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Genetics

PrlA is important for the translocation of exported proteins across the cytoplasmic membrane of *Escherichia coli*

(*lacZ*/β-galactosidase fusion proteins/overproduction lethality/signal sequence/protein secretion)

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ABSTRACT **Strains of *Escherichia coli* in which *lacZ* (specifies β-galactosidase) is fused to genes that specify exported proteins such as LamB (λ receptor) exhibit unusual phenotypes. In particular, such strains are killed by high-level expression of the LacZ hybrid protein. Previous results suggest that this overproduction phenotype is the consequence of a lethal jamming of the cellular protein export machinery and this hypothesis is supported by the observed accumulation of the precursor forms of many noncytoplasmic proteins within the moribund cell. Under conditions in which protein export is compromised, biochemical and immunocytochemical analyses indicate that these hybrid proteins can be found in transmembrane orientation. To identify the cellular component rendered rate-limiting by the LacZ hybrid protein under jamming conditions we have utilized signal sequence mutations, which block entry of the hybrid protein into the export pathway, and a dominant suppressor of these lesions, *prlA4*. Data obtained with a series of merodiploids heterozygous and homozygous for *prlA*$^+$ and *prlA4* show that PrlA is the component sequestered by hybrid jamming. Taken together, these results suggest that PrlA is a component of the export machinery that functions in the translocation of proteins across the cytoplasmic membrane.**

In *Escherichia coli*, *lacZ* (specifies β-galactosidase) fusions have been particularly useful in the study of protein export, because strains in which this gene is fused to sequences specifying a noncytoplasmic protein often exhibit unusual phenotypes. First, the function of the β-galactosidase moiety is inhibited if the chimera is directed efficiently to a membrane location. Presumably in this context, the enzyme cannot oligomerize into the active tetrameric species. Second, the cellular protein export machinery cannot deal effectively with sequences of this large cytoplasmic enzyme. Accordingly, high-level synthesis of a LacZ hybrid protein can lead to a lethal "jamming" of the export apparatus. These characteristic phenotypes have been exploited to obtain export-defective signal sequence mutations in the gene to which *lacZ* is fused and to identify components of the export machinery such as *secA* (a peripheral inner membrane protein) and *secB* (a cytoplasmic protein) (for review see ref. 1).

The signal sequence mutations obtained using selections based on gene fusions have been employed to further our knowledge of the components of the export apparatus by utilizing the technique of interactive suppressors. For example, mutations that alter the *lamB* (encodes the outer membrane protein LamB) or *malE* [encodes the periplasmic maltose-binding protein (MBP)] signal sequence can confer a negative phenotype when recombined onto the corresponding wild-type gene. This permits selection for suppressors that alter a component of the export machinery and restore recognition of the mutant signal sequence. One gene identified by this method is *prlA* (also known as *secY*, encodes a 49,000-dalton integral inner membrane protein) (2, 3).

Recently, biochemical evidence supporting a direct role for PrlA and SecA in protein export has been presented (4, 5). Despite these advances the mechanistic role of these proteins in the export process is presently unclear and a number of fundamental questions remain to be addressed. We have exploited a suppressor allele of *prlA* and a *lamB–lacZ* gene fusion carrying a signal sequence mutation to investigate the LacZ-mediated jamming of the export apparatus. Our results indicate that the fusion protein sequences sequester PrlA rendering its function rate-limiting for export and they support the view that PrlA is a component of the translocator—i.e., the part of the export apparatus through which proteins cross the inner membrane.

MATERIALS AND METHODS

Media and Chemicals. Media and chemicals have been described elsewhere (6).

Strains and Plasmids. All strains are derivatives of *E. coli* K-12 strain MC4100 and are described in Table 1 and *Results* (2, 7). Strain constructions were done by using standard genetic techniques (6). The plasmid pMLB1107 is a pBR322-derived vector in which transcription of an inserted fragment can be regulated by the *lac* promotor. In addition, this vector contains the Lac repressor gene (Mike Berman, personal communication). pRLA41 was constructed by cloning a single 2440-base-pair fragment carrying the 3′ region of the P*spc* operon and a small 5′ segment of the Pα operon into a unique *Pst* I site at codon 11 of *lacZ* on pMLB1107. In addition to the *prlA4* gene, the inserted fragment carries the upstream ribosomal protein genes specifying L15 and L30 (8). In pRLA41, *prlA4* expression is controlled by the *lac* promoter and thus inducible with isopropyl β-D-thiogalactoside (IPTG).

Maltose Sensitivity Assay. Sensitivity to maltose was quantitated by the disk assay as described (9) except that 10-μl samples of 0.5%, 1%, 2%, 5%, and 10% maltose were applied to the disks. To induce the plasmid-borne *prlA4* gene, 20 mM IPTG was added to the F-top agar. All disk assays were repeated a minimum of 10 times with the appropriate controls.

Pulse-Labeling and Immunoprecipitation. Cells were grown and LamB and MBP synthesis was induced as described (10) except that cells were induced with 0.2% maltose for 60 min. Under this condition, the pleiotropic defects exhibited by maltose-sensitive *lamB–lacZ* fusion strains are minimal. Cell death, for example, is not evidenced until induction times approach 3 hr. Pulse–chase assays and immunoprecipitations were done as described (11).

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Abbreviations: MBP, maltose-binding protein; IPTG, isopropyl β-D-thiogalactoside.

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Table 1. Maltose-sensitive disk assay

Strain	Genotype	Diameter of sensitivity, mm
	Assayed with 0.5% maltose	
pop3186	*prlA*$^+$ *lamB–lacZ42-1*	9.8
pop3186/pRLA41	*prlA*$^+$ *lamB–lacZ42-1*/ *prlA4* (uninduced)	5.2
pop3186/pRLA41	*prlA*$^+$ *lamB–lacZ42-1*/ *prlA4* (induced)	0.3
pop3186/pMLB-1107.2	*prlA*$^+$ *lamB–lacZ42-1*/ *lacZ* (induced)	10.1
	Assayed with 5.0% maltose	
pop3186	*prlA*$^+$ *lamB–lacZ42-1*	22
SE1073	*prlA*$^+$ *lamB17D–lacZ42-1*	0
BKR73A4	*prlA4 lamB17D–lacZ42-1*	10
SE1073/pRLA41	*prlA*$^+$ *lamB17D–lacZ42-1/prlA4* (uninduced)	0
BKR73A4/pRLA41	*prlA4 lamB17D–lacZ42-1/prlA4* (uninduced)	10
BKR73A4/pRLA41	*prlA4 lamB17D–lacZ42-1/prlA4* (induced)	6
BKR73A4/F′141	*prlA4 lamB17D–lacZ42-1/F′ prlA*$^+$	0

Data shown are from a representative series of assays described in the text. Uninduced or induced in parentheses refers to the absence or presence of IPTG in the top agar to vary the level of *prl4* expression.

SDS/PAGE and Autoradiography. Sample electrophoresis was performed by using 10% polyacrylamide gels that were then fluorographed. Autoradiograms were scanned and peaks were integrated as described (10).

RESULTS

Background and Rationale. High-level synthesis of certain LamB–LacZ and MalE–LacZ hybrid proteins is lethal to the cell (7). Several lines of evidence indicate that this lethality is the consequence of a general block in protein export. First, synthesis of these hybrids causes the cytoplasmic accumulation of the precursor form (contains the signal sequence) of many different exported proteins (12, 13). Second, signal sequence mutations that prevent hybrid protein export relieve this lethality and high-level synthesis no longer results in generalized precursor accumulation (7).

Although the molecular mechanism of the block in export is not yet clear, the phenomenon has been studied in some detail in several different laboratories. Results of these studies indicate that the cell recognizes the hybrid protein as an exported protein and initiates the localization process. Export proceeds efficiently to a stage at which the signal sequence is removed by leader peptidase at the periplasmic face of the inner membrane and sequences of the hybrid molecule are accessible to externally added proteases (refs. 14 and 15; B. Rassmussen and T.J.S., unpublished data). Jamming of the export machinery occurs subsequent to signal sequence cleavage but prior to complete translocation of the entire molecule leaving the hybrid stuck in transmembrane fashion. Given this topology, it is reasonable to propose that jamming occurs at the level of the translocator, and although this has not been proven directly, we think it likely. Indeed, Voorhout *et al.* (16) have reached the same conclusion based on immunocytochemical analysis.

If the hybrid protein is incompletely translocated and thus jammed part way through the export apparatus of the inner membrane, then it follows that specific cellular components must become limiting for protein translocation from the cytoplasm under jamming conditions. These components must be those that comprise the part of the export machinery used for the actual translocation reaction.

Overproduction of PrlA Relieves Maltose Sensitivity. The overproduction lethality conferred by *lamB–lacZ* fusions such as 42-1 (contains the signal sequence plus 181 codons of mature LamB) is evidenced phenotypically as maltose sensitivity because maltose induces high-level synthesis of the hybrid protein. Maltose sensitivity can be simply scored by using a disk test analogous to that employed for determining antibiotic sensitivity, and the degree of sensitivity can be quantitated by measuring the diameter of the zone of growth inhibition. Accordingly, this test provides a means to identify conditions in which maltose sensitivity is altered.

We reasoned that increased production of the export component that is rendered rate-limiting by hybrid protein jamming would result in decreased maltose sensitivity. If so, then it should be possible to identify the component in question by introducing the relevant gene on a multicopy plasmid vector into the *lamB–lacZ* fusion strain and scoring maltose sensitivity using the disk assay. We began our studies using *prlA*, as its gene product is the only component of the export machinery that is known to be located within the cytoplasmic membrane, the location of the partially translocated hybrid protein.

To investigate the possible role of PrlA as a limiting factor in hybrid jamming, the maltose-sensitive phenotype of pop3186 (*lamB–lacZ42-1*) was assayed under conditions of PrlA4 overproduction. Overproduction of PrlA4 was attained by using the high-copy plasmid pRLA41, in which expression of the suppressor allele is driven by the *lac* promoter. As can be seen in the upper part of Table 1, the presence of the plasmid greatly reduced sensitivity to maltose under uninduced and induced conditions, resulting in a decrease by a factor of 2 in the diameter of sensitivity to 0.5% maltose under uninduced conditions and almost complete resistance to this concentration of maltose under conditions of *prlA4* induction. The plasmid pMLB1107, lacking the *prlA4* insert, had no effect on maltose sensitivity.

The observation that overproduction of PrlA relieves maltose sensitivity suggests that PrlA is the limiting export component under conditions of high-level hybrid protein synthesis. However, from these data alone, it is impossible to rule out a decrease in maltose sensitivity caused by overproduction of other proteins encoded within the plasmid insert or an indirect effect on other components of the export apparatus caused by the overproduction of PrlA. We do not think the presence of the suppressor allele affects the maltose sensitivity in these experiments since the presence of the *prlA4* mutation in single copy has no known effects on proteins with wild-type signal sequences or on the maltose sensitivity conferred by the fusion with the wild-type signal sequence (data not shown).

The prlA4 Suppressor Can Restore Maltose Sensitivity. To more selectively probe the role of PrlA in hybrid jamming we used a genetic approach combining an export-defective signal sequence mutation with the *prlA4* suppressor allele of *prlA*. Signal sequence mutations such as *lamB17D*, when present in a *lamB–lacZ* gene fusion, block entry of the hybrid protein into the export pathway and cause the hybrid protein to accumulate in the cytoplasm (7). Accordingly, they confer a maltose-resistant phenotype to the fusion strain. As shown in the lower part of Table 1, this phenotype is reversed by the *prlA4* suppressor—i.e., maltose sensitivity is restored. The

decreased maltose sensitivity that is observed under suppressing conditions (strain BKR73A4) relative to the wild-type (strain pop3186) is probably the result of incomplete suppression of the 17D signal sequence mutation by the *prlA4* suppressor. Indeed, strains that carry *lamB–lacZ* hybrid genes with signal sequence mutations that cause a more pronounced export defect than *lamB17D* and/or that are suppressed less well by *prlA4* remain maltose-resistant in the presence of the suppressor (unpublished data).

Maltose Sensitivity Is Recessive in a prlA$^+$/prlA Diploid. Initial characterization of *prlA4* showed that the suppressor is dominant to the wild-type gene in diploid analysis (2). This dominance reflects the fact that the suppressor mutation is a missense mutation that results in a functionally altered gene product (10). An experiment demonstrating this dominance is shown in Fig. 1. Here it can be seen that the signal sequence mutation *lamBS78* blocks export of an otherwise wild-type LamB protein causing the accumulation of the precursor form of the molecule. When the *prlA4* suppressor was provided in trans on the plasmid pRLA41, processing of the LamBS78 precursor to mature product occurs with nearly wild-type kinetics. This result verifies the dominance of *prlA4* and demonstrates that the suppressor gene on the plasmid is fully functional. Indeed, the suppression observed with *prlA4* on the plasmid is better than that observed in haploid strains that carry *prlA4* at the normal chromosomal locus. Presumably this reflects significant basal level production of PrlA from the multicopy plasmid (note that this occurs even in the absence of induction with IPTG). When a similar dominance test (*prlA$^+$/prlA4*) was performed with a strain carrying the *lamB17D–lacZ* fusion, we found that *prlA4* behaved in a recessive manner—i.e., maltose sensitivity was not restored and the diploid strain remained completely maltose-resistant (lower part of Table 1). This result is striking because we predict that the signal sequence mutation present in the gene fusion should be suppressed in the diploid strain. Indeed, in a *prlA4/prlA4* diploid, maltose sensitivity is observed (lower part of Table 1). The maltose sensitivity of the *prlA4/prlA4* diploid indicates that the recessive nature of *prlA4* in the diploid test cannot be explained by simple overproduction of PrlA. In addition, we can conclude that the maltose resistance observed in the *prlA$^+$/prlA4* strain is not due to effects of protein products other than PrlA encoded by pRLA41. Further support for these conclusions comes from the observation that the maltose-sensitive strain BKR73A4 (*lamB17D–lacZ, prlA4*) can be rendered maltose-resistant by the introduction of an F′ factor (F′141) that carries *prlA$^+$* (lower part of Table 1).

Maltose Sensitivity Correlates with Precursor Accumulation. To confirm that the maltose-sensitive and -resistant phenotypes observed in the *lamB–lacZ* fusion strains carrying different alleles of *prlA* accurately reflect the presence or absence of a jammed export complex, we measured the translocation of a representative wild-type protein, MBP, using a standard pulse–chase assay. This assay monitors the rate of processing of the precursor form of MBP to the mature form, a reaction catalyzed by leader peptidase at the periplasmic face of the inner membrane. Normally, translocation and processing are quite rapid; however, if the export apparatus is jammed by the LamB–LacZ hybrid protein, the precursor form of MBP accumulates in the cell.

The pulse–chase data for the various strains following a 60-min induction with maltose are presented in Fig. 2. The degree of precursor accumulation in the parent strain pop3186 (*lamB–lacZ prlA$^+$*) is shown in Fig. 2*A*. Under these conditions the precursor form of MBP predominates up to the 2-min time point. When the 17D signal sequence mutation is present on the hybrid gene (strain SE1073, Fig. 2*B*), precursor accumulation is virtually abolished as would be expected since the hybrid protein fails to enter the export pathway. When the suppressor allele *prlA4* is present together with the *lamB17D–lacZ* fusion (strain BKR73A4) precursor MBP accumulates to the same degree as that caused by the wild-type fusion in the *prlA$^+$* background (compare Fig. 2 *C* with *A*). This finding, in combination with the maltose-sensitive phenotypes described above, indicates that the block in export normally relieved by the signal sequence mutation has been restored in the presence of the suppressor allele *prlA4*. Fig. 2*D* shows the results obtained with the *lamB17D–lacZ prlA$^+$/prlA4* diploid. Again, as predicted from the maltose-resistant phenotype of the strain, nearly normal export of MBP is observed. Thus, the maltose-sensitive phenotype correlates with precursor accumulation and the recessive nature of *prlA4* with respect to this phenotype is confirmed.

A Plausible Model. To account for the seemingly paradoxical behavior of *prlA4* in diploid analysis (compare Figs. 1 and 2), we offer the explanation shown in Fig. 3. According to this view, protein translocation across the inner membrane occurs via PrlA or a complex that includes PrlA. LacZ hybrid proteins interfere with the translocation reaction by direct interaction with, and sequestration of, PrlA (Fig. 3*A*). Signal sequence mutations would prevent this interaction by preventing entry of the hybrid protein into the export pathway (Fig. 3*B*) and consequently, no general block to export would be predicted. When the suppressor allele, *prlA4*, is present, recognition of the fusion protein by PrlA would be restored and a general block to the export of noncytoplasmic proteins would be observed (Fig. 3*C*). Fig. 3*D* shows the predicted situation for a strain that carries the suppressor and wild-type alleles of *prlA*. Under these conditions the hybrid protein with an altered signal sequence may sequester the suppressor PrlA4, creating a jammed complex, as is the case in Fig. 3*C*. However, because the wild-type PrlA fails to recognize the mutant hybrid, it would remain free to participate in general protein export, as is the case in Fig. 3*B*. The continued availability of wild-type PrlA would allow expression of a

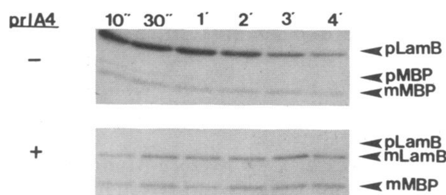

FIG. 1. Effect of the *prlA4* suppressor on the processing of LamB with an altered signal sequence. Cells were pulse labeled with [^{35}S]-methionine and immunoprecipitations were performed. The strain BKR104 was assayed with the vector pMLB1107.2 (*Upper*) and the plasmid carrying the suppressor allele of *prlA*, pRLA41 (*Lower*). In both cases, plasmid-borne genes are not induced with IPTG. "p" denotes the precursor form of the designated protein; "m" designates the mature product. Time points shown indicate the time of the trichloroacetic acid precipitation following the addition of chase.

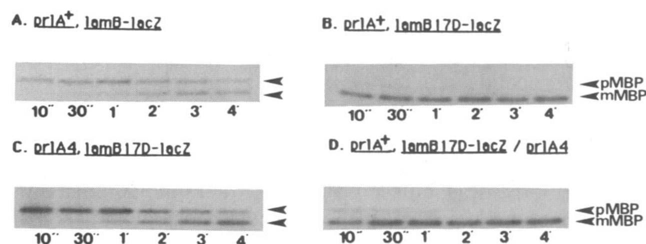

FIG. 2. Processing of MBP following induction of the *LamB–LacZ* fusions with maltose. The samples were prepared as described in the legend to Fig. 1. Strains are as follows: pop3186 (*A*); SE1073 (*B*); BKR73A4 (*C*); SE1073/pRLA41 (*D*).

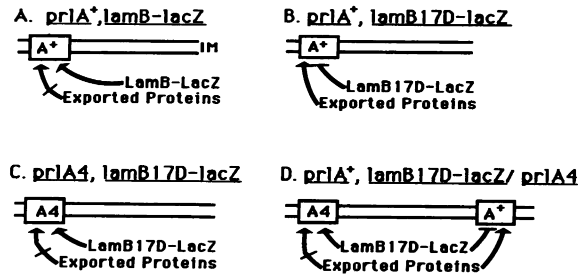

FIG. 3. Model for the maltose sensitivity phenotypes observed in the presence of *lamB–lacZ* fusion alleles when combined with wild-type and suppressor alleles of *prlA*. →, Recognition by PrlA; ⊣, no recognition by PrlA; ↛, recognition by PrlA blocked due to fusion jamming. The corresponding precursor accumulation assays are shown in Fig. 2. IM, inner membrane.

maltose-resistant phenotype and thus account for the recessive nature of the suppressor with respect to this phenotype. Moreover, because this model separates jamming from the suppression of signal sequence mutations, it can also account for the dominant suppression of signal sequence mutations carried on an otherwise wild-type *lamB* gene (Fig. 1).

The LamB17D–LacZ Hybrid Protein Selectively Blocks Export at PrlA4. The model shown in Fig. 3 predicts that the LamB17D–LacZ hybrid protein would specifically block export of proteins with mutant signal sequences at PrlA4 but that proteins that can be translocated via the wild-type PrlA would be unhindered. To test this hypothesis we took advantage of a second signal sequence mutation in *lamB, lamBS78*, which is not exported in wild-type, *prlA*+, strains but is efficiently exported in a *prlA*+/*prlA4* diploid (Fig. 1). Strain BKR7313/pRLA41 harbors the *lamB17D–lacZ* fusion and the *lamBS78* allele and both the wild-type and suppressor alleles of *prlA*. By using this strain we can monitor the effects of the LamB17D–LacZ hybrid protein on export via the PrlA4 suppressor (by assaying processing of LamBS78) and export via wild-type PrlA (by assaying the processing of MBP). This experimental scheme is shown in Fig. 4*A*.

The top autoradiogram in Fig. 4*B* shows the data obtained in a pulse–chase assay of LamBS78 and MBP export following a 60-min induction with maltose of strain BKR7313/pRLA41 (*lamBS78 lamB17D-lacZ prlA*+/*prlA4*). Even at the 4-min time point no processing of LamBS78 was observed, indicating that LamBS78 did not reach the periplasmic face of the inner membrane under these conditions. This is in contrast to MBP, whose processing is apparent at 10 sec and complete by the 2-min time point in the same strain under identical conditions. It is also in marked contrast to the rapid processing of LamBS78 that is observed in the isogenic strain lacking the fusion (BKR104/pRLA41, Fig. 1). Taken together, these results support the model presented in Fig. 4*A* and demonstrate that the LamB17D–LacZ hybrid protein specifically blocks export at PrlA4.

Strain BKR7313/pRLA41 is diploid for two different chromosomal regions: *lamB* (*lamBS78 lamB17D–lacZ*) and *prlA* (*prlA*+ *prlA4*). Accordingly, appropriate controls for the experiment described in the preceding paragraph involve strains in which each of these regions is altered in turn. In the first control, a strain that is isogenic except for the presence of *lamB*+ instead of *lamBS78* was used. In this strain (BKR7310/pRLA41), mature LamB and MBP were observed at the 10-sec time point with nearly complete processing of both proteins by 2 min (Fig. 4*B*). As expected, this strain is maltose-resistant and we presume that the slight export defect observed for both proteins relates to the inherent leakiness of the 17D signal sequence mutation (10). The second control involves a strain isogenic to BKR7313/pRLA41 except that both *prlA* alleles are *prlA4*. Pulse–chase

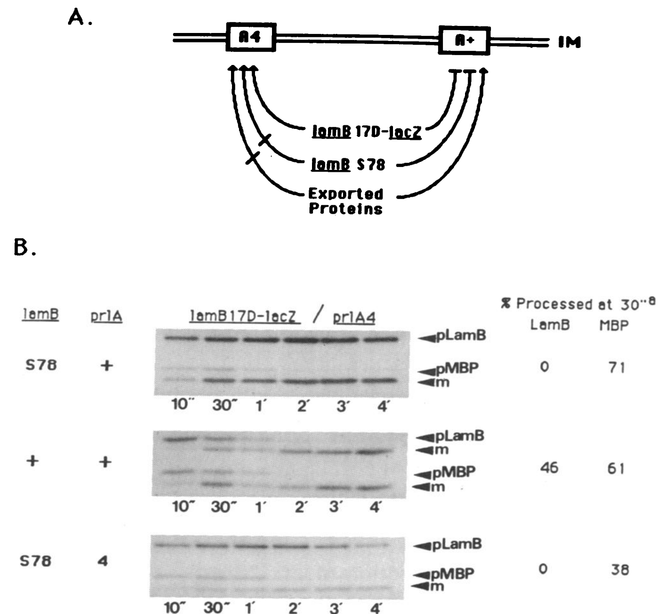

FIG. 4. Experimental design and assay for selective precursor accumulation in strains diploid for *prlA* and *lamB*. (*A*) The predicted situation following maltose induction of the strain BKR7313/pRLA41. Symbols are as described in the legend to Fig. 3. (*B*) The samples were prepared as described in the legend to Fig. 1. Strains assayed are (top to bottom) BKR7313/pRLA41, BKR7310/pRLA41, and BKR7314/pRLA41. The *lamB* and *prlA* alleles indicated on the left are chromosomal; the *lamB17D–lacZ* fusion is present on the phage λSE73 (7) and *prlA4* is carried on the plasmid pRLA41 as indicated across the top of the figure. Superscript a, % of LamB and MBP processed to the mature form was determined by densitometry.

assays reveal a complete block to LamBS78 export and a decrease by a factor of 2 in the amount of mature MBP that is present at the 30-sec time point relative to BKR7313/pRLA41. Possible explanations for the differential sensitivity of LamBS78 and wild-type MBP to jamming by the LamB17D–LacZ hybrid protein are considered in the *Discussion*. It is of note, however, that if induction time (with maltose) is increased to 2 hr instead of 1 hr, no processing of MBP is seen before the 2-min time point, and it is reasonable to expect that increased levels of the mutant hybrid protein are required to effectively jam a strain that contains higher amounts of PrlA4 (see Table 1). In any event, this strain is maltose-sensitive, as predicted, since all available export sites can be jammed.

DISCUSSION

A central question posed by the process of protein export is the mechanism by which the large hydrophilic molecules are translocated across a hydrophobic lipid bilayer. One necessary step in the elucidation of this mechanism is the identification of the cellular components that participate in this translocation reaction. In the *E. coli* system, genetic and biochemical approaches have been successfully used to address this issue. However, most of the cellular components identified are soluble cytoplasmic proteins and, in the few cases where a function can be assigned, this role appears to involve antifolding and perhaps piloting activities (17, 18). Because PrlA is the only truly integral inner membrane protein yet identified and because a role in translocation would seem to necessitate such a cellular location, it is commonly assumed that PrlA is a component of the translocator. The data we present provide evidence that this assumption may be correct.

The approach we used to probe the nature of the translocator is genetic and rests heavily on *lamB–lacZ* gene fusions and *prlA4*, a dominant suppressor of signal sequence mutations. The *lamB–lacZ* gene fusion used in these studies is known to jam the export apparatus of the cell, causing a generalized accumulation of precursors to exported proteins (7, 12). Evidence summarized in "Background and Rationale" suggests that this jamming occurs at the level of the translocator, leaving the hybrid protein stuck in a transmembrane fashion.

Because PrlA is clearly an integral inner membrane protein and since *prlA4* is a dominant suppressor of signal sequence mutations, we can use this allele to confer selectivity to that portion of the export machinery that is located in the membrane. Such selectivity is possible because PrlA4 will recognize proteins with altered signal sequences as being destined for export, whereas the wild-type counterpart, PrlA, will not. This selectivity permits meaningful interpretation of the diploid analysis described.

Two lines of evidence are presented to show that PrlA is the cellular component that is sequestered by hybrid jamming. First, overproduction of PrlA decreases the maltose sensitivity of *lamB–lacZ* fusion strains. Second, and perhaps more convincingly, *prlA4* will restore the maltose-sensitive phenotype conferred by a *lamB–lacZ* fusion carrying a signal sequence mutation. However, this restoration is masked if the cell also contains a *prlA*$^+$ allele. Because *prlA4* is a dominant suppressor of signal sequence mutations we conclude that its recessive nature with respect to maltose sensitivity reflects the fact that wild-type PrlA is insensitive to jamming by the mutant hybrid protein and therefore operates in normal fashion for protein export. Indeed, we can show a selective block in protein export via PrlA4 with little effect on PrlA$^+$ in the *prlA*$^+$/*prlA4* diploid strain (Fig. 4). Given these results and the topology of the hybrid protein discussed in "Background and Rationale," we propose that PrlA participates directly in the translocation reaction. Biochemical analysis will ultimately be required to verify our proposal directly.

Our results further suggest that PrlA is the only cellular component that becomes rate-limiting for protein export under conditions of hybrid jamming. This does not mean that PrlA is the only component of the translocator, although this is one intriguing possibility. Other proteins, if present in sufficient quantities, could be part of the translocator complex as well. It has been observed that synthesis of SecA is increased substantially under jamming conditions (19) and therefore may always be present in excess. Such regulation could exist for other components of the translocator as well.

We have also found that the export of proteins with altered signal sequences via PrlA4 is more sensitive to the jamming effects of hybrid proteins than is the export of wild-type proteins (Fig. 4). Several explanations for this result can be offered; we present but one. It seems likely that signal sequences perform multiple functions during the export process (10) and the signal sequence mutations we employ may well be multiply defective. For example, the signal sequence may be recognized by a cytoplasmic export factor. Since the *prlA4* suppressor would not restore interaction of the mutant signal sequence with such a cytoplasmic factor, the export of the mutant protein under suppressing conditions would be compromised and therefore more susceptible to interference from the hybrid molecule.

Genetic analysis of export components such as PrlA is hindered because of the essential nature of the function performed. Accordingly, the types of mutations that can be studied are limited to conditional lethals or suppressor alleles. Our results show that in *prlA*$^+$/*prlA4* diploids, the function of PrlA4 can be examined specifically by following the export of proteins (including LacZ hybrid proteins) with altered signal sequences. Since PrlA4 is not essential under these conditions, the types of mutations that can be identified and studied are greatly expanded and we are hopeful that this will provide a means to probe the complex functions of this protein in greater detail.

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