The Presence of Both the Signal Sequence and a Region of Mature LamB Protein Is Required for the Interaction of LamB with the Export Factor SecB*

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In the accompanying paper (Altman, E., Bankaitis, V. A., and Emr, S. D. (1990) J. Biol. Chem. 265, 18148–18153) a putative SecB binding site was identified in the mature LamB protein. The export of wild-type LamB was unperturbed when this region was removed, however, suggesting the presence of a second site of interaction between SecB and LamB. In this paper we show that the interference caused by export of defecti

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1 The abbreviations used are: MBP, maltose binding protein; SDS, sodium dodecyl sulfate; IPTG, isopropyl β-D-thiogalactoside.

It has become increasingly clear that the secB gene codes for a component of the _Escherichia coli_ export machinery. However, unlike other export components which are encoded by the secA and prlA/secY genes, secB is a nonessential gene (for a general review see Bankaitis et al., 1986). How SecB interacts with exported proteins remains an intriguing question since SecB only appears to be necessary for the efficient export of specific LamB under normal conditions, when the signal sequence is mutated. The presence of both the LamB signal sequence and the interfering region which maps to amino acids 320–380 of mature LamB. Although the interfering region is not necessary for the export of wild-type LamB under normal conditions, when the signal sequence is mutated the interfering region is required to promote the efficient export of LamB protein. Also, deletion of the interfering region eliminates the ability of wild-type LamB precursor to be maintained in an export competent conformation in vivo. Collectively, our results indicate that efficient export of the LamB protein is achieved by an interaction with SecB that involves both the LamB signal sequence and the interfering region in mature LamB.

**Materials and Methods**

**Bacterial Strains**—All strains used in this study are isogenic derivatives of MC4100: ΔlacA169 araU139 rpsL150 thi relA1 flaB5301 deoL1 ptaF35 (Casadaban, 1976) and the relevant genotypes are listed in Table I. When necessary, the secB::Tn10 lesion was moved into strains by P1 transduction, while the F'lacY'Z'Y'A' episome was moved into strains by mating with S9100 (Altman et al., 1990).

**lamB Plasmids**—The construction of pLamBwt, pLamBIIR, pUClamB880, and pUClamB860AIIR are described in the accompanying paper (Altman et al., 1990). pLamB1LR was constructed by replacing the EcoRI-Smal fragment containing lamB’s signal sequence in pLamB74 with the equivalent EcoRI-Smal fragment from pSE73. pSE73 was constructed by replacing the SuvI fragment containing lamB’s signal sequence in pSE80 with the equivalent SuvI fragment from pSP73 (Emr and Silhavy, 1984). By exchanging a Smal-BamHI fragment that included the lamB interfering region between pLamBIIR and pLamB1LR, pLamB172AIIR was constructed.

**Anti-SecB Affinity Chromatography**—Bacterial cells were labeled for 15 s at 37 °C with Tran[^-35]S]label (ICN), 60 μCi/ml[^-35]S]methionine, 90 nM nethionine. Cells were extracted as described previously (Kumamoto, 1989), and the extract was divided into three equal portions. One portion was precipitated with trichloroacetic acid and

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OmpF are absolutely dependent on SecB for their efficient export, the periplasmic ribose binding protein (RBP), alkaline phosphatase, β-lactamase, and M13 coat protein, as well as the outer membrane lipoprotein can be efficiently exported independent of SecB (Kumamoto and Beckwith, 1983, 1985; Collier et al., 1988).

Several observations have made it clear that the synthesis of export-defective SecB-dependent proteins interferes with the normal export process, and that this interference is due to the limitation of a single component of the export machinery, SecB. First, the subset of exported proteins affected by interference is identical to the subset of proteins that require SecB for export (Collier et al., 1988). Second, overproduction of SecB suppresses interference (Collier et al., 1988). Third, interference does not increase the export defect observed in secB mutant cells (Collier et al., 1989). Finally, interference is caused by the limitation of a single export component (Altman et al., 1990).

In the accompanying paper (Altman et al., 1990), the interfering region of an export-defective LamB protein was mapped and found to reside between amino acids 320 and 380 of the mature protein. Surprisingly, when this region was deleted from a wild-type LamB protein export was unperturbed even though it is well documented that LamB export is crippled in a secB null background (Kumamoto and Beckwith, 1985; Trun et al., 1988). The result suggested that LamB contained an additional site of SecB interaction. In this study we show that the efficient interaction of SecB with LamB protein is dependent on the presence of both the LamB signal sequence and the interfering region in mature LamB.
Immunoprecipitated with anti-LamB antiserum (Kumamoto and Gannon, 1988), to determine the total amount of radioactive LamB precursors in the sample. The second portion was applied to an affinity column containing anti-SecB antibody that was prepared as described (Kumamoto and Gannon, 1988). The third portion was mixed with an excess of purified SecB protein (prepared as described previously: Kumamoto, 1980) and then applied to an anti-SecB column. After washing with phosphate-buffered saline/0.5% Tween 20, the columns were eluted (Kumamoto, 1989) and the LamB precursor that bound to the columns in association with SecB was detected by immunoprecipitation, SDS-polyacrylamide gel electrophoresis, and fluorography (Kumamoto and Gannon, 1988). Quantitation of the relative amounts of precursor LamB that bound to the columns was determined by densitometry of fluorograms as described previously (Altman et al., 1990).

Post-translational Assay—By varying the amount of carbonyl cyanide m-chlorophenylhydrazone and β-mercaptoethanol used in the assay conditions described by Zimmerman and Wickner (1986), we were able to reverse the carbonyl cyanide m-chlorophenylhydrazone block directly with the addition of β-mercaptoethanol without the need of a prior wash step to remove excess carbonyl cyanide m-chlorophenylhydrazone. This allowed us to easily monitor posttranslational export in E. coli. Vigorously aerated cultures were grown to logarithmic phase (.30Ds0 to .40Ds0 in M63 minimal medium (Miller, 1972) at 30 °C. Carbonyl cyanide m-chlorophenylhydrazone was added to a final concentration of 50 μM 1 min prior to pulse labeling (a stock solution of carbonyl cyanide m-chlorophenylhydrazone was prepared at a concentration of 5 mM in 55% ethanol and stored at −90 °C). Samples were then pulse labeled for 1 min with the addition of 10 μCi of [35S]methionine/ml of culture and chase was initiated by the addition of unlabeled methionine to a final concentration of 0.5% (w/v). One min later the carbonyl cyanide m-chlorophenylhydrazone block was released by the addition of β-mercaptoethanol to a final concentration of 0.05% (w/v). To terminate the assay, 1-ml aliquots were removed and immediately dispensed into 150 μl of ice-cold 50% trichloroacetic acid.

We find that under the assay conditions described, the translocation of both MBP and LamB can be blocked, but that when the block is released 100% of the proteins are exported. If higher concentrations of carbonyl cyanide m-chlorophenylhydrazone were used, however, the translocation block cannot be released by β-mercaptoethanol. Although degradation of unexported LamB is sometimes a problem in most of the LamB experiments reported here, some degradation of unexported wild-type LamB protein is 100% stable and no degradation occurs. We found that unexported wild-type LamB protein was degraded at an average rate of 2%/min of chase, while unexported LamB17D protein was degraded at an average rate of 3%/min of chase.

Regents—DNA restriction and modifying enzymes were obtained from New England BioLabs, Inc. [35S]Methionine came from American Corp., Trans[35S]label came from ICN Radiochemicals, while Safety-Solve came from Research Products International Corp. Carbonyl cyanide m-chlorophenylhydrazone and β-mercaptoethanol used in the posttranslational assay were purchased from Sigma. Anti-LamB, MBP, and OmpA rabbit serum were generous gifts of Tom Silhavy, Phil Bassford, and Paul Ray, respectively.

RESULTS

Interference Is Influenced by the LamB Signal Sequence—In the accompanying paper (Altman et al., 1990), the interfering region of the export-defective LamB560 protein was mapped to between amino acids 320 and 380 of the mature protein. We suggested that this region might be responsible for SecB binding. However, deletion of the interfering region from an otherwise wild-type LamB protein did not affect its export, suggesting that SecB was still able to associate with the deleted derivative. Because the interfering region was mapped using export-defective LamB proteins that contained a large deletion in the signal sequence, it is possible that normally there is a signal sequence-dependent interaction between LamB and SecB that was not detected in the mapping study. To test this, we scored the interference caused by six different export-defective LamB proteins (Fig. 1). Since Bankaitis and Bassford (1984) had shown that interference was directly proportional to the strength of an export-defective mutation (weaker signal sequence mutations that cause incomplete export blocks do not interfere as much as stronger signal sequence mutations that cause complete export blocks), we only examined LamB proteins containing signal sequence mutations that caused total export blocks as judged by their inability to secrete any LamB protein after a 4-min chase (Stader et al., 1986). Although all of the export-defective LamB proteins examined were totally export-incompetent and synthesized LamB at equivalent rates, their interference levels varied. The export-defective proteins showed a general pattern in that the interference levels decreased as increasing amounts of the signal sequence was removed from the export-defective protein.

Previous analysis of LamB's signal sequence has indicated that a core of 4 residues is absolutely critical for export, since all point mutations which abolish LamB's export lie in either amino acids 14, 15, 16, or 19 of the signal sequence (Emr and Silhavy, 1982). Closer examination of the data in Fig. 1 indicates that maximal interference occurs when amino acids

### TABLE I

| Strain       | Relevant genotype | Reference            |
|--------------|------------------|----------------------|
| MC1140       | Wild type, rpsL150, F- | Casadaban (1976)     |
| ES2060       | lamB560          | Emr and Silhavy (1980) |
| ES2068       | lamB568          | Emr and Silhavy (1980) |
| ES2069       | lamB515D         | Emr and Silhavy (1980) |
| SE2071       | lamB14D          | Emr and Silhavy (1980) |
| SE2073       | lamB17D          | Emr and Silhavy (1980) |
| SE2078       | lamB578          | Emr and Silhavy (1980) |
| ES2087       | lamB587          | Emr and Silhavy (1980) |
| ES3030       | malK Δ1 lamB     | Emr and Silhavy (1980) |
| ES3090       | rpsL6 θpsI F lacI Q Z+Y+A | Altman et al. (1990) |
| CK1953       | secB:nls5       | Kumamoto and Beckwith (1985) |

*The original nomenclature of Emr and Silhavy (1980) is used in this study to describe export-defective LamB proteins that result from deleting parts of the LamB signal sequence.

*The nomenclature suggested by Stader et al. (1986) is used in this study to describe export-defective LamB proteins that are the result of single amino acid changes in LamB's signal sequence.
SecB Interacts with Precursor LamB

**LamB signal sequence processing site**

|    | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| wt | met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |
| 14D| met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |
| 19R| met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |
| S78| met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |
| S87| met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |
| S60| met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |
| S68| met | met | ile | thr | leu | arg | lys | leu | pro | leu | ala | val | ala | ala | gly | val | met | ser | ala | gln | ala | met | ala | val | asp | phe |

Fig. 1. Interference of MBP caused by different export-defective LamB proteins. Maltose-grown cells of MC4100 (lamB+), SE2071 (lamB14D), SE2069 (lamB19R), SE2078 (lamBS78), SE2087 (lamBS87), SE2060 (lamBS60), or SE2068 (lamBS68) were pulse-labeled for 30 s with no chase, and the interfered MBP protein was assayed by immunoprecipitation using anti-MBP serum. The signal sequence alteration for each of the export-defective proteins is indicated. A reduced level of interference is caused by lamB14D, lamBS60, or lamBS87 and occurs at an average of 14% MBP precursor, while an increased level of interference is caused by lamB19R or lamBS87 and occurs at an average of 40% MBP precursor. The interference caused by lamBS78 occurs at an intermediate level, which is consistent with the model proposed by Emr and Silhavy (1983), that the effect of the lamBS78 mutation is an indirect one, and is due to the proximal location of the deletion to the critical amino acid core defined by residues 14, 15, 16, and 19 of the LamB signal sequence (reviewed in Silhavy et al., 1983). wt, wild-type.

14, 15, and 16 of the signal sequence are preserved (interference caused by lamB19R or lamBS87). Amino acid 19 can be either mutated (lamB19R) or deleted (lamBS87) and interference is still maximal. However, when amino acids 14, 15, or 16 are either mutated or deleted, only a reduced level of interference is observed (interference caused by lamB14D, lamBS60, or lamBS87). These results could be explained if the region around amino acids 14–16 contributed to the interaction between SecB and the LamB protein.

SecB Association with Intracellular LamB Precursors Is Dependent upon the Presence of Both the LamB Signal Sequence and the Interfering Region between Amino Acids 320 and 380 of Mature LamB—The results described above and in the accompanying paper (Altman et al., 1990) suggested that the interaction of SecB with the LamB protein might depend upon multiple regions of LamB. To test this hypothesis directly, we used anti-SecB affinity chromatography to determine whether SecB was capable of association with precursor LamB molecules containing or lacking the interfering region, and containing or lacking a functional signal sequence.

It had previously been demonstrated that SecB complexes containing SecB and wild-type precursor LamB could be detected using anti-SecB affinity chromatography of labeled cell extracts (Kumamoto, 1989). To determine the effect of the deletions that were used in the previous study (Altman et al., 1990), we labeled secB+ cells harboring plasmids that encoded either wild-type LamB (wtLamB), wild-type LamB lacking the interfering region (LamBAIR), export-defective LamB containing a deletion in the signal sequence (LamBS60), and export-defective LamB containing both a deletion in the signal sequence as well as a deletion of the interfering region (LamBS60AIR). Extracts of the labeled cells were prepared and split into three equal portions. One portion was immunoprecipitated directly to determine the total amount of labeled precursor LamB present in the extract. The remaining two portions were applied to anti-SecB columns in the presence or absence of excess SecB competitor. After elution, the amount of precursor LamB that was complexed with SecB was determined by immunoprecipitation of the bound fraction and compared to the total amount of labeled precursor LamB that was present in the extract. Consistent
with previous results, we observed that 50% of the intracellular precursor could be obtained as a SecB complex, when extracts containing wild-type precursor LamB were analyzed (Fig. 2). When excess SecB was present during chromatography to compete with the binding of SecB complexes, precursor LamB was not detected in the bound fraction, demonstrating that binding of precursor LamB to the affinity column was dependent upon its association with SecB.

Deletion of either the LamB interfering region or the signal sequence resulted in a marked decrease in the amount of precursor that was recovered as a SecB complex, and deletion of both regions almost completely eliminated the ability of LamB to associate with SecB (Fig. 2). When excess SecB was present during chromatography of the extracts, LamB species were not detected in the anti-SecB bound fraction (Fig. 2) demonstrating that LamB binding occurred by virtue of association with SecB. We conclude from these studies that under physiological conditions, both the signal sequence and the interfering region are necessary for the efficient association of SecB with precursor LamB.

LamB17D’s Export Kinetics Are Altered When the Interfering Region Is Removed or When SecB Is Absent—The above results suggested that the SecB association mediated by the interfering region might be more critical for export if the signal sequence was compromised. Although it is not possible to demonstrate the importance of the interfering region using signal sequence mutations that prevent export, we reasoned that the interfering region might be important for the export of a LamB protein containing a weak signal sequence mutation.

All of the LamB signal sequence mutants were isolated by employing lamB-lacZ fusions that resulted in a maltose-sensitive phenotype upon induction with maltose. Maltose-resistant mutants were obtained and then crossed from the lamB-lacZ fusion back to wild-type lamB to examine the effect of the mutation on LamB protein export. While most of the mutations rendered both the LamB-LacZ hybrid protein and the wild-type LamB protein export-defective, mutations that introduced a charged residue at amino acid 17 of the LamB signal sequence only caused the LamB-LacZ hybrid protein to become export-defective (Emr and Silhavy, 1980, 1982). Interestingly, the lamB-lacZ gene fusion used in the selection process coded for a LamB-LacZ hybrid protein that lacked the interfering region of mature LamB. It is possible that silent mutants such as LamB17D, which contains an aspartic acid residue at amino acid 17 instead of the wild-type glycine residue, were isolated for this reason. To test this, the export kinetics of the LamB17D protein with or without the interfering region were examined. As shown in Fig. 3, the export kinetics of the LamB17D protein without the interfering region were 2-fold slower than that of the LamB17D protein containing the interfering region; furthermore, 25% of the LamB17D protein lacking the interfering region appears to be export-incompetent, yet no such defect is seen with the LamB17D protein containing the interfering region. Thus, a significant defect in the export of LamB protein can be seen if removal of the interfering region is combined with a weak signal sequence mutation such as lamB17D.

This result suggests that the LamB17D protein is extremely dependent on the presence of SecB for its efficient export. To analyze this further, we compared the export kinetics of

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\text{FIG. 2. Association of LamB precursors with SecB in vivo.} \\
\text{The LamB protein in glycerol grown cells of SE3001 F' lacQ'/Z'/Y'/A' (ΔlamB), containing either plasmids plamBwt, plamBAIR, pUClamBS60, or pUClamBS60ΔIR, was induced to chromosomal levels by the addition of IPTG 2 h prior to labeling. Cells were then pulse-labeled for 15 s and extracted as described under "Materials and Methods." The total amount of labeled LamB precursor in each extract (TOTAL) was determined by immunoprecipitation of a portion of the extract prior to affinity chromatography. After affinity chromatography of the labeled extract on an anti-SecB column, the amount of total LamB precursor was determined by elution and immunoprecipitation (BOUND). As a control, chromatography was also performed in the presence of excess SecB competitor and the amount of bound LamB precursor under these conditions was determined as above (CONTROL). Equivalent amounts of all immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. To quantitate accurately the amount of bound precursor, 1/10 or 1/5 dilutions of the total samples were also analyzed (data not shown). A map of the four lamB constructs analyzed in this experiment is shown in the upper part of the figure. The signal sequence is indicated by SS, while IR denotes the sequence coding for the interfering region which maps to between amino acids (aa) 320 and 382 of the mature protein. Deletions in either the signal sequence or the interfering region are indicated by a striped box.}
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\text{FIG. 3. Export kinetics of LamB17D versus LamB17DΔIR.} \\
\text{Glycerol-grown cells of SE3001 F' lacQ'/Z'/Y'/A' (ΔlamB), containing either plasmid plamB17D or plamB17DΔIR, were induced with 20 μM IPTG 2 h prior to labeling. The cells were pulse-labeled for 30 s and chased as indicated. LamB17D data points are shown as open squares, while LamB17DΔIR data points are shown as open circles.}
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LamB17D versus wild-type LamB in both secB+ and secB null backgrounds (Fig. 4, A and B). Although the export rate of LamB17D was only three times slower than wild-type LamB in a secB+ background, the export rate of LamB17D was more than thirty times slower than wild-type LamB in a secB null background. After a 20-min chase in a secB null background, 65% of the wild-type LamB protein was exported, while less than 2% of the LamB17D protein was exported. This synergistic effect demonstrates that the efficient export of LamB17D is absolutely dependent on the presence of SecB. When SecB is limited, either by removing the interfering region or by removing SecB, the export kinetics of LamB17D are drastically affected. Collectively, these data indicate that if the signal sequence is altered, interaction of SecB with the interfering region is necessary for the efficient export of the LamB protein.

If the Interfering Region Is Removed LamB Precursor Is Not Maintained in an Export-competent Conformation—Although the export of wild-type LamB is unperturbed under normal conditions when the interfering region is removed, our results indicate that the presence of the interfering region is necessary if proper association of SecB with precursor LamB is to occur. We reasoned that if wild-type LamB's export could be temporally delayed, a dependence on the interfering region might be uncovered. To accomplish this, we employed a posttranslational export assay. Cells containing wild-type LamB with or without the interfering region were pulse-labeled in the presence of the uncoupler carbonyl cyanide m-chlorophenylhydrazone, which causes a translocation block (Randall, 1986). Chase was then initiated and the translocation block was released by the addition of β-mercaptoethanol. As shown in Fig. 5, while wild-type LamB can be efficiently exported posttranslationally, LamB protein that lacks the interfering region cannot. Thus, it appears that an interaction between SecB and the interfering region is necessary if the LamB precursor is to be maintained in an export-competent form.

Role of SecB in the Export of Three SecB-dependent Proteins—SecB appears to interact with the exported protein in a complex manner, as evidenced by the presence of two sites of interaction in the LamB protein. Toward a better understanding of how SecB promotes the export of SecB-dependent proteins, we compared the export rates of LamB, MBP, and OmpA in both secB+ and secB null backgrounds (Fig. 6). While all three proteins are exported with swift kinetics in a secB+ background, there is a severe export block in a secB null background at early chase points. At later chase points, however, there is a clear difference in the three proteins' dependence on SecB; OmpA is 100% exported, LamB is 65% exported (this number might be greater except that 35% of LamB was degraded before it could be exported), yet only 40% of MBP ever gets exported. This result indicates that the requirement for SecB differs from protein to protein.

Fig. 4. Export kinetics of wild-type LamB versus LamB17D in SecB+ and SecB- backgrounds. A, SecB+. Maltose-grown cells of MC4100 (lamB+) or SE2073 (lamB17D) were pulse-labeled for 15 s and chased as indicated. Wild-type LamB data points are shown as open squares while LamB17D data points are shown as open circles. If the slopes of the linear region of the export curves of these two proteins are examined wild-type LamB's slope, is three times greater than LamB17D's slope. Since wild-type LamB is completely exported by 1 min of chase the slope comparison would predict that LamB17D should be completely exported by 3 min of chase. LamB17D appears to be completely exported somewhere between 2 and 4 min of chase (LamB17D is 82% exported at the 2-min chase time and 100% exported at the 4-min chase time). B, SecB-. Maltose-grown cells of MC4100 secB::Tn5 (lamB+, secB-) or SE2073 secB::Tn5 (lamB17D, secB-) were pulse-labeled for 30 s and then chased as indicated. Wild-type LamB data points are indicated with open squares, while LamB17D data points are indicated with open circles. The predicted data points (based on the fact that export of LamB17D is three times slower than wild-type LamB) for LamB17D are indicated with open triangles.

Fig. 5. Posttranslational export of the wild-type (wt) LamB protein with or without the interfering region. Glycerol-grown cells of SE3001 F' raciQ'Z'Y'A' (ΔlamB), containing either plasmid planBwt or planBΔIR, were induced with 20 μM IPTG 2 h prior to labeling. Carbonyl cyanide m-chlorophenylhydrazone was added 1 min prior to labeling, to introduce a translocation block. The samples were then pulse-labeled for 1 min and chased by the addition of excess cold methionine. The translocation block was released 1 min later by the addition of β-mercaptoethanol. Control samples were pulse-labeled for 1 min with no chase in the absence of carbonyl cyanide m-chlorophenylhydrazone. See "Materials and Methods" for complete experimental details.
Fig. 6. Export kinetics of LamB, MBP, and OmpA in SecB+ or SecB− backgrounds. Maltose-grown cells of either MC4100 (lamB+, mbl+, ompA+, secB+) or MC4100 secB− Tn5 (lamB−, mbl−, ompA−, secB−) were pulse-labeled for 30 s and chased as indicated. LamB, MBP, and OmpA data points in a secB null background are shown as open circles, triangles, and squares, respectively. Since the LamB, MBP, and OmpA data points were almost identical in a secB− background, a single averaged export profile for the three proteins in a secB− background is indicated by a dashed line (the only variance in the data points were at the 0-min chase point, where the percent exported values were 86% for LamB, 97% for MBP, and 90% for OmpA).

**Discussion**

It is now apparent that the export interference phenomenon in *E. coli* is caused by the limitation of SecB (Collier et al., 1988; Altman et al., 1990). In the accompanying paper, we mapped the interfering region in an export-defective LamB protein to between amino acids 320 and 382 of the mature protein. However, when the interfering region was removed from the wild-type LamB protein, no export defect was observed, even though it has been shown that export of the wild-type LamB is crippled in a secB− mutant background (Kumamoto and Gannon, 1988; Trun et al., 1988). These results raised the possibility that SecB might interact with the LamB signal sequence. Consistent with this hypothesis, we found that the interference caused by export-defective LamB proteins was weakest when a large portion of the signal sequence was deleted. Previous analysis of mutations in the LamB signal sequence that rendered the LamB protein export-defective have indicated that amino acids 14–16 might be part of a critical core of amino acids within the LamB signal and sequence (Emr and Silhavy, 1982). Interestingly, we found that interference is greater when the export-defective LamB protein contains amino acids 14–16 of the signal sequence than when it lacks them. Taken together, these observations suggest that the region of the LamB signal sequence is necessary for the interaction of LamB with SecB.

Using anti-SecB affinity chromatography, it was possible to test directly whether the efficient interaction of SecB with LamB precursor was dependent on the presence of either the LamB signal sequence or the interfering region in mature LamB. Consistent with previous results (Kumamoto, 1989), we observed that SecB precursor LamB complexes were efficiently formed *in vitro*. However, if the LamB precursor contained a deletion in the signal sequence or a deletion of the interfering region, the association of LamB with SecB was reduced 5-fold. When both the signal sequence and the interfering region were removed from the LamB protein the association of LamB with SecB was reduced 25-fold. The results of these binding studies show that the interaction of SecB with the LamB protein requires that both the signal sequence and the interfering region be intact. The simplest interpretation of our data is that SecB protein binds directly to both the LamB signal sequence and the interfering region between amino acids 320 and 382 of mature LamB, and that maximum binding depends on the presence of both sites.

Although deletion of the interfering region does not alter the normal export kinetics of wild-type LamB, two observations demonstrate that the interfering region is required for the efficient export of the LamB protein: 1) the export of a LamB protein harboring a weak signal sequence mutation is significantly slower when the interfering region is removed, and 2) the removal of the interfering region drastically reduces the ability of wild-type LamB precursor to be maintained in an export-competent conformation. Thus, the efficient action of SecB appears to require the presence of both the LamB signal sequence and the interfering region in mature LamB. It is well documented that SecB functions as an antifolding agent during the export process (Collier et al., 1988; Kumamoto and Gannon, 1988; Liu et al., 1989) and that SecB forms a tetrameric complex in the cytoplasm (Weiss et al., 1988; Kumamoto et al., 1989; Watanabe and Blobel, 1989a). In light of our results, it is possible that a single SecB tetramer acts simultaneously at both the signal sequence and a region in the mature portion of the exported protein. This would provide a simple model for how the SecB protein acts as an antifolding factor during the export process.

The data from several labs suggest that MBP may interact with SecB in a manner similar to LamB. First, it is apparent that SecB interacts with the mature region of MBP (Collier et al., 1988; Gannon et al., 1989; Liu et al., 1989) and that this interaction promotes the antifolding activity of SecB (Collier et al., 1988; Kumamoto and Gannon, 1988; Liu et al., 1989). Second, Watanabe and Blobel (1989b) have demonstrated that while precursor MBP species that contain an intact signal sequence are bound by SecB, precursor MBP species that contain a deletion in the signal sequence are not bound by SecB. Together, these data indicate that the interaction of SecB with MBP involves both the signal sequence and a region of mature MBP. Considerable controversy exists, however, as to whether the signal sequence is directly or indirectly involved in the binding of SecB to the exported protein. While Watanabe and Blobel (1989b) have proposed that SecB binds directly to the signal sequence of the exported protein, Randall and colleagues favor a model in which SecB does not bind to the signal sequence of the exported protein, and have proposed that the signal sequence plays an indirect role in modulating the binding of SecB to the mature protein (Liu et al., 1989; Randall et al., 1990). Although it will be extremely difficult to delineate between these two possibilities, the data presented in this and the accompanying paper favors a more direct role for the signal sequence during the association of SecB with the exported protein.

The larger question of how SecB facilitates the export of the bound protein remains to be answered. It could be simply that SecB functions as an antifolding agent and maintains SecB-dependent proteins in an export competent unfolded form. In this scenario, SecB’s only function would be to keep the protein unfolded so that it could productively interact with the export complex represented by other components, such as SecA and PrlA/SecY. However, two observations suggest that SecB interacts directly with both SecA and PrlA/SecY; first, a synergistic response is seen at the permissive temperature when a secB mutant is combined with the secAts allele (Kumamoto and Beckwith, 1983), and second, the suppressor function of the prlA4 allele is disabled in a secB null background (Trun et al., 1988). Because of these data, we believe that SecB has two functions in the export process: 1)
a recognition function in which SecB presents the exported protein to the SecA-PrlA/SecY complex, a process that might involve an interaction with the signal sequence, and 2) an antifolding function which retards the folding of the exported protein into an export-incompetent form.

Our finding that three SecB-dependent proteins vary widely in their dependence for SecB is also consistent with SecB having two functions. Although the export of MBP, LamB, and OmpA is blocked shortly after synthesis in a secB null background, the final export rates of these proteins are different, as OmpA and LamB eventually are exported, but MBP is not. Very similar results have been obtained when SecB is limited due to the interfering effects of synthesizing an export-defective MBP protein. At early chase times precursors of MBP, LamB, and OmpA accumulate as a result of interference. At later chase times however, all of the LamB and OmpA precursor are exported, while a significant portion of the MBP precursor is rendered export-incompetent (Collier et al., 1986; Bankaitis and Bassford, 1984). While MBP, LamB, and OmpA would all be dependent on SecB to recognize and deliver them to the SecA-PrlA/SecY complex, MBP also would be dependent on SecB's antifolding function for export, since MBP folds rapidly into an export-incompetent form (Randall and Hardy, 1986; Collier et al., 1988; Kumamoto and Gannon, 1988). OmpA and LamB, although requiring SecB for efficient delivery to the export complex might not have the same requirements for SecB's antifolding function, since being largely hydrophobic membrane proteins they may not tend to fold into export-incompetent forms.

It appears likely that SecB affects the interaction of SecA-PrlA/SecY with the signal sequence by stabilizing a productive interaction of the signal sequence with the export complex and/or triggering the active translocation of the bound exported protein. In fact, Watanabe and Blobel (1989b) have proposed that SecB is the signal recognition particle of E. coli. Although this is consistent with our data, we feel it is more likely that SecB belongs to a family of chaperones that facilitate the export of certain proteins, for the following reasons. First, trigger factor and GroEL, two chaperones not as well characterized as SecB, have been shown to function in a manner similar to SecB by maintaining OmpA in an export-competent form (Lecker et al., 1989). Second, SecB is only required for the efficient export of a subset of the secreted proteins that are dependent on the E. coli export apparatus (Kumamoto and Beckwith, 1983, 1985; Collier et al., 1988). Third, one of the SecB-independent proteins, β-lactamase, has been shown to be dependent on GroEL for its efficient export (Kusukawa et al., 1989). Collectively, these data suggest that multiple export factors such as SecB, trigger factor, and GroEL act in concert to facilitate the recognition and delivery of secreted proteins. Some proteins such as OmpA might utilize all of the factors for their efficient export, while the export of other proteins might only require some of the factors. Clearly, further work will be needed to elucidate precisely how SecB and the rest of the export machinery of E. coli efficiently translocates secreted proteins across the cytoplasmic membrane.

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