A Mutation in the *Escherichia coli* secY Gene That Produces Distinct Effects on Inner Membrane Protein Insertion and Protein Export*

John A. Newitt and Harris D. Bernstein‡

From the Genetics and Biochemistry Branch, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

E. coli strains that contain the secY40 mutation are cold-sensitive, but protein export defects have not been observed even at the nonpermissive temperature. Here we describe experiments designed to explain the conditional phenotype associated with this allele. We found that combining the secY40 mutation with defects in the signal recognition particle targeting pathway led to synthetic lethality. Since the signal recognition particle is required for the insertion of inner membrane proteins (IMPs) into the cytoplasmic membrane but not for protein export, this observation prompted us to examine the effect of the secY40 mutation on IMP biogenesis. The membrane insertion of all IMPs that we tested was impaired at both permissive and nonpermissive temperatures in secY40 cells grown in either rich or minimal medium. The magnitude of the insertion defects was greatest in cells grown at low temperature in rich medium, conditions in which the growth defect was most pronounced. Consistent with previous reports, we could not detect protein export defects in secY40 cells grown in minimal medium. Upon growth in rich medium, only slight protein export defects were observed. Taken together, these results suggest that the impairment of IMP insertion causes the cold sensitivity of secY40 strains. Furthermore, these results provide the first evidence that the protein export and membrane protein insertion functions of the translocon are genetically separable.

Protein translocation across the bacterial inner membrane (IM) and the eukaryotic endoplasmic reticulum are closely related processes (reviewed in Ref. 1). A highly conserved, membrane-embedded heterotrimer called the SecYEG complex in bacteria and the Sec61p complex in eukaryotes (2, 3) forms the core component of the translocation machinery or “translocon” in both systems. Genetic studies in *Escherichia coli* and *Saccharomyces cerevisiae* have demonstrated the importance of the SecYEG-Sec61p complex for protein translocation *in vivo* (4, 5). The *E. coli* SecYEG complex and the peripheral membrane protein SecA, which provides an ATP-driven push, are sufficient to support translocation of preproteins into proteoliposomes *in vitro* (6). Likewise, the mammalian Sec61p complex can be cross-linked to nascent secretory proteins (2) and is sufficient to mediate translocation into lipid vesicles (7).

A variety of studies have indicated that in addition to catalyzing protein translocation, the Sec61p-SecYEG complex facilitates the insertion of membrane proteins into the endoplasmic reticulum or IM. Genetic and biochemical studies have demonstrated that the Sec61p complex plays a central role in the insertion process in both yeast and mammals (7–9). Several studies using conditional alleles of secY isolated in screens for protein export mutants have suggested that the insertion of model inner membrane proteins (IMPs) is SecY-dependent (10–13). Consistent with these results, depletion of SecE was recently shown to block the insertion of the maltose transporter MalF (14). However, several other studies that also used conditional alleles of secY have suggested that the insertion of some IMPs is SecY-independent (10, 12, 15) or that SecY dependence is related to the length of cytoplasmic loops (16). Although the discrepancies in these studies may be due in part to the use of different assays for IMP insertion, they nevertheless raise the possibility that some mutant alleles may not affect the transport of all proteins equally.

Protein insertion into and translocation across a phospholipid bilayer are two processes that are likely to imply very different functional requirements on the translocon. The mechanism by which the SecYEG-Sec61p complex directs both of these processes is still poorly understood. Available evidence indicates that the mammalian translocon forms an aqueous channel that permits translocation of hydrophilic polypeptides (17–19) and also opens laterally to allow the exit of transmembrane IMPs (20, 21). One study indicated that cleavable signal peptides of secreted proteins and signal-anchor domains of transmembrane proteins are positioned differently within the eukaryotic translocon, suggesting that secreted and membrane proteins might be handled differently (22). In addition, the *E. coli* translocon may be able to recognize the longer hydrophobic segments of signal anchors or transmembrane regions of IMPs and allow them to partition into the lipid bilayer (23). A recent study suggests that during co-translational targeting, the mammalian ribosome can detect the presence of an emerging membrane-spanning domain and use this information to influence the activity of the translocon (24).

Given the complexity of the tasks performed by the translocon, it is possible that the passage of secreted proteins and the insertion of integral membrane proteins are facilitated by distinct regions of the heterotrimer. Thus, some mutations may preferentially affect the transport of one of these two classes of proteins. We hypothesized that one such mutation might be the *E. coli secY40* allele, which was isolated in a screen for secretion mutants (25, 26) based on the observation that protein export defects up-regulate secA expression (27, 28). Although the mutation gives rise to a moderate increase in secA synthe-
sis and a clear cold-sensitive phenotype, no significant defects in maltose-binding protein (MBP) or OmpA export were observed even after the cells were incubated at the nonpermissive temperature for prolonged periods (25, 26).

In this study, we tested the possibility that the secY40 mutation selectively affects IMP insertion using a combination of genetic and biochemical experiments. We exploited the observation that secreted proteins and IMPs are targeted to the IM of *E. coli* by different pathways. At least some IMPs are targeted by the signal recognition particle (SRP) (29, 30), a ribonucleoprotein that is composed of homologs of the mammalian 54-kDa signal sequence binding protein (“Ffh”) and 7SL RNA (“4.5 S RNA”) (31, 32). In contrast, exported proteins are targeted by the chaperone SecB (33) or by other SRP-independent mechanisms (29). Although SRP is essential for viability (34, 35), we found that secY40 strains were hypersensitive to perturbations of the SRP pathway. This result suggested that the secY40 mutation exacerbates the effect of SRP mutations by having a similar effect on IMP biogenesis. Direct examination revealed that the secY40 mutation blocked IMP insertion but, as expected, had little or no effect on the export of most proteins. These results confirm that secY is required for IMP biogenesis and provide the first evidence that mutations in secY can have different effects on protein export and IMP insertion.

**EXPERIMENTAL PROCEDURES**

*Reagents, Media, and Bacterial Manipulations—*Antisera against various proteins were obtained from 5 Prime → 3 Prime (alkaline phosphatase (AP) and β-lactamase (BLA)), New England Biolabs (MBP), Dr. Jon Beckwith (ribose binding protein (RBP) and DegP), and Dr. Stephen Pollitt (OmpA). Basic media preparation and bacterial manipulations were performed using standard methods (36). Selective media contained 100 μg/ml ampicillin or 40 μg/ml chloramphenicol. The bacterial strains used in this study and their genotypes are listed in Table I.

**Plasmid Construction—**Derivatives of pBR322 and pHD83 that overexpress IMPs and derivatives of pACYC and pUC19 containing AP fusions to IMPs have been described previously (29). For the experiments described here, a BamHI–SalI fragment of plasmid pJS310 containing the *H.丹* 310-AP fusion was cloned into a derivative of pLG288 (37). The AcrB 576-AP fusion was placed under control of the *lac* promoter by transferring a *BglII*–*HindIII* fragment containing the fusion into the *lacZ*–containing plasmid pUB11 (obtained from Carol Gross). A *HindIII*–*BglII* fragment from plasmid pH-l (38) containing the AP gene was cloned into pWJC12 (39) to create plasmid pAP-1. To obtain plasmid pPFF (wild type), the *BglII* site immediately downstream of the *ffh* coding sequence was first converted to a *BamHI* site. A *SalI*–*BamHI* fragment containing the *ffh* gene was then cloned into the corresponding sites in pACYC184. Leucine residues 38 and 39 in Ffh were changed to alanines by PCR using the “megaprimer” technique (40) to create the *ffh* cs101 mutant.

**Pulse-chase Labeling and Immunoprecipitation—**Pulse-chase labeling in M9 medium was performed essentially as described (29) except that 0.4% maltose was substituted for glucose in experiments in which MBP export was assayed. For experiments in rich medium, saturated cultures were diluted into fresh LB to an optical density (A660) of 0.005. When the cultures reached an A660 of 0.5 they were labeled for 5 min with 380 μCi/ml Tran35S-label (ICN, specific activity 1490 Ci/mmol). In temperature shift experiments, cultures were grown to an A660 of 0.4–0.5 at 37 °C and then transferred to 23 °C for varying lengths of time prior to labeling. Samples were processed as described (29), and immunoprecipitations were performed with the following modifications. Tri- chloroacetic acid-precipitated samples were solubilized in IP1 (5% SDS, 500 mM Tris-HCl, pH 9.5, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride) and heated at 50 °C for 30 min and then diluted with an equal volume of IP2 (2.17% Triton X-100, 163 mM NaCl, 50 mM Tris-HCl, pH 8.5, 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride). After binding the antibody-antigen complexes to protein A-agarose, the beads were washed three times with IP3 (0.1% Triton X-100, 0.02% SDS, 150 mM NaCl, 50 mM Tris-HCl, pH 8, 5 mM EDTA) and once with IP4 (150 mM NaCl, 50 mM Tris-HCl, pH 8, 1 mM EDTA).

| Table I | Bacterial strains |
|--------|------------------|
| Strain | Genotype | Source/Reference |
| MC4100 | F- araD139 Δ(argF-lac)U169 rpsL | ATCC |
| CU165 | MC4100 secY40 zhd33::tet | Ref. 25 |
| WAM113 | MC4100 ara+ fkh::kan-1 Ap<sup>+</sup> fkh | Ref. 34 |
| HDB45 | MC1100 ffh::kan-1 pHD84 | Ref. 29 |
| HDB58 | MC4100 zhd33::tet | This study |
| HDB61 | MC4100 fkh::kan-591 pHD85 | Ref. 29 |
| HDB74 | WAM113 zhd33::tet | This study |
| HDB78 | WAM113 secY40 zhd33::tet | This study |
| HDB84 | HDB85 ffh::kan-1 pHD84 | This study |
| HDB85 | HDB84 secY40 zhd33::tet | This study |
| HDB86 | HDB86 ffh::kan-1 pHD84 | This study |
| HDB87 | HDB86 secY40 zhd33::tet | This study |

**RESULTS**

The secY40 Mutation Is Lethal in Strains That Have SRP Deficiencies—We reasoned that if the secY40 mutation inhibits the insertion of IMPs at the permissive temperature, then a second genetic change that also impairs IMP biogenesis might have a synergistic effect and lead to a synthetic lethal phenotype. Slight SRP deficiencies can be tolerated by wild-type *E. coli*, but a 5–8-fold decrease in the intracellular Ffh concentration becomes growth-limiting (29), presumably because the biogenesis of IMPs is reduced below a critical level. Therefore, assessment of the viability of secY40 strains that have reduced SRP activity should provide an indication as to whether the mutation impedes IMP insertion. In support of this notion, we found that the secY39 mutation, which has been shown to inhibit IMP insertion (16), is lethal in strains carrying SRP deficiencies (data not shown).

To determine whether the secY40 allele affects the viability of cells that have reduced levels of SRP, we constructed strains HDB84 (sec<sup>Y</sup>+) and HDB85 (sec<sup>Y</sup>40) in which expression of *ffh* is regulated by the *trc* promoter. Cells were plated on LB agar containing various amounts of IPTG and incubated at 37 °C. Previous work demonstrated that the parent strain (HDB45) produces near normal levels of Ffh in the presence of 10 μM IPTG (29). HDB84 grew at all IPTG concentrations between 0 and 20 μM, although noticeably smaller colonies were produced below 2 μM IPTG (Fig. 1A, diamonds). In contrast, HDB85 showed an exquisite sensitivity to the level of Ffh. Reduction in the IPTG concentration from 20 to 10 μM resulted in the production of slightly smaller colonies (Fig. 1A, circles); at 5 μM IPTG, a ~50-fold decrease in plating efficiency was observed. Similar results were obtained using strains in which expression of the gene encoding 4.5 S RNA (*ffs*) is regulated by the *trc* promoter. HDB86 (*sec<sup>Y</sup>*) grew normally at all IPTG concentrations between 50 and 100 μM (Fig. 1B, triangles). HDB87 (sec<sup>Y</sup>39) however, produced progressively smaller colonies below 100 μM IPTG, and at 50 μM IPTG the plating efficiency dropped sharply (Fig. 1B, squares). These results show that even a slight decrease in SRP concentration below the wild-type level inhibits the growth of secY40 strains and indicate that, even at the permissive temperature, the secY40 mutation compounds the effect of perturbing the SRP pathway.

Similar results were obtained when the secY40 mutation was combined with an Ffh Cs mutation that is likely to reduce substrate binding activity. The *ffh* cs101 allele contains a mutation in a highly conserved motif that does not affect protein stability (data not shown) but that has been shown to reduce the signal sequence binding activity of canine SRP54 (the mammalian SRP 54-kDa protein) (41). Cells containing the mutation grow at 37 °C or above but do not grow at 30 °C. To test the ability of cells to tolerate both the *ffh* and *secY* muta-
The secY40 Allele Selectively Blocks IMP Insertion

Fig. 1. secY40 strains are hypersensitive to reductions in Ffh and 4.5 S RNA concentration. HDB84 (fkh::kan Ffh secY40; diamonds) and HDB85 (HDB84 secY40; circles) (panel A) and HDB86 (fkh::kan Ffh secY40; triangles) and HDB87 (HDB86 secY40; squares) (panel B) were grown to saturation in LB containing 20 and 100 μM IPTG, respectively. Cells were diluted in LB, and approximately 300 colony-forming units were plated on LB agar containing various concentrations of IPTG and incubated at 37 °C for 14 h. Relative colony size was scored as follows: | colony size indistinguishable from that observed at highest concentration of inducer; 2, colony diameter >75% of maximum; 3, colony diameter between 50 and 75% of maximum; 4, colony number reduced >95%.

Fig. 2. IMP insertion is impaired in secY40 cells grown in minimal medium. HDB58 (secY40; lanes 1 and 2) and CU165 (secY40; lanes 3 and 4) transformed with a plasmid expressing LctP 426-AP (A), AcrB 576-AP (B), MalF 350-AP (C), or MtlA 310-AP (D) were grown to midlog phase at 37 °C in M9-glucose medium supplemented with amino acids. Insertion of IMP-AP fusions was assessed by pulse-chase analysis followed by immunoprecipitation with anti-AP antibodies (see “Experimental Procedures”). The length of the chase is indicated. The mobilities of the intact fusion proteins and the protease-resistant AP domain are indicated.

We found that CU165 grown at the permissive temperature (37 °C) had a small but reproducible defect in the insertion of all IMPs tested. Small amounts of full-length LctP 426-AP (lactate permease fusion) were observed in CU165 pulse-labeled for 30 s (Fig. 2A, lane 3). Significant amounts of full-length LctP 426-AP and AcrB 576-AP (multidrug efflux pump fusion) also remained in the cytoplasm after a 5-min chase (Fig. 2A and B, lanes 4) and at later time points (data not shown). A larger amount of full-length fusion protein was consistently observed after the chase presumably because the synthesis of the C-terminal AP tag on many molecules was not completed within the pulse labeling period. Substantial amounts of protease-protected MalF 350-AP (maltooligosaccharide transport fusion) and MtlA 310-AP (mannitol permease fusion) were observed in pulse-labeled CU165, but less of the intact fusion protein remained after a 5-min chase (Fig. 2, C and D). Thus, the insertion of these proteins may be delayed rather than completely blocked by the secY40 mutation. Insertion defects were also observed for all of these proteins in CU165 after shifting to 23 °C for 90 min, but the magnitude of the defects did not increase under these growth conditions (data not shown).

Consistent with previous reports (25, 26), we found that protein export was normal in CU165 grown in defined minimal medium. The SecB-dependent proteins MBP and OmpA, and the SecB-independent proteins RBP, BLA, and AP were immunoprecipitated from radiolabeled cells generated in the experiments described above or from cells transformed with pAP-1, a plasmid that encodes native AP. In each case, a portion of the sample that had not been treated with protease was used (see “Experimental Procedures” and Ref. 29). The prepolytein form of each of these proteins was converted to the mature form in CU165 as rapidly as in HDB58 (Fig. 3, lanes 1–4), indicating that the secY40 allele did not affect protein export. In agreement with previous reports (25, 26), incubation at the nonpermissive temperature (23 °C) also did not reveal a secretion defect (data not shown). In contrast, the addition of sodium azide, an inhibitor of SecA function, effectively blocked protein...
were treated with 2 mM sodium azide for 10 min prior to pulse labeling.

The fact that the secY40 allele inhibits only IMP insertion.

Elevated IMP Insertion Defects Are Observed under Conditions in Which secY40 Cells Exhibit Strong Growth Defects—

The secY40 allele was originally isolated in rich medium (25, 26) and the observation that IMP insertion defects in cells grown in minimal medium did not show a clear temperature dependence prompted us to reexamine the viability of a secY40 strain in different growth media. MC4100, HDB58, and CU165 were streaked onto LB agar, M9-glucose agar, and M9-glycerol agar and incubated at 37 or 23 °C. As expected, CU165 did not grow at 23 °C on LB agar (Fig. 4). On enriched M9-glucose agar, however, CU165 grew at 23 °C, but the colonies had an abnormal, mucoid appearance. On the poorest medium, M9-glycerol agar, CU165 grew as well as HDB58 and MC4100 and formed normal colonies at both 37 and 23 °C. These results suggest that the severity of the Cs phenotype associated with the secY40 allele correlates with the rate of cell growth.

To determine whether the secY40 mutation caused a more substantial IMP accumulation at 23 °C than at 37 °C under conditions of rapid growth, we developed a pulse labeling method to analyze the fate of prepolypeptides synthesized by cells grown in rich medium. HDB58 and CU165 were transformed with pAP-1. Lanes 1 and 2, HDB58; lanes 3 and 4, CU165. In lane 5, HDB58 cells were treated with 2 mM sodium azide for 10 min prior to pulse labeling (HDB58 + N3). Preproteins (p) and mature proteins (m) are indicated.

FIG. 3. Protein export is unaffected by the secY40 mutation in cells grown in minimal medium. BLA, RBP, AP, OmpA, and MBP were immunoprecipitated from samples generated in the experiment shown in Fig. 2 that had not been subjected to proteolysis. AP was immunoprecipitated from cells that had been transformed with pAP-1. Lanes 1 and 2, HDB58; lanes 3 and 4, CU165. In lane 5, HDB58 cells were treated with 2 mM sodium azide for 10 min prior to pulse labeling (HDB58 + N3). Preproteins (p) and mature proteins (m) are indicated. translocation and led to substantial preprotein accumulation (Fig. 3, lane 5). Hence, under the growth conditions used in these experiments, the secY40 allele inhibits only IMP insertion.

FIG. 4. The cold-sensitive phenotype of the secY40 allele depends upon growth conditions. Cells were streaked from cultures that had just reached stationary phase onto duplicate agar plates containing LB, M9-glucose supplemented with amino acids, or M9-glycerol medium. Plates were incubated at 37 °C for 17 h (LB), 24 h (M9 + glucose + AA), or 48 h (M9 + glycerol) and at 23 °C for 36 h (LB), 48 h (M9 + glucose + AA), or 108 h (M9 + glycerol).

FIG. 5. IMP insertion in secY40 cells grown in rich medium. HDB58 (lanes 1 and 2) and CU165 (lanes 3 and 4) transformed with a plasmid expressing MalF 350-AP were grown in LB medium at 37 °C to midlog phase. A, a portion of the culture was pulse-labeled at 37 °C for 5 min as described (see “Experimental Procedures”). The remainder was shifted to 23 °C and incubated for 48 min (B) or 114 min (C) prior to removing an aliquot for pulse labeling. Cells were spheroplasted and treated with (lanes 2 and 3) or without (lanes 1 and 4) proteinase K. Samples were immunoprecipitated with anti-AP antibody.

labeled MalF 350-AP was retained in the cytoplasm (lanes 3 and 4 in Fig. 5, B and C). In contrast, HDB58 showed no significant accumulation of MalF 350-AP at 37 °C (Fig. 5A, lanes 1 and 2) or at 23 °C (Fig. 5, B and C, lanes 1 and 2). Thus, the secY40 mutation exerts a greater effect on IMP insertion at low temperature when cells are grown in rich medium. In contrast, the secY40 mutation had little or no effect on the export of most proteins when cells were grown in LB. Protein export was assessed by immunoprecipitating OmpA, RBP, BLA, AP, and DegP from pulse-labeled HDB58 and CU165.
transformed with a plasmid expressing an IMP-AP fusion or with pAP-1. In these experiments, the rate of protein translocation in the two strains at 37 °C was comparable (Fig. 6, lanes 1 and 2), although the precursor form of several exported proteins could be detected in CU165 by Western blot (data not shown). Similar results were obtained at 23 °C (Fig. 6, lanes 3 and 4) except that RBP export was clearly impaired (Fig. 6, RBP, lane 4). The marked effect on RBP export at low temperature was unique, however, and we cannot determine from these data whether the export block was a direct consequence of the mutation or an indirect effect of the decreased efficiency of IMP insertion.

**DISCUSSION**

In this report, we show that the secY40 mutation selectively blocks the insertion of IMPs. Initially, we found that combining the secY40 mutation with SRP deficiencies produced a synergistic effect and reduced cell viability. The genetic data suggested that the secY40 mutation blocks the same biological process as ffh and ffh mutations. Consistent with these results, direct examination of the insertion of several individual IMPs revealed that the secY40 mutation has a broad effect on IMP biogenesis. Moderate IMP insertion defects were observed in cells grown at 37 °C in both rich and minimal media. Shifting cells grown in LB to 23 °C led to more severe IMP insertion defects. In contrast, no defects in the export of SecB-dependent proteins (which had been examined previously) or several SecB-independent proteins could be detected in cells grown in minimal medium. The export of most proteins was not notably affected by growth in rich medium either. Taken together, these results suggest that the marked cold sensitivity of secY40 strains grown in rich medium is attributable to an increased impairment of IMP insertion defects at low temperature.

The data presented here help to clarify two issues regarding SecY function. First, the observation that the insertion of several unrelated IMPs was blocked by the secY40 mutation supports the view that the SecYEG translocon is required for IMP biogenesis. The observation that the mutation affects the insertion of both MalF, which has a long periplasmic loop, and MtiA, which has only short periplasmic loops, also suggests that the translocon facilitates the insertion of a broader range of IMPs than has been previously proposed (18). Second, the finding that the secY40 mutation inhibits IMP insertion links the mutation to the protein transport function of SecY and argues against the proposal that it affects a postulated second function of SecY (25). Our data do not fully explain, however, why the secY40 mutation was isolated in a screen for mutations that increase secA expression. One possibility is that an inhibition of IMP insertion, like a protein export block, leads to an increase in SecA synthesis. This possibility is consistent with several studies that indicate that SecA is required for the insertion of at least some IMPs (10, 14, 16, 43). Alternatively, the slight protein export defects observed in secY40 strains when cells are grown in rich medium may be sufficient to cause a moderate up-regulation of secA expression. This explanation is consistent with the observation that the secY40 mutation increases secA expression to a much smaller degree than secY alleles that produce strong effects on protein export (26).

An important point that arises from this study is that it cannot be assumed that translocon mutations have equal effects on the transport of all proteins. For this reason, the use of a single sec allele to test the "Sec dependence" of transport of a given protein may yield misleading results. Another implication of this study is that transport defects may be sensitive to growth conditions. The observation that both the phenotype and the biochemical defects associated with the secY40 allele varied in different media demonstrates that it can be misleading to extrapolate from one growth condition to another. Slowing the growth rate of secY40 cells by culturing in minimal medium may suppress the cold-sensitive phenotype by reducing the burden of polypeptides that the translocon must handle in a given time period. This notion is consistent with the observation that the secY40 Cs phenotype can be suppressed by simply overproducing a cytoplasmic protein (44), which presumably reduces the rate of synthesis of IMP proteins. Sensitivity to biosynthetic rates may be a general property of sec mutations, since the growth defects of several Cs alleles of secY, secE, secD, and secF can all be suppressed by overproduction of cytoplasmic proteins (44).

The most intriguing implication of this study is that translocon mutations can have distinct effects on protein secretion and membrane protein insertion. Our results suggest that different regions of SecY/Sec61p may be specialized to facilitate two fundamentally distinct processes, the translocation of largely hydrophilic proteins and the membrane insertion of more hydrophobic IMPs. Although a variety of export-defective alleles of secY have been obtained based on the up-regulation of secA expression, the mutations tend to cluster in specific re-

| Table II | Synthetic lethal interaction between secY40 and ffh mutant alleles |
|----------|------------------------------------------------------------------|
| HDB74 (sec Y¹) or HDB78 (secY40) were transformed with a plasmid that expresses either a mutant ffh allele (pFFH (cs101)) or wild-type ffh (pFFH (WT)) as a control, plated on LB agar containing 50 μg/ml chloramphenicol, and incubated at 37 °C for 16 h. Colony formation was scored as follows: +++, colonies indistinguishable from control; +++, colony diameter between 50 and 100% of control; +, colony diameter <50% of control; –, colony number reduced >95%. |

| pFFH (WT) | HDB74 (sec Y¹) | HDB78 (secY40) |
|-----------|----------------|----------------|
| ++        | +++            | +              |
| pFFH (cs101) | ++            | –              |

**Fig. 6.** Protein export in secY40 cells grown in rich medium. Proteins were immunoprecipitated from HDB58 (lanes 1 and 3) and CU165 (lanes 2 and 4) transformed with a plasmid expressing AP (OmpA, AP, DegP) or an IMP-AP fusion (RBP, BLA). Cells were grown at 37 °C in LB to midlog phase. One portion of the culture was pulse-labeled at 37 °C (lanes 1 and 2), and another portion was incubated at 23 °C for 48 min prior to pulse labeling (lanes 3 and 4). In lane 5, HDB58 cells were treated with 2 mM sodium azide for 10 min prior to pulse labeling.
regions of the protein (26). Indeed, no mutations have been iso-
lated in some highly conserved regions that might conceivably
participate in IMP insertion. For these reasons, it should be
interesting to develop additional screening methods to isolate
new secY alleles that impair the transport of specific classes of
proteins. Characterization of these alleles as well as further
analysis of existing sec mutations should provide a great deal of
insight into the mechanism by which the translocon performs a
complex set of tasks.

Acknowledgments—We thank Carol Gross, Koreaki Ito, and Jon
Beckwith for gifts of plasmids, strains, and antibodies and Gisela Storz
for critical reading of the manuscript.

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