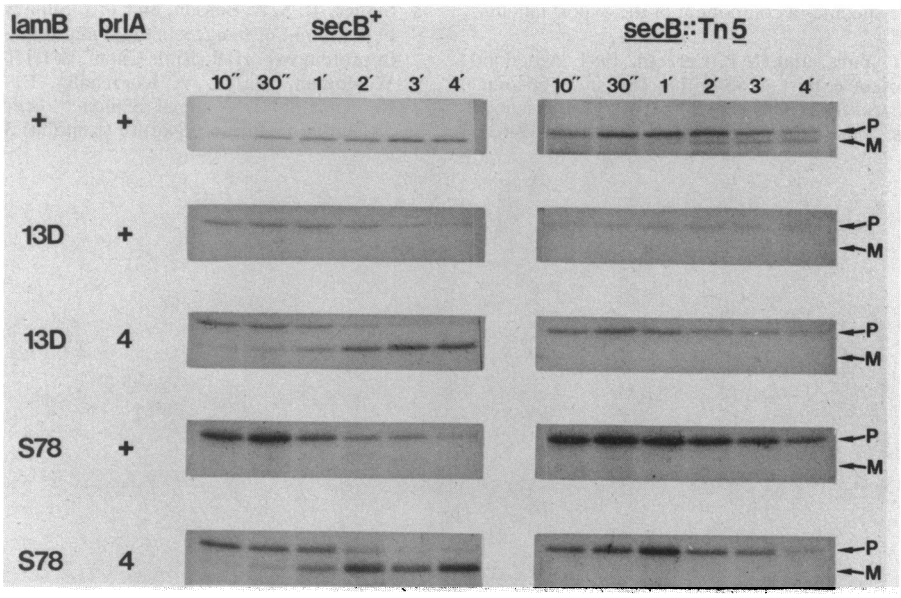

FIG. 1. Autoradiograms of immunoprecipitates of pulse-chase samples from strains carrying various combinations of *lamB*, *prlA*, and *secB* mutations. The *lamB* and *prlA* genotypes of the strains are indicated on the left, and the *secB* genotypes are indicated above the autoradiograms. P, Precursor LamB; M, mature LamB.

signal sequence mutations, we conclude that SecB, either directly or indirectly, is capable of recognizing the mutant LamB precursors. SecB and PrlA cannot recognize the same sequence determinant because signal sequence mutations that block interaction with wild-type PrlA do not affect recognition by SecB. Two explanations can be offered. First, it may be that these mutations do not alter the portion of the signal sequence which is recognized by SecB. Alternatively, it may be that SecB does not recognize the signal sequence at all. If the second explanation is correct, then LamB must contain an export signal that is located within sequences that correspond to the mature protein.

Collier et al. (2) have shown that internalized maltose-binding protein interferes with the export of wild-type proteins by depleting the cells of SecB, and they have exploited this observation to map the SecB-binding site to a region of the mature protein between residues 150 and 186. We have analyzed SecB recognition of LamB by using a different approach; our results strengthen the conclusion that SecB recognition involves sequences outside the signal sequence. Since this conclusion seems to apply to both periplasmic and outer membrane proteins, recognition of mature sequences by SecB may apply in general. The nature of the recognition determinant(s) in LamB, however, is not yet clearly defined.

The more critical requirement for SecB under conditions where LamB (Fig. 1) or maltose-binding protein (2) export is largely posttranslational can be explained by the antifolding activity of SecB (2); SecB would maintain cytoplasmic precursor in an export-competent state until translocation could occur. Such a role, however, does not adequately account for the effect of *secB* null mutations in an otherwise wild-type strain. Under these conditions, LamB export was clearly posttranslational (Fig. 1), as is the export of OmpA (2), another outer membrane protein. In addition, studies of Kumamoto and Gannon (C. A. Kumamoto and P. M. Gannon; J. Biol. Chem., in press) show that SecB is essential for cotranslational export of maltose-binding protein. We suspect, as did Collier et al. (2), that SecB plays an additional role in targeting the precursor molecule to the export machinery in the cytoplasmic membrane. Viewed in this manner, the antifolding activity of SecB may simply reflect the fact that SecB is binding the precursor molecule.

It is paradoxical that signal sequences, which are quite diverse, appear to direct export efficiently with great specificity. The results obtained with SecB and PrlA address this apparent paradox by suggesting that two different components which interact with different sequence determinants participate in the recognition of precursor forms of exported proteins. The combinatorial aspect of this model could contribute substantially to the overall specificity of the localization process.

We thank Mark Rose, Spencer Benson, and Phil Bassford for helpful discussions.

This work was supported by a Public Health Service grant from the National Institutes of Health to T.J.S. and by an American Cancer Society postdoctoral training grant to J.S.

LITERATURE CITED

1. **Cerretti, D. P., D. Dean, G. R. Davis, D. M. Bedwell, and M. Nomura.** 1983. The *spc* ribosomal protein operon of *Escherichia coli*: sequence and cotranscription of the ribosomal protein genes and a protein export gene. Nucleic Acids Res. **11**:2599–2616.
2. **Collier, D. N., V. A. Bankaitis, J. B. Weiss, and P. J. Bassford, Jr.** 1988. The antifolding activity of SecB promotes the export of the *E. coli* maltose-binding protein. Cell **53**:273–283.
3. **Emr, S. D., S. Hanley-Way, and T. J. Silhavy.** 1981. Suppressor mutations that restore export of a protein with a defective signal sequence. Cell **23**:79–88.
4. **Fandl, J. P., and P. C. Tai.** 1987. Biochemical evidence for the *secY24* defect in *Escherichia coli* protein translocation and its suppression by soluble cytoplasmic factors. Proc. Natl. Acad. Sci. USA **84**:7448–7452.
5. **Kumamoto, C. A., and J. Beckwith.** 1985. Evidence for specificity at an early step in protein export in *Escherichia coli*. J. Bacteriol. **163**:267–274.
6. **Schultz, J., T. J. Silhavy, M. L. Berman, N. Fiils, and S. D. Emr.** 1982. A previously unidentified gene in the *spc* operon of

Escherichia coli K12 specifies a component of the export machinery. Cell **31**:227–235.

7. **Shiba, K., K. Ito, T. Yura, and D. P. Cerretti.** 1984. A defined mutation in the protein export gene within the *spc* ribosomal protein operon of *Escherichia coli*: isolation and characterization of a new temperature-sensitive *secY* mutant. EMBO J. **3**:631–635.
8. **Stader, J., S. A. Benson, and T. J. Silhavy.** 1986. Kinetic analysis of *lamB* mutants suggests the signal sequence plays multiple roles in protein export. J. Biol. Chem. **261**:15075–15080.
9. **Weidmann, M., T. V. Kurzchalia, E. Hartmann, and T. A. Rapoport.** 1987. A signal sequence receptor in the endoplasmic reticulum membrane. Nature (London) **328**:830–833.