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# Synthesis and Export of the Outer Membrane Lipoprotein in *Escherichia coli* Mutants Defective in Generalized Protein Export

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**Export of the outer membrane lipoprotein in *Escherichia coli* was examined in conditionally lethal mutants that were defective in protein export in general, including *secA*, *secB*, *secC*, and *secD*. Lipoprotein export was affected in a *secA*(Ts) mutant of *E. coli* at the nonpermissive temperature; it was also affected in a *secA*(Am) mutant of *E. coli* at the permissive temperature, but not at the nonpermissive temperature. The export of lipoprotein occurred normally in *E. coli* carrying a null *secB*::Tn*5* mutation; on the other hand, the export of an OmpF::Lpp hybrid protein, consisting of the signal sequence plus 11 amino acid residues of mature OmpF and mature lipoprotein, was affected by the *secB* mutation. The synthesis of lipoprotein was reduced in the *secC* mutant at the nonpermissive temperature, as was the case for synthesis of the maltose-binding protein, while the synthesis of OmpA was not affected. Lipoprotein export was found to be slightly affected in *secD*(Cs) mutants at the nonpermissive temperature. These results taken together indicate that the export of lipoprotein shares the common requirements for functional SecA and SecD proteins with other exported proteins, but does not require a functional SecB protein. SecC protein (ribosomal protein S15) is required for the optimal synthesis of lipoprotein.**

Protein export is a complex process which requires the sequential participation of a number of gene products, including the enzymes for the processing of precursor proteins. Genetic analysis has revealed that at least four genes are required for protein export in general: *secA*, *secB*, *secD*, and *secY* (*prlA*). *secA*, *secD*, and *secY* mutants have been isolated as conditionally lethal mutants of *Escherichia coli* and have been found to accumulate precursors of a number of exported proteins at the nonpermissive temperature (4, 14, 18). On the other hand, a null mutation in the *secB* gene is not lethal, but it affects the export of a subset of outer membrane and periplasmic proteins (9). Mutations in the *secC* gene have been isolated as suppressor mutations of a *secA*(Ts) allele (3). *secC* mutants were found to be cold sensitive in growth; at the nonpermissive temperature, the synthesis of several outer membrane and periplasmic proteins was severely curtailed. It has been proposed that the *secC* gene product (ribosomal protein S15) interacts with the SecA protein and participates in the coupling of protein synthesis and protein secretion (3).

The major outer membrane lipoprotein differs from most of the other exported proteins in *E. coli* in that the precursor protein (prolipoprotein) is modified with glycerol and fatty acids, and is subsequently processed by prolipoprotein signal peptidase (SPase II) during the course of its export. We have previously shown (5) that the export of lipoprotein is affected in *secA*(Ts) and *secY*(Ts) mutants, resulting in the accumulation of unmodified prolipoprotein in the cytoplasmic membrane at the nonpermissive temperature. These observations suggest that the early steps in protein export are shared by lipoproteins and nonlipoproteins and that the modification and processing of prolipoproteins represent late events in protein export. On the other hand, we have previously shown (5) that lipoprotein export is not affected in a *secB* mutant strain.

To further define the export pathway of lipoprotein compared with that of nonlipoproteins in *E. coli*, we studied lipoprotein export in other mutants that were defective in generalized protein export. In the present study we compared the export of the major outer membrane lipoprotein with those of nonlipoproteins in individual *E. coli* mutants containing an amber allele of *secA*, a Tn*5* insertion mutation in *secB*, a *cs* allele of *secC*, or a *cs* allele of *secD*. Our results indicate that lipoprotein export in *E. coli* requires functional SecA protein, SecD protein, as well as SecY protein but that it is not affected by the null mutation in *secB*. In addition, our results indicate that the *secC* mutation affects the synthesis of lipoprotein and maltose-binding protein (MBP), but does not affect the synthesis of OmpA.

## MATERIALS AND METHODS

**Phage, bacterial strains, and medium.** λDO20 phage, which carries the wild-type *secA* gene, was a generous gift from D. Oliver (State University of New York at Stony Brook, Stony Brook, N.Y.) (15). The bacterial strains used in this study are listed in Table 1. The media used in this study include proteose peptone beef extract (PPBE) broth medium (20) and M9 minimal medium (13) supplemented with thiamine (100 μg/ml), 0.4% glycerol–0.4% maltose, or 0.4% glucose.

**Isolation of *E. coli* (λDO20) lysogen.** Strain MM52 [*secA*(Ts)] and strain MM113 [*secA*(Am)] cells were grown at 30°C overnight in PPBE broth. Five microliters of λDO20 phage lysate was spotted onto a lawn of strain MM52 or MM113. Following incubation at 30°C overnight, phage-infected cells from the clear zone were streaked onto a PPBE plate to obtain single colonies. Ten colonies from each strain were tested for immunity against superinfection with a clear

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TABLE 1. Bacterial strains and plasmid used in this study

| Strain or plasmid | Relevant genotype | Source or reference |
|---|---|---|
| *E. coli* | | |
| MC4100 | $F^-$ *lacU169 relA rpsL thi araD139* | M. J. Casadaban (2) |
| MM52 | MC4100 *secA52*(Ts) | J. Beckwith (14) |
| MM113 | MC4100 *secA*(Am) *supF*(Ts) *zci*::Tn*10* *trp*(Am) *malT*$^c$ | J. Beckwith (10) |
| SFN194 | MC4100 *malT*$^c$ *zhe*::Tn*10* | J. Beckwith |
| MM151 | MC4100 *malT*$^c$ *zhe*::Tn*10* *secB7* | J. Beckwith (9) |
| MM152 | MC4100 *malT*$^c$ *zhe*::Tn*10* *secB*::Tn*5* | J. Beckwith (9) |
| SFN194W | MC4100 *malT*$^c$ *lpp*::Tn*10* | This study |
| MM151W | MC4100 *malT*$^c$ *secB7* *lpp*::Tn*10* | This study |
| WR19-1 | *Hfr*C *gpsA glpD glpR glpK phoA fadE rel-1 ton*A *lpp*::Tn*10* | V. Braun (17) |
| MM123 | MC4100 *cs-2124* | J. Beckwith (3) |
| CG-1 | MC1000 *phoR secD1* | J. Beckwith (4) |
| CG-2 | MC1000 *phoR* | J. Beckwith (4) |
| Plasmid pHF100 | Amp$^r$ *ompF-lpp* chimeric gene | S. Mizushima (21) |

mutant of phage λ, and λDO20 lysogens of strain MM52 and MM113 were obtained.

**Construction of strains carrying *lpp*::Tn*10*.** To eliminate the wild-type lipoprotein structural gene from the *secB* strain and its parental wild-type strain, strains SFN194W and MM151W carrying *lpp*::Tn*10* were obtained by transduction. The *zhe*::Tn*10* in SFN194 and MM151 were first cured as described by Bochner et al. (1). *lpp*::Tn*10* was then introduced into these cured strains with a P1 lysate derived from strain WR19-1, which contained *lpp*::Tn*10*. Tet$^r$ colonies were analyzed by the Ouchterlony double diffusion test with antiserum against purified lipoprotein and were found to lack lipoprotein. The *lpp*::Tn*10* derivatives of strains SFN194 and MM151 were transformed with plasmid pHF100 (which contained an *ompF-lpp* hybrid gene and an *amp* gene), and Tet$^r$ Amp$^r$ colonies were isolated. These strains were designated SFN194W(pHF100) and MM151W(pHF100), respectively.

**Labeling experiments.** Pulse-labeling was used to study lipoprotein export in various *sec* mutants grown at permissive and nonpermissive temperatures. For *secA*(Ts), *secA* (Am), and *secC* mutants, the overnight cultures grown at the permissive temperature were inoculated into fresh M9-glycerol-maltose medium, and the incubation was continued at the permissive temperature. At the mid-logarithmic phase of growth ($A_{600}$, 0.4 to 0.5), the cultures were shifted to the nonpermissive temperature; at regular time intervals, 2-ml portions of cultures were withdrawn and added to prewarmed tubes containing 40 μCi of [$^{35}$S]methionine. After 2 min of labeling with vigorous shaking, trichloroacetic acid (final concentration, 10%) was added and the tubes were immediately placed in an ice bath.

For the *secD* mutant, labeling was carried out in M9-glycerol-maltose medium for 2 min at both permissive and nonpermissive temperatures with [$^{35}$S]methionine. For *secB* mutants, 2 min of labeling with [$^{35}$S]methionine was used to study lipoprotein export, and 30 s of labeling was used to study the export of the OmpF-Lpp hybrid protein. The trichloroacetic acid precipitates were collected by centrifugation, washed with acetone, and solubilized with 1% sodium dodecyl sulfate (SDS) in 50 mM Tris hydrochloride (pH 8) containing 1 mM EDTA. After this solution was heated at 100°C for 5 min, portions were immunoprecipitated (7) with antisera against lipoprotein, OmpA, MBP, or OmpF. [$^{35}$S] methionine-labeled immune precipitates were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) for lipoprotein (6) and for other proteins (11). The experimental details regarding incubation temperatures and the length of incubation at the nonpermissive temperature are described in the legends to the figures.

**Chemicals.** [$^{35}$S]Methionine (specific activity, 300 Ci/mmol) was purchased from Dupont, NEN Research Products (Boston, Mass). Fixed *Staphylococcus aureus* cells were purchased from Calbiochem-Behring (La Jolla, Calif.).

## RESULTS

**Export of lipoprotein in a *secA*(Am) mutant.** It has been shown previously that a *secA*(Ts) mutant accumulates unmodified prolipoprotein as well as precursors of a number of other exported proteins in the cytoplasmic membrane at the nonpermissive temperature (5, 12). In this study we compared the export of lipoprotein in a *secA*(Am) mutant (16) with that in the wild-type and a *secA*(Ts) mutant. When this *secA*(Am) mutant strain is grown at 42°C, the level of SecA protein is severely reduced, and this reduction in SecA protein results in the failure to synthesize certain exported proteins, such as MBP (16). Significant amounts of pre-MBP and pre-OmpA were detected in the *secA*(Ts) mutant even at the permissive temperature, but no prolipoprotein was detected (Fig. 1, part 1). This result is in agreement with the previous finding that about 10% of pre-OmpA, but no prolipoprotein, accumulated at 30°C. No accumulation of precursor forms of MBP and OmpA was seen in the wild-type strain. These data suggest that the mutant SecA(Ts) protein is not fully active even at the permissive temperature. Accumulation of prolipoprotein, pre-MBP, and pre-OmpA was observed 2 h after a shift to the nonpermissive temperature, as reported previously (5, 12).

In contrast to the results obtained with the *secA*(Ts) mutant, there was significant accumulation of prolipoprotein, as well as pre-MBP and pre-OmpA, in the *secA*(Am) mutant at the permissive temperature (Fig. 1, part 3). Upon a shift to the nonpermissive temperature, lesser accumulation of prolipoprotein and pre-OmpA was observed with increasing time of incubation at this temperature (Fig. 1, part 3). For MBP, an increase in the time of incubation at the nonpermissive temperature resulted in a drastically reduced synthesis of MBP. On the other hand, the defect in the export of MBP, which was manifested by the accumulation of pre-MBP at the permissive temperature, was alleviated after the temperature shift, as was found for lipoprotein and OmpA. These results suggest that there is a general defect in the export of lipoprotein, MBP, and OmpA in the *secA*(Am)

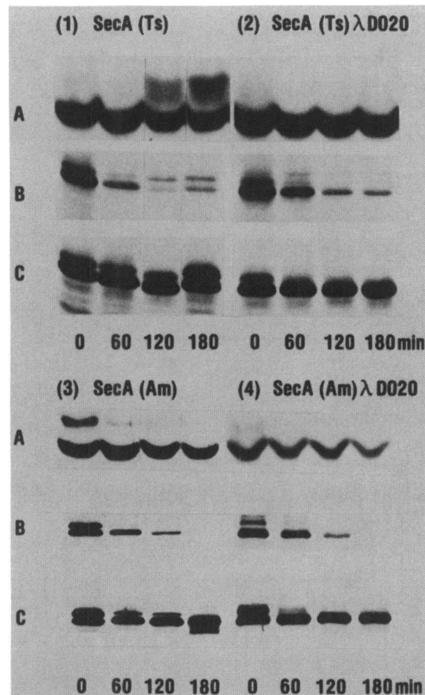


FIG. 1. Effect of the wild-type *secA* gene on the synthesis or export of lipoprotein, OmpA, and MBP in *secA*(Ts) and *secA*(Am) mutants of *E. coli*. MM52 [*secA*(Ts)], MM52(λDO20), MM113 [*secA* (Am)], and MM113(λDO20) cells were grown in M9-glycerol-maltose medium at 30°C and were then shifted to 42°C at the mid-logarithmic phase of growth. Portions (2 ml) were taken from the culture at the time intervals indicated in the figure and were labeled for 2 min with 40 μCi of [$^{35}$S]methionine. Lipoprotein, MBP, and OmpA were immunoprecipitated with the specific antisera, and the immune precipitates were analyzed by SDS-PAGE, as described in the text. 1, MM52 [*secA*(Ts)]; 2, MM52(λDO20); 3, MM113 [*secA* (Am)]; and 4, MM113(λDO20). (A) Lipoprotein; (B) MBP; (C) OmpA protein.

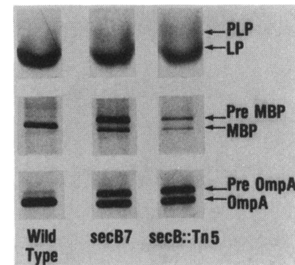


FIG. 2. Lipoprotein export in *secB* mutants. The wild-type strain SFN194, the *secB7* mutant MM151, and the *secB* knockout mutant MM152 (*secB*::Tn*5*) were grown in M9-glycerol-maltose medium at 30°C. At the mid-logarithmic phase of growth, cells were labeled for 2 min with 40 μCi of [$^{35}$S]methionine at 30°C. Lipoprotein, MBP, and OmpA protein were analyzed by SDS-PAGE following immunoprecipitation with specific antisera. Abbreviations: PLP, prolipoprotein; LP, lipoprotein; Pre-MBP, pre-maltose-binding protein; MBP, maltose-binding protein; Pre-OmpA, pre-outer membrane protein A; OmpA, outer membrane protein A.

mutant at the permissive temperature. At the nonpermissive temperature, the amber mutation resulted in the decreased synthesis of the inducible MBP without affecting the synthesis of such constitutive proteins as lipoprotein and OmpA. The apparent defect in the export of lipoprotein, MBP, and OmpA seen at the permissive temperature was actually alleviated at the nonpermissive temperature.

**Effect of wild-type *secA* gene on the lipoprotein export in *secA*(Ts) and *secA*(Am) mutants.** Prolipoprotein accumulated in the *secA*(Ts) mutant at the nonpermissive temperature and in the *secA*(Am) mutant at the permissive temperature but not at the nonpermissive temperature. These results led us to consider the possibility that the SecA protein may not be required for lipoprotein export. The apparent defect in lipoprotein export in *secA*(Ts) and *secA*(Am) mutants may be due to an interference of lipoprotein export by the presence of a structurally altered SecA protein found in the *secA*(Ts) mutant at the nonpermissive temperature and in the *secA*(Am) mutant at the permissive temperature. In the latter case, reduction in the synthesis of the SecA(Am) protein at the nonpermissive temperature might result in the restoration of the export of lipoprotein and OmpA.

To test this hypothesis, we examined the effect of the wild-type *secA* gene on lipoprotein export in both *secA*(Ts) and *secA*(Am) mutants. The defect in lipoprotein export in the *secA*(Ts) mutant, which was manifested as the accumulation of prolipoprotein, was complemented by the wild-type *secA* gene on the λDO20 phage (Fig. 1, part 2). Likewise, the defect in the export of OmpA and MBP in the *secA*(Ts) mutant was also complemented by the wild-type *secA* gene. The effect of the wild-type *secA* gene on the export defect of lipoprotein, OmpA, and MBP in the *secA*(Am) mutant is shown in Fig. 1, part 4. While significant amounts of precursors of all three exported proteins were present in the *secA*(Am) mutant at the permissive temperature (Fig. 1, part 3), the level of accumulation of prolipoprotein was significantly reduced in the *secA*(Am) mutant carrying the λDO20 phage (Fig. 1, part 4). Upon a shift to the nonpermissive temperature, the export defect was restored at an earlier time in the *secA*(Am) (λDO20) lysogen than in the mutant without the cloned wild-type *secA* gene. The apparent complementation of the lipoprotein export defect in both *secA* (Ts) and *secA*(Am) mutants by the wild-type *secA* gene suggests that the SecA protein is required for lipoprotein export. If interference by a structurally abnormal SecA protein was based on prolipoprotein accumulation in the *secA*(Ts) or *secA*(Am) mutants, one might have expected a lack of complementation by the wild-type *secA* gene. While the presence of the wild-type *secA* gene restored the export of lipoprotein, MBP, and OmpA in *secA*(Ts) or *secA*(Am) mutants, it did not restore the synthesis of MBP in the *secA* (Am) mutant at the nonpermissive temperature. These results suggest that the block in the synthesis of MBP is not directly related to the export defect in this mutant.

**Export of lipoprotein in *secB* mutants.** The *secB* mutants exhibited a low-level secretion defect and accumulated precursors of certain exported proteins (MBP and OmpF) (8). We have reported that one of the *secB* mutants, strain *secB7*, is not affected in lipoprotein export but is slightly affected in the export of OmpA protein (5). A knockout mutation in *secB* has been obtained, and this mutant is viable, even though it cannot grow in enriched medium (L broth) (9). We reexamined lipoprotein export in this *secB* null mutant. Strain SFN194 (wild type) and strains MM152 (*secB*::Tn*5*) and MM151 (*secB7*) were labeled for 2 min with [$^{35}$S]-methionine. There was no accumulation of prolipoprotein in either the *secB*::Tn*5* mutant or the *secB7* mutant (Fig. 2), although significant amounts of pre-MBP and pre-OmpA were detected in both mutants.

To investigate further the divergence of secretory path-

**Prolipoprotein**

−20 −1 +1 +8 +58

Met Lys-----Ala Gly Cys Ser-----Lys Ile Asp Gln-----Arg Lys

Processing site

**Hybrid Protein**

−22 −1 +1 +11 +12 (+8) +62 (+58)

Met Met-----Asn Ala Ala Glu-----Lys Val Asp Gln-----Arg Lys

OmpF Fusion site Lpp

**Pre OmpF Protein**

−22 −1 +1 +12 +362

Met Met-----Asn Ala Ala Glu-----Lys Val Asp Leu-----Gln Phe

FIG. 3. Structure of OmpF-Lpp hybrid protein. The hybrid protein encoded by the *ompF-lpp* gene fusion on plasmid pHF100 contained the intact signal sequence of pre-OmpF protein plus 11 amino acid residues of the mature OmpF protein, followed by 51 amino acid residues (+8 to +58) of the mature lipoprotein. Numbers with minus signs indicate the positions of the amino acids in the OmpF signal sequence, and numbers with plus signs indicate those in the mature protein. The processing site in the hybrid protein is presumably the same as that in the wild-type pre-OmpF protein (21).

ways, as defined by the requirement of the SecB protein, we have studied the effect of the *secB* mutations on the export of a hybrid protein which comprises the signal sequence of OmpF plus 11 amino acid residues from the mature portion of OmpF and the COOH-terminal 51 amino acid residues from the mature form of the lipoprotein (21). The detailed amino acid sequence around the fusion junction is shown in Fig. 3. This hybrid protein contains an authentic processing site for signal peptidase I (Ala-X-Ala-) of the OmpF protein, but it does not contain the prolipoprotein modification and processing site (Leu-Ala-Gly-Cys-). The molecular weight of the processed form of this hybrid protein is similar to that of lipoprotein, and they migrate to the same position in a SDS-polyacrylamide gel. In order to facilitate the analysis of the hybrid protein in the *secB* mutant, we constructed derivatives of the wild-type and *secB7* mutant strains in which the *lpp* gene was inactivated by the insertion of Tn*10*. Plasmid pHF100 carrying the hybrid *ompF-lpp* gene was then transformed into these strains as described above. The effect of the *secB* mutation on the export of this hybrid protein was studied by pulse-labeling at 30°C. The export of the OmpF-Lpp hybrid protein was affected by the *secB7* mutation, resulting in the accumulation of a significant amount of the precursor of the hybrid protein (Fig. 4B). The export of OmpA and OmpF was strongly affected by the *secB7* mutation (Fig. 4C), while the export of lipoprotein was not affected (Fig. 4A). These results indicate that the SecB protein directly or indirectly interacts with the signal sequence plus 11 amino acid residues of the mature OmpF that is present in the precursor of the Omp-Lpp hybrid protein.

**Synthesis of lipoprotein in the *secC* mutant.** It has been reported previously that a mutation in the *secC* gene affects the synthesis of a number of exported proteins such as MBP, LamB, ribose-binding protein, and OmpF; but it does not affect the synthesis of nonexported proteins such as elongation factor G and SecA at the nonpermissive temperature (23°C) (3). At the nonpermissive temperature (23°C), the relative incorporation of [$^{35}$S]methionine into periplasmic proteins and outer membrane proteins was reduced to about 40% of that at 37°C in the *secC* mutant, whereas it was unchanged in the *secC*$^+$ strain. Moreover, the incorporation of [$^{35}$S]methionine into soluble proteins and inner membrane proteins was not affected in the *secC* mutant at the nonpermissive temperature (data not shown).

The effect of the *secC* mutation on the synthesis of lipoprotein is shown in Fig. 5. While the synthesis of MBP was completely curtailed 1 h after it was shifted to the nonpermissive temperature, the synthesis of OmpA was not affected. Lipoprotein synthesis was significantly affected by

(A) (B) (C)

PLP LP

Pre OmpF-Lpp Mature OmpF-Lpp

Pre OmpF OmpF PreOmpA OmpA

secB+ secB− secB+ secB− secB+ secB−

FIG. 4. Export of OmpF-Lpp hybrid protein in wild-type and *secB* mutant cells. Strains SFN194 (wild-type), MM151 (*secB7* mutant), and SFN194W(pHF100) and MM151W(pHF100) (isogenic strains of SFN194 and MM151, except that they lacked lipoprotein because of *lpp*::Tn*10* and carried the plasmid encoding the OmpF-Lpp hybrid protein) were used to study the effect of the *secB* mutation on the export of the OmpF-Lpp hybrid protein. Each strain was grown in M9-glycerol-maltose medium at 30°C. At the mid-logarithmic phase of growth, cells were pulse-labeled for 30 s with 40 μCi of [$^{35}$S]methionine. Lipoprotein and the OmpF-Lpp hybrid protein were immunoprecipitated with antiserum against purified lipoprotein. OmpF and OmpA proteins were immunoprecipitated with the mixed serum of anti-OmpF and anti-OmpA. The proteins were analyzed by SDS-PAGE after immunoprecipitation. (A) Lipoprotein (LP), SFN194 (*secB*$^+$), and MM151 (*secB*); (B) OmpF-Lpp hybrid protein, SFN194W(pHF100) (*secB*$^+$), and MM151W(pHF 100) (*secB*); (C) OmpF and OmpA proteins, SFN194W(pHF100) (*secB*$^+$), and MM151W(pHF100) (*secB*). PLP, Prolipoprotein

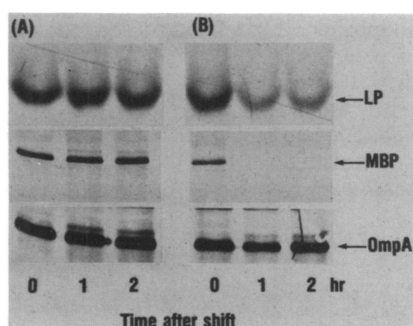


FIG. 5. Reduced synthesis of lipoprotein (LP) in the *secC* mutant. Strain MC4100 (wild type) and strain MM123 (*secC*) were grown in M9-glycerol-maltose medium at 37°C. At the mid-logarithmic phase of growth, the cultures were shifted to 23°C. Portions were taken at the time intervals indicated in the figure and were labeled with 40 μCi of [$^{35}$S]methionine for 2 min at 23°C. Lipoprotein, OmpA, and MBP were analyzed by SDS-PAGE following immunoprecipitation. (A) MC4100; (B) MM123.

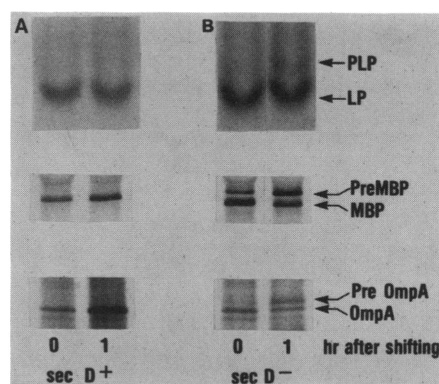


FIG. 6. Defective lipoprotein (LP) export in a *secD* mutant. Strains CG-2 (wild type) and CG-1 (*secD*) were grown in M9-glycerol-maltose medium at 37°C. At the mid-logarithmic phase of growth, cells were shifted to 23°C and were labeled for 2 min with 50 μCi of [$^{35}$S]methionine at time zero and 1 h after the shift. OmpA, MBP, and lipoprotein were analyzed by SDS-PAGE following immunoprecipitation. (A) CG-2 (wild type); (B) CG-1 (*secD*). PLP, Prolipoprotein.

the *secC* mutation, albeit not as severely as that of MBP (Fig. 5B). In the wild-type strain, there was no change in the synthesis of MBP, OmpA, and lipoprotein on a shift to 23°C (Fig. 5A). These results indicate the selective nature of the *secC* mutation in affecting the synthesis of exported proteins; it affects the synthesis of the constitutively expressed lipoprotein as well as the inducible MBP, albeit to different extents, but has little effect on the synthesis of OmpA.

**Export of lipoprotein in the *secD* mutation.** The *secD* mutant is cold sensitive for growth and accumulates precursor forms of several exported proteins such as alkaline phosphatase, MBP, OmpF, and ribose-binding protein at the nonpermissive temperature (23°C) (4). We studied the effect of the *secD* mutation on the lipoprotein export. The export of OmpA and MBP was affected in the *secD* mutant at the nonpermissive temperatures (Fig. 6), resulting in the accumulation of the precursor forms. The export of lipoprotein was also affected by the *secD* mutation, albeit to a lesser extent.

## DISCUSSION

In the present study we investigated the export of lipoprotein in various *E. coli* mutants that were defective in protein export in general. Lipoprotein export differs from the export of other precursor proteins in that it is processed by a distinct signal peptidase, prolipoprotein signal peptidase, or SPase II. The processing of prolipoprotein is preceded by a series of modification reactions which convert the unmodified prolipoprotein to glyceride-modified prolipoprotein. Inasmuch as modification and processing of prolipoprotein represent late events in the export of lipoprotein to the outer membrane, it is reasonable to assume that the early steps in the export process are shared by precursors of lipoproteins and nonlipoproteins. Based on results of this study, together with earlier findings that the export of lipoprotein is affected in *secA* and *secY* mutants, we conclude that three of the four genes required for protein export in general are also essential for the export of lipoprotein in *E. coli*, i.e., *secA*, *secD*, and *secY*.

The requirement of a functional SecB gene product divides the exported proteins into two groups: *secB*-dependent and *secB*-independent proteins. Lipoprotein appears to belong to the latter group. A functional SecB protein is required, however, for the optimal export of a hybrid protein consisting of the signal sequence plus 11 amino acid residues of OmpF and the mature protein of lipoprotein. This hybrid protein is presumably processed by SPase I due to the absence of the modification and processing site unique to prolipoprotein (21). The requirement of the SecB protein for the export of this hybrid protein cannot be attributed to a change in the specificity of the processing enzyme from SPase II to SPase I, since precursors of other such *secB*-independent proteins as ribose-binding protein and alkaline phosphatase are also processed by SPase I. Our results suggest that the SecB protein interacts directly or indirectly with the precursor of the OmpF-Lpp hybrid protein, presumably via the signal sequence and the 11 amino acid residues of OmpF. Since the defect in the export of the hybrid protein is quantitatively much less than that of OmpF, the putative interaction of the SecB protein with the precursors of the exported proteins must be dependent on the overall structure and conformation of the precursor protein rather than on that of the signal sequence of the precursor protein alone.

We also showed that the synthesis of lipoprotein, which is constitutive rather than inducible, is also affected by the mutation in the *secC* gene, as was found for the synthesis of an inducible exported protein, MBP. On the other hand, synthesis of OmpA was not affected by the *secC* mutation. Thus, the apparent requirement of a functional SecC protein further divides the exported proteins into two subsets: *secC*-dependent and *secC*-independent proteins. The role of the SecC protein (ribosomal protein S15) in the synthesis of exported proteins or in the coupling of protein synthesis with protein export remains unclear.

The notion that the synthesis of an exported protein is somehow coupled to its export is not only implicit in the revised model of signal hypothesis vis à vis the signal recognition particle in the eucaryotic cell but it is also suggested by results of earlier studies on the synthesis of MBP in *secA*(Am) mutants of *E. coli* (16). Results of our studies on the synthesis and export of lipoprotein, OmpA, and MBP in *secA*(Ts) and *secA*(Am) mutants revealed a rather complicated picture. Our results suggest that a functional SecA protein is essential for the export of all three

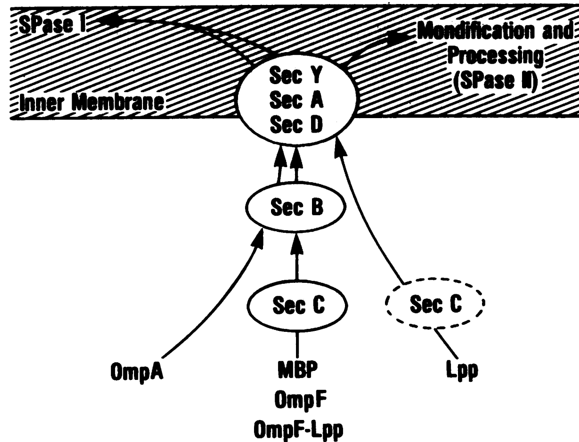


FIG. 7. A common export pathway for prolipoprotein and nonlipoprotein precursors in *E. coli*. Optimal synthesis of prolipoprotein appears to require a functional SecC protein. Export of prolipoprotein requires functional SecA, SecD, and SecY proteins; but it does not require a functional SecB protein.

proteins. This conclusion is based on the finding that precursors of MBP, OmpA, and lipoprotein accumulated in the *secA*(Ts) mutant at the nonpermissive temperature and in the *secA*(Am) mutant at the permissive temperature. Similar observations have been reported by Liss and Oliver (12). The unexpected finding in this study was the decrease in the accumulation of all three precursor proteins in the *secA*(Am) mutant at the nonpermissive temperature. This observation is consistent with the notion that the export of lipoprotein and OmpA requires lesser amounts of the functional SecA protein than other exported proteins. It has been noted previously that the *secA*(Ts) mutation does not affect the synthesis of MBP compared with the *secA*(Am) allele (12, 14, 16). It has been suggested that these two *secA* mutations may only differ quantitatively, with the *secA*(Am) mutation being a tighter one than the *secA*(Ts) mutation (19). Our results on the defective export of lipoprotein and OmpA in *secA*(Ts) and *secA*(Am) mutants do not support this interpretation. The different phenotypes of the *secA*(Ts) and *secA*(Am) mutants remain to be explained.

While the defect in the export of lipoprotein, MBP, and OmpA in the *secA*(Am) mutant was complemented by the wild-type *secA* gene, the defect in the synthesis of MBP in the *secA*(Am) mutant at the nonpermissive temperature was not restored in the lysogen containing λDO20. Results of previous studies (19) have led to the conclusion that the inhibition of MBP synthesis in the *secA*(Am) mutant at the nonpermissive temperature is a secondary consequence of the primary export defect, since the addition of cyclic AMP reverses the synthesis defect without affecting the export defect of MBP in the *secA*(Am) mutant. Our data show that in the presence of the wild-type *secA* allele, the synthesis block remains while the export defect is restored. Thus, the reduction in the synthesis of MBP in the putative *secA*(Am) mutant is not a consequence of the export defect in this mutant; rather, it may be a consequence of a defect in a neighboring gene adjacent to the *secA* gene, the expression of which is reduced by the amber mutation because of a polarity effect. Further work is needed to clarify this paradox.

Figure 7 summarizes our current understanding of the export of lipoproteins, as defined by the requirements of the gene products encoded by *secA*, *secB*, *secC*, and *secD* compared with the requirements of such nonlipoproteins as OmpA, OmpF, and MBP. It is clear that further work in which both genetic and biochemical approaches are used will be needed to elucidate further the export pathway of proteins in *E. coli*.

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