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The First Gene in the *Escherichia coli secA* Operon, Gene X, Encodes a Nonessential Secretory Protein

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Tn*phoA* insertions in the first gene of the *Escherichia coli secA* operon, gene X, were isolated and analyzed. Studies of the Gene X-PhoA fusion proteins showed that gene X encodes a secretory protein, since the fusion proteins possessed normal alkaline phosphatase activity and a substantial portion of this activity was found in the periplasm. In addition, the Gene X-PhoA fusion proteins were initially synthesized with a cleavable signal peptide. A gene X::Tn*phoA* insertion was used to construct a strain containing a disrupted chromosomal copy of gene X. Analysis of this strain indicated that gene X is nonessential for cell growth and viability and does not appear to play an essential role in the process of protein export.

In order to elucidate the molecular mechanisms of protein export in *Escherichia coli*, genetic selections have been used to isolate mutants which are in this process. One of the earliest mutants isolated defined a new essential gene, *secA*, which encodes a 102-kDa peripheral membrane protein which has been shown to be required both in vivo and in vitro to promote precursor protein translocation across the plasma membrane (5, 17, 18). Molecular cloning and sequence analysis of the *secA* gene revealed that it appears to be the second gene in a three-gene operon, flanked upstream by a gene predicted to encode a 147-amino-acid-residue polypeptide of unknown function, termed gene X, and downstream by *mutT*, a gene involved in DNA replication fidelity (1, 28). The existence of gene X had been suggested originally by molecular genetic analysis and transcriptional mapping studies of this region (19, 30). That gene X and *secA* are cotranscribed is supported by the facts that gene X sequences are needed for proper *secA* expression (22) and that gene X insertion or nonsense mutations are strongly polar on *secA* expression (8, 28). Analysis of *mutT-lacZ* fusions indicates that gene X and *secA* sequences are also required for proper *mutT* expression (26).

Expression of the *secA* gene is coordinated with the protein secretion capability of the cell, since the level of SecA protein synthesis increased more than 10-fold when protein export was blocked by genetic or physiological means (20, 23). This regulation has been shown to operate at the translational level, with SecA protein repressing its own translation during conditions of normal protein export (27). More recently, we have shown that the regulated site, the secretion response element, is located near the end of gene X and the beginning of *secA* and that SecA protein bound its mRNA only if the secretion response element was genetically intact (6, 26). These studies suggest that gene X sequences play important roles in the *cis* configuration in promoting SecA protein-mRNA binding and in ribosome delivery to *secA*. However, the studies do not address the question of whether Gene X protein plays a role (aside from its mere translation) in *secA* regulation or in the protein export process. To begin to address these questions, we describe the isolation of Tn*phoA* insertion mutations in gene X, which revealed that Gene X is a secretory protein. The construction of a strain containing a disrupted copy of gene X allowed us to determine whether this gene is essential for cell growth and protein export.

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MATERIALS AND METHODS

Media and reagents. Media used for the growth of bacterial cultures have been described previously (14). The following antibiotics and indicators at the concentrations given were used in solid media: ampicillin, 100 μg/ml; kanamycin, 30 μg/ml; tetracycline, 20 μg/ml; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 20 μg/ml; and 5-bromo-4-chloro-3-indolyl-phosphate (X-P), 20 μg/ml. DNA restriction and modifying enzymes were obtained from New England Biolabs, Inc. (Beverly, Mass.). All enzymes were used as recommended by the supplier. Radiochemicals were purchased as follows: [γ-^{32}P]ATP (4,500 Ci/mmol) and [α-^{32}P]dATP (600 to 800 Ci/mmol) from ICN Radiochemicals (Irvine, Calif.) and Tran-^{35}S-label (~1,100 Ci/mmol) from Amersham Corp. (Arlington Heights, Ill.). IgSorb was purchased from the New England Enzyme Center (Boston, Mass.). The Sequenase DNA sequencing kit was purchased from United States Biochemical Corp. (Rockville, Md.). pZ523 columns were purchased from 5′→3′, Inc. (West Chester, Pa.), and Geneclean kits were purchased from Bio 101, Inc. (La Jolla, Calif.). M13mp18 and M13mp19 replicative-form DNAs were purchased from New England Biolabs, Inc. The *phoA* primers used for sequence analysis of the Tn*phoA* fusion joints, CAGAACAGGGCAAAACGG and CGCTAAGAGAATCACGC, were synthesized on an Applied Biosystems (Foster City, Calif.) 380B DNA synthesizer. Nitrocellulose was obtained from Schleicher & Schuell (Keene, N.H.). All other reagents used were standard laboratory grade.

Strains and plasmids. MC4100 (F$^-$ Δ*lacU169 araD136 relA rspL thi*) and four derivatives, MM171 [Φ(*secA-lacZ*)*f181* (Hyb) *leu*::Tn*5*(λPR9)], MM171.2 [Φ(*secA-lacZ*)*f181*(Hyb) (λPR9)], BA13.1 [*secA13*(Am) *trp*(Am) *supF*(Ts) *recA1 srl*::Tn*10*], and DO1151.100 [*secA51*(Ts) *srl*::Tn*10*], have been described previously (10, 22). CC118 [F$^-$ Δ(*leu-ara*)*7697 araD139* Δ*lacX74 phoA*Δ*20 galE galK rpsE rpsL rpoB argE* (Am) *recA1 thi*], used as a host for Tn*phoA* mutagenesis, has been described previously (13). XPh43 [Δ*lacU169* Δ(*brnQ phoA proC aroL aroM phoB phoR*)*24 trp rpsL267 crp-72 thi*] and BW360 (*polA1 zig*::Tn*10*) were used in the construction

of a gene X null strain and were obtained from B. Wanner and W. Wickner, respectively. MGS68 (*recA1 srl*::Tn*10 secA22*) is a derivative of MM18 [Φ(*malE-lacZ*)72-*47*(Hyb)] (9) which contains a mutation in the *secA* gene that alters the electrophoretic mobility but not the translocation activity of the gene product. BL21(λDE3), containing the bacteriophage T7 RNA polymerase gene under the control of the *lac* promoter, and its derivative, BL21.10 (*leu*::Tn*10 azi-4*), have been described previously (20, 29). A pBR322 derivative plasmid, pMF8, containing the gene X-*secA* operon has been described previously (27). Plasmid pT7secA2 is a derivative of pT7secA (27), which was constructed as follows: a 600-bp *Eco*RI-*Bam*HI fragment containing the bacteriophage fl DNA packaging sequence was isolated from pUC-fl (Pharmacia, Piscataway, N.J.) and inserted into the T7 expression vector, pET-5 (24), that had been cleaved with *Eco*RI and *Bam*HI and gave rise to plasmid pCO1; a 1.88-kb *Bam*HI-*Kpn*I fragment from pW2 (27) and a 1.15-kb *Kpn*I-*Bam*HI fragment from pAW13 (a derivative of pMF8 containing a *Bam*HI linker at the *Mlu*I site in *mutT*), which together compose the end of gene X, the entire *secA* gene, and the beginning of the *mutT* gene, were inserted into pCO1 that had been cleaved with *Bam*HI to give rise to pT7secA2.

Isolation of gene X-*phoA* fusions. CC118(pMF8) was subjected to Tn*phoA* mutagenesis (13) with λTn*phoA* as described previously (25). In this procedure, nine separate cultures were infected with the phage, >5,000 kanamycin-resistant transductants per culture were pooled, and plasmid DNAs were prepared. The separate plasmid DNAs were used to transform CC118 to kanamycin resistance, and blue colonies arising at a 7% frequency were isolated and purified on TYE Kan XP plates. Approximately 50% of these isolates were resistant to ampicillin and were studied further. Plasmid DNAs were prepared from 19 ampicillin-resistant isolates and used to transform *secA13*(Am) and *secA51*(Ts) mutants at 30°C, and after purification these transformants were tested for growth at 42°C. Four plasmids, pCB2, pCB9, pCB12, and pCB19, that showed poor or no complementation of these strains were found and were studied further. Restriction enzyme analysis with *Eco*RI and *Nco*I was used to determine the approximate position of the Tn*phoA* element in these plasmids. The precise position of the *phoA* fusion joint was determined by DNA sequence analysis. For this purpose, 3.52-, 3.73-, 3.49-, and 3.51-kb *Hpa*I-*Bgl*II fragments containing the fusion joints were isolated from pCB2, pCB9, pCB12, and pCB19, respectively, and cloned into M13mp19 that had been cleaved with *Hinc*II and *Bam*HI. Each M13 transducing phage was subjected to DNA sequence analysis with oligonucleotides complementary to the early region of the *phoA* gene as described previously (28).

Construction of a gene X disruption. In order to integrate pCB19 into the gene X-*secA* locus, a P1*vir* lysate was grown on BW360 and used to transduce XPh43(pCB19) to tetracycline resistance on TYE plates containing ampicillin and tetracycline, and *polA* transductants were identified by screening colonies for sensitivity to methyl methanesulfonate. A P1*vir* lysate was prepared from one such strain and used to transduce MM171.2 to kanamycin resistance on TYE X-Gal plates containing kanamycin, and colorless colonies which had lost the *secA-lacZ* fusion were isolated. After two rounds of purification on TYE X-P plates containing kanamycin, a stable transductant, DO322 [gene X::Tn*phoACB19*(λPR9)], that gave a light blue color on these plates but was no longer ampicillin resistant, indicating the retention of the gene X::Tn*phoA* insertion but the loss of the other plasmid sequences, was found. A P1*vir* lysate prepared on DO322 was used to transduce BL21.10 (pT7secA2) to kanamycin resistance to give rise to BL21.16 [gene X::Tn*phoACB19*(λDE3) (pT7secA2)]. An isogenic strain, BL21.17 [*leu*::Tn*5* Φ(*secA-lacZ*)*f181*(Hyb) (λDE3) (pT7secA2)], was constructed by using a P1*vir* lysate grown on MM171 and transducing BL21.10(pT7secA2) to kanamycin resistance and scoring for the presence of the *secA-lacZ* fusion on media containing X-Gal. The gene X disruption in BL21.16 was confirmed by Southern blot analysis as described by Maniatis et al. (12) with the following modifications: an oligonucleotide to gene X nucleotides 433 to 453 (28) was kinased with [γ-^{32}P]ATP, mixed with the nitrocellulose filter immersed in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt's solution–0.05% sodium dodecyl sulfate (SDS), and incubated at 37°C for 36 h with gentle shaking; the nitrocellulose filter was removed, washed in 2× SSC at room temperature for 10 min with gentle shaking four consecutive times, dried, and used to expose X-ray film with screens at −80°C.

RESULTS

Isolation of gene X-*phoA* fusions. It has been shown previously that SecA protein binds to liposomes composed of anionic phospholipids (11). We have found recently that a fraction of the cellular SecA protein is associated with the plasma membrane in an integral fashion and that purified SecA protein can insert itself into liposomes composed of anionic phospholipids (21, 31). While attempting to use the Tn*phoA* genetic system (13) to identify a transmembrane segment of SecA protein that may exist in a dynamic fashion, we obtained Tn*phoA* insertions in gene X that identify its gene product as an envelope protein. The existence of the gene X-*phoA* fusions allowed us to do a more detailed study of gene X function, which we describe in this report.

Plasmid pMF8, containing the gene X-*secA* operon, was subjected to Tn*phoA* mutagenesis and screened for derivatives which showed defects in complementation of *secA* (Am) or *secA*(Ts) mutants (see Materials and Methods for details). Four isolates were obtained, and the positions of the fusion joints were determined by restriction enzyme mapping and DNA sequencing. Figure 1 shows that three of the fusions were located in gene X, while one was located early in the *secA* gene (after amino acid residue 34). The fact that Tn*phoA* insertion mutations in gene X displayed poor *secA* complementation activity is consistent with the polarity seen previously in this operon (8, 28). Alkaline phosphatase activity assays (4) of CC118 containing pCB9, pCB12, and pCB19 gave 115, 139, and 122 U of activity, respectively. However, it was unclear whether these levels of enzymatic activity corresponded to efficient secretion of a lower level of fusion protein or inefficient secretion of a higher level of fusion protein, most of which would remain inactive. Therefore, we estimated the specific activities of these fusion proteins by comparing their levels of synthesis with their activities, using wild-type alkaline phosphatase as a standard. These results are shown in Fig. 2. First, it is apparent that the mobilities of the Gene X-PhoA fusion proteins correspond well to their predicted molecular weights. Second, the specific activities of these fusion proteins were similar to those of wild-type alkaline phosphatase, since similar units of enzymatic activity yielded protein bands of similar intensity. While there was some proteolysis of the fusion proteins that was detected during steady-state growth

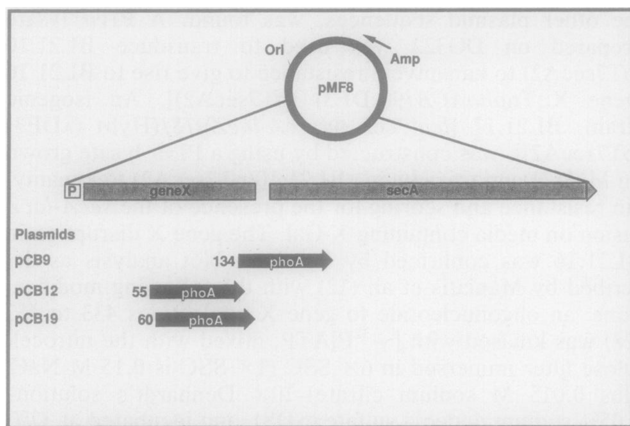

FIG. 1. Gene X-*phoA* fusions isolated. Plasmid pMF8, containing a promoter (P), gene X, *secA*, and the proximal portion of *mutT*, is shown in the upper portion of the figure. The plasmids used in this study are indicated below the plasmid, along with the amino acid residue of gene X immediately before *phoA*. Ori, origin of replication; Amp, ampicillin resistance.

conditions (data not shown), these results indicate that the export signal(s) located in gene X must be efficient in allowing most of the alkaline phosphatase moieties to achieve a location outside of the plasma membrane which is required for normal enzymatic activity.

To determine in which of the three envelope layers, the inner membrane, outer membrane, or periplasmic space, the

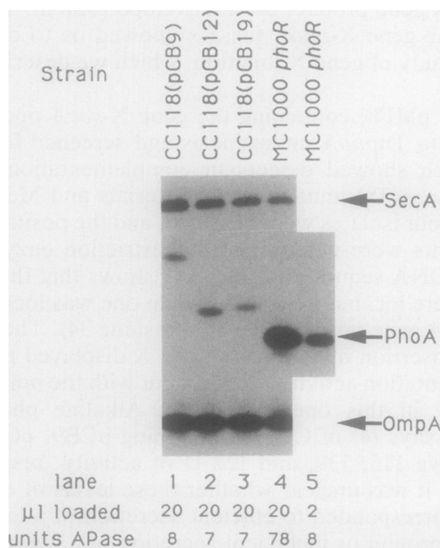

FIG. 2. Visualization of Gene X-PhoA fusion proteins. Strains were grown in M63 minimal medium containing 0.4% glucose, 25 μg each of 17 amino acids (lacking methionine, cysteine, and tryptophan) per ml, and 20 μg of ampicillin per ml when needed at 37°C to an A_{600} of 0.7. Aliquots (0.5 ml) of each culture were labeled with 10 μCi of Tran-^{35}S-label for 5 min, and then 0.5 ml of ice-cold 10% trichloroacetic acid was added. Proteins were analyzed by immunoprecipitation with antisera to alkaline phosphatase (APase), SecA, and OmpA, followed by polyacrylamide gel electrophoresis and autoradiography as described previously (28). The sample size (μl) and alkaline phosphatase activity (units) loaded in each lane are given. The positions of SecA protein (SecA), alkaline phosphatase (PhoA), and OmpA (OmpA) are indicated.

TABLE 1. Partial periplasmic locations of Gene X-PhoA fusion proteins[a]

Strain	% APase activity		
	Periplasmic		High-speed supernatant
	Method 1	Method 2	
CC118(pCB9)	37	26	93
CC118(pCB12)	48	29	96
CC118(pCB19)	56	25	95
MC1000 *phoR*	95	93	98

[a] Strains were grown in LinA medium (5) supplemented with 0.4% glucose and 40 μg of ampicillin per ml when needed at 37°C to an A_{600} of 0.8. Cultures were poured over an equal volume of crushed ice, sedimented at 10,000 × *g* for 5 min, and resuspended in 1/50 volume of ice-cold 25 mM Tris-acetate (pH 7.6)–100 mM KCl–10 mM magnesium acetate–1 mM dithiothreitol. The periplasmic contents were isolated by the cold osmotic shock method of Neu and Heppel (16) (method 1) or the chloroform release method of Ames et al. (2) (method 2). Cells were also subjected to sonication (three pulses of 16 s each) and were sedimented at 65,000 rpm for 20 min in the TL100 ultracentrifuge to give rise to a high-speed supernatant and pellet. The alkaline phosphatase (APase) activity of each fraction was determined by the method of Brickman and Beckwith (4).

Gene X-PhoA fusion proteins were located, we performed subcellular fractionation experiments. One such experiment is described in Table 1. Release of the periplasmic contents by either cold osmotic shock (16) or chloroform (2) yielded significant but incomplete release of alkaline phosphatase activities from these strains but nearly quantitative release of such activity from an isogenic strain constitutive for wild-type alkaline phosphatase production. Comparison of the periplasmic fractions from these different strains by analysis on SDS-polyacrylamide gels followed by staining with Coomassie blue showed that similar profiles of assorted periplasmic proteins were present in all cases and revealed no evidence of cell lysis (data not shown). We found that the strains containing the gene X-*phoA* fusions were more susceptible to cell lysis during spheroplasting. When cells from these strains were broken by sonication and membranes were pelleted by sedimentation, more than 93% of the alkaline phosphatase activity was recovered in the high-speed supernatant in each case. These data are consistent with a cell envelope location of the fusion proteins, with some weak association with either the inner or outer membrane, which is perturbed during sonication. The interpretation that the nonshockable alkaline phosphatase activity is cytoplasmic in nature is inconsistent with its high specific activity (data not shown) as well as with the fact that the signal peptides of the Gene X-PhoA fusion proteins are processed rapidly and completely (see Fig. 3). Given the abnormal nature of the fusion proteins, we have not attempted to define the precise conditions for their release from the cell envelope fraction. Definitive localization studies of Gene X protein must await the availability of an appropriate antiserum.

Gene X encodes a protein with a cleavable signal peptide. Since the Gene X-PhoA fusion proteins were recovered in the periplasmic and soluble fractions, consistent with the lack of any predicted membrane-spanning segment in Gene X protein, we reasoned that Gene X is likely to possess a cleavable signal peptide. Therefore, we determined whether precursor forms of the Gene X-PhoA fusion proteins could be detected when protein export was blocked. MGS68 containing the gene X-*phoA* fusions was grown under conditions to foster either normal protein export or a protein export block due to the production of an export-defective

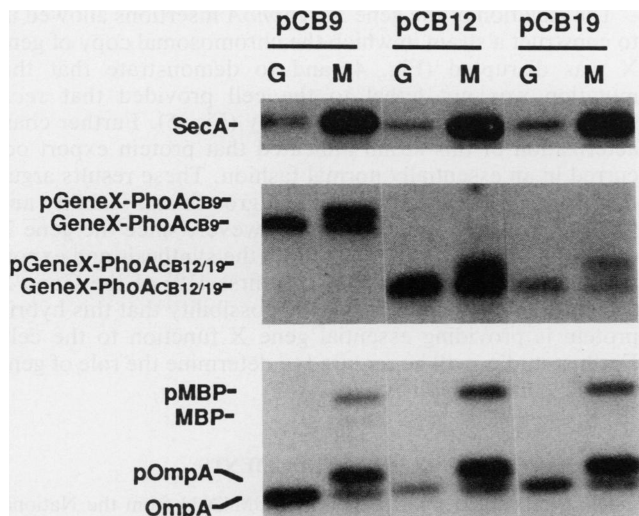

FIG. 3. Gene X encodes a protein with a cleavable signal peptide. MGS68 containing the plasmid indicated was grown in M63 minimal medium containing 0.4% glycerol, 25 μg each of 17 amino acids (lacking methionine, cysteine, and tryptophan) per ml, and 20 μg of ampicillin until an A_{600} of 0.3 was reached, at which time the culture was divided into two equal portions and one was supplemented with 0.4% glucose (G) and the other was supplemented with 0.4% maltose (M). Growth was continued for an additional 2 h, after which the cultures were pulse-labeled for 1 min with Tran-^{35}S-label. Gene X-PhoA, SecA, MalE, and OmpA protein synthesis was analyzed as indicated in the legend to Fig. 2 with the appropriate antisera. The positions of SecA (SecA) and the precursor and mature forms of the Gene X-PhoA fusion proteins from MGS68 (pCB9) (pGeneX-PhoACB9 and GeneX-PhoACB9, respectively) and MGS68(pCB12) and MGS68(pCB19) (pGeneX-PhoACB12/19 and GeneX-PhoACB12/19, respectively), of maltose-binding protein (pMBP and MBP, respectively), and of OmpA (pOmpA and OmpA, respectively) are indicated.

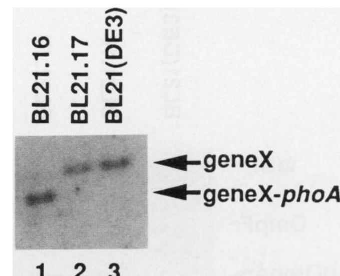

FIG. 4. Verification of the disruption of gene X. Chromosomal DNA was isolated from the strains indicated, digested with *Pvu*II, and subjected to electrophoresis on a 0.85% agarose gel and the Southern blotting procedure with a ^{32}P-labeled oligonucleotide to gene X nucleotides 433 to 453 (28). The positions of the 1.33-kb gene X$^+$ and 1.1-kb gene X-*phoA* *Pvu*II fragments are given.

MalE-LacZ fusion protein after maltose induction (9). Figure 3 shows that imposition of the protein export block led to the accumulation of precursor forms of the three different Gene X-PhoA fusion proteins as well as precursor forms of two other secretory proteins, maltose-binding protein and OmpA. These results clearly indicate that Gene X protein is initially synthesized with a cleavable signal peptide. Furthermore, since the protein export block led to a greater accumulation of precursor forms of maltose-binding protein and OmpA than of those of the Gene X-PhoA fusion proteins, it appears that gene X must encode a signal peptide that is utilized efficiently. This conclusion is supported by the fact that no precursor forms of the Gene X-PhoA fusion proteins were observed under the export-proficient conditions in this experiment despite the rapid radiolabeling conditions employed (1-min pulse-label).

Gene X disruption results in normal growth and protein export. To address whether gene X encodes an essential envelope protein, we constructed a strain containing a Tn*phoA* disruption of the chromosomal copy of gene X (see Materials and Methods for details). This strain, DO322 [gene X::Tn*phoACB19* (λPR9)], contains the same gene X::Tn*phoA* insertion in the gene X chromosomal locus as is found on pCB19; the strain also contains an additional copy of the gene X-*secA* operon on λPR9 in the event that gene X is essential for cell viability and the gene X::Tn*phoA* insertion is strongly polar on *secA* expression. To determine whether gene X function was essential for cell growth and viability, we compared the transductions of the gene X::Tn*phoA* chromosomal disruption into two different strains: one in which only *secA* function would be supplied in *trans* from pT7secA2 and one in which both gene X and *secA* functions would be supplied in *trans* from pMF8. Approximately equal numbers of light-blue, kanamycin-resistant transductants were obtained in both cases, indicating that disruption of gene X was not deleterious to cell viability as long as sufficient *secA* function was supplied in *trans*. One gene X-disrupted strain, BL21.16 [gene X::Tn*phoACB19*(λDE3) (pT7secA2)], was chosen for further characterization. Since the gene X::Tn*phoA* insertion is strongly polar on *secA* expression (data not shown) and pT7secA2 produces levels of SecA protein slightly lower than wild-type levels without induction of the *lac* promoter (15), we needed to construct a strain isogenic to BL21.16 that produced similar levels of SecA protein. Accordingly, BL21.17, which was disrupted in the chromosomal *secA* gene and contained the pT7secA2 plasmid, was constructed. To confirm that BL21.16 contains a disruption of the single copy of gene X in the cell, we subjected these strains to Southern blot analysis. These results are given in Fig. 4. BL21.16 lacks a wild-type copy of gene X and instead contains the gene X::Tn*phoACB19* copy as indicated by the absence of the 1.33-kb gene X$^+$ *Pvu*II fragment and its substitution by a 1.1-kb gene X-*phoA* *Pvu*II fragment in the Southern blot shown in Fig. 4.

The growth and cell viabilities of BL21.16 and BL21.17 were similar to those of the wild-type strain, BL21(λDE3), indicating that gene X is not essential for these functions. In order to determine whether Gene X plays a role in catalyzing protein export, we measured the rates of protein secretion for these three strains. The results are shown in Fig. 5. Protein secretion was nearly normal for BL21.16 and BL21.17, although both strains accumulated a small amount of pro-OmpA under these conditions (presumably, because of the subnormal levels of SecA protein made by these two strains). A small amount of precursor to maltose-binding protein was also detectable in these two strains in certain experiments performed identically to the one described in the legend to Fig. 5. The small amounts of precursors to these two proteins were quickly processed during pulse-chase analysis (data not shown). These findings imply that Gene X protein plays no major role in the export of these proteins. However, since BL21.16 synthesizes a fusion protein containing the first 60 amino acid residues of Gene X

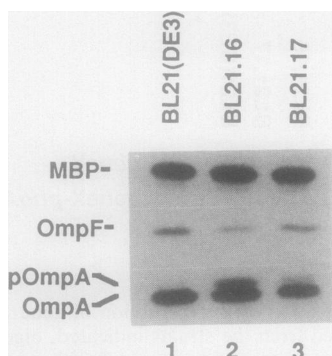

FIG. 5. Normal protein export in the absence of Gene X protein. Strains were grown in M63 minimal medium containing 0.4% maltose, 25 μg of any required amino acid per ml, and 20 μg of ampicillin per ml when needed at 37°C to an A_{600} of 0.7. Aliquots (0.5 ml) of each culture were labeled with 10 μCi of Tran-^{35}S-label for 1 min, and then 0.5 ml of ice-cold 10% trichloroacetic acid was added. Proteins were analyzed by immunoprecipitation with the appropriate antisera, polyacrylamide gel electrophoresis, and autoradiography as described previously (28). The positions of maltose-binding protein (MBP), OmpF/OmpC (OmpF), and the precursor and mature forms of OmpA (pOmpA and OmpA, respectively) are indicated.

fused to PhoA, we cannot rule out that this hybrid protein is providing essential gene X function.

DISCUSSION

While performing Tn*phoA* mutagenesis on the cloned gene X-*secA* operon, we found gene X-*phoA* fusions which demonstrated that gene X encodes a secretory protein. This theory was supported by the facts that (i) strains containing these fusions produced alkaline phosphatase with high specific activity (Fig. 2), indicating the presence of an efficient topogenic signal on the fusion proteins, (ii) a substantial portion of this activity was released into the periplasmic fraction (Table 1), and (iii) each fusion protein appears to contain a cleavable amino-terminal signal peptide which is processed rapidly (Fig. 3), the hallmark of a true secretory protein. In our original sequence analysis of gene X, we did not speculate that the predicted gene product might encode a secretory protein, since a classical amino-terminal signal sequence was not discernible. This sequence lacks a basic amino terminus, although this feature has been shown to be more important in facilitating rapid protein export kinetics than in maintaining a minimal level of protein export function (32). Furthermore, the Gene X signal sequence contains a glutamic acid residue in the 17th position, although the absence of charged amino acids in front of this position allows this sequence to meet a minimum hydrophobic-core requirement of approximately 12 amino acid residues (3). The protein sequence up to this region is rich in two amino acid residues, leucine and alanine, which are found preferentially in hydrophobic-core regions of signal peptides (7). Finally, the Gene X signal sequence does contain a potential consensus-processing site, Ala-Lys-Ala (7, 33), giving rise to a signal peptide of 23 amino acid residues. While it is tempting to speculate that these features of the Gene X signal peptide may be significant in delaying the export of this protein and in allowing it to play some role in modulating the protein export process itself, we have yet to obtain any evidence for such a role.

The isolation of the gene X::Tn*phoA* insertions allowed us to construct a strain in which the chromosomal copy of gene X was disrupted (Fig. 4) and to demonstrate that this mutation was not lethal to the cell provided that *secA* function was provided appropriately (Fig. 5). Further characterization of this strain indicated that protein export occurred in an essentially normal fashion. These results argue that gene X is nonessential for cell growth and viability and for the protein export process. However, since the gene X disruption that we examined allows the synthesis and export of a fusion protein containing the first 41% of the Gene X sequence, we cannot rule out the possibility that this hybrid protein is providing essential gene X function to the cell. Further studies will be required to determine the role of gene X in *E. coli* cell biology.

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