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MINI-REVIEW

The *sec* and *prl* Genes of *Escherichia coli*

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Abstract

Two general approaches have been used to define genetically the genes that encode components of the cellular protein export machinery. One of these strategies identifies mutations that confer a conditional-lethal, pleiotropic export defect (*sec*, *sec*retion). The other identifies dominant suppressors of signal sequence mutations (*prl*, *pr*otein *l*ocalization). Subsequent characterization reveals that in at least three cases, *prlA*/*secY*, *prlD*/*secA*, and *prlG*/*secE*, both types of mutations are found within the same structural gene. This convergence is satisfying and provides compelling evidence for direct involvement of these gene products in the export process.

Key Words: Protein secretion; signal sequence; suppressors; hybrid proteins; gene fusions.

Introduction

Genetic analysis of protein export in *Escherichia coli* began with the demonstration that β-galactosidase could be targeted to a membrane location by gene fusion (Silhavy *et al.*, 1976). This illustrated that intragenic information specifying cellular location is cis-dominant and contained in a region of the gene corresponding to the amino terminus of the protein. Early work with *lacZ* fusions appeared shortly after the publication of the "Signal Hypothesis" (Blobel and Dobberstein, 1975) and the demonstration that bacterial proteins are also made initially in precursor form with a hydrophobic (signal) sequence at the amino terminus (Inouye and Beckwith, 1977). Taken together, these results suggested a conservation of mechanism

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throughout biology, a fact that continues to stimulate productive interchange between scientists working with many diverse organisms.

Because gene fusions are deletion substitutions, they permit identification of discrete sequences that function in the targeting process. The logic of this method has since been applied for the identification of location-specific targeting signals in several different organisms. In bacteria, however, the chief benefits of gene fusion technology stem from the observation that the function of certain enzymes is dependent upon their particular cellular location and from the discovery that *lacZ* fusions, in particular, can confer novel phenotypes. Here we will outline how the properties of gene fusions have been exploited to gain insights into the composition and nature of the cellular export machinery in *E. coli.*

Enzymatic Markers for Cellular Location

E. coli alkaline phosphatase (PhoA) is a dimer found naturally in the periplasmic space. Several lines of evidence establish that this enzyme does not function in the cytoplasm, apparently because the reducing nature of this environment prevents disulfide bond and dimer formation. Accordingly, *phoA* fusions will exhibit enzymatic activity only if the gene to which *phoA* is fused contains properly positioned export signals (Manoil and Beckwith, 1986). Thus, vectors for the construction of *phoA* fusions, either by genetic transposition or by recombinant DNA, can be used to probe DNA sequences for export signals simply by screening for constructs that specify active PhoA enzyme. For example, Tn*phoA* can be used in a variety of bacteria to identify genes that specify exported proteins (Manoil *et al.*, 1990).

Cytoplasmic membrane proteins typically contain multiple membrane-spanning segments and are often depicted in snakelike fashion as two-dimensional cartoons. Because the amino acid sequence of these proteins is distinctive, such a topological cartoon can often be deduced from the amino acid sequence (Kyte and Doolittle, 1982). PhoA fusions have been used extensively as a relatively simple test for topology. If *phoA* is fused to a target gene at a position corresponding to a periplasmic domain, PhoA sequences will be directed to the periplasm and the resulting construct will specify active enzyme. Conversely, fusions to a cytoplasmic domain fail to export PhoA and little or no activity results (Manoil and Beckwith, 1986). A detailed description of this and other uses of *phoA* fusions is provided in the recent review by Manoil *et al.* (1990).

β-Galactosidase functions selectively in the cytoplasm and, as such, behaves in a manner opposite PhoA (Froshauer *et al.*, 1988; Georgiou *et al.*, 1988). If by gene fusion, LacZ is directed from the cytoplasm, little or no

activity results (Oliver and Beckwith, 1981; Hall *et al.*, 1982). As discussed in more detail in the following section, it seems that LacZ cannot be exported to the periplasm and it is difficult to predict how it would behave if this could be accomplished. However, it is clear that if LacZ sequences are embedded in a membrane, activity is compromised and this again appears to reflect a problem in quaternary structure (Oliver and Beckwith, 1981).

LacZ and PhoA provide examples where mislocalization interferes with enzyme activity; cytoplasmic PhoA and membrane-embedded LacZ are inactive. To the geneticist this is valuable because in each case a selection for mutations that alter the export process is provided. Methods for selecting either $LacZ^+/PhoA^+$ or $LacZ^-/PhoA^-$ are known and thus, with the appropriate fusion strain, mutations that either restore or prevent LacZ or PhoA export can be obtained. Of these possibilities, only the $LacZ^+$ selection has been successfully applied for mutants in which protein export is altered. In particular, starting with a *lacZ* fusion strain in which the hybrid protein is membrane localized, selection for $LacZ^+$ has yielded mutations that cause a generalized block in protein export. As described below, most of the *sec* (*sec*retion) genes were identified in this manner.

Overproduction Lethality

For more than a decade we have known that the cellular export machinery cannot deal effectively with sequences of LacZ. Consequently, if *lacZ* is fused to a gene specifying a periplasmic or an outer membrane protein and high-level synthesis of the hybrid protein is induced, the cell dies owing to a lethal jamming of the export machinery. This is evidenced by the cytoplasmic accumulation of the precursor form of exported proteins within the moribund cell (Emr and Silhavy, 1980; Bassford *et al.*, 1979). For example, with appropriate *malE* (encodes the periplasmic maltose-binding protein)-*lacZ* or *lamB* (encodes the outer membane maltoporin LamB, the receptor for phage λ)-*lacZ* fusion strains, overproduction lethality is observed upon maltose addition since maltose induces high-level synthesis of both hybrid proteins. Accordingly, this phenotype is commonly referred to as maltose-sensitivity (Mal^s). Again, this is valuable to the geneticist because mutations affecting export can be obtained simply by selecting for maltose-resistance (Mal^r).

Why Does LacZ Jam?

What is the problem with the export of LacZ? First, it is not a question of general incompatibility between cytoplasmic proteins and the export

machinery; other cytoplasmic proteins can be exported (Bedouele and Duplay, 1988). Second, it is not simply a question of size; truncated forms of LacZ such as amber fragments jam as well (Bassford *et al.*, 1979). Nor is it the presence of a specific "poison" sequence; various nonoverlapping fragments of LacZ can cause problems too (Lee *et al.*, 1989). Insight into this question has come from the analysis of environmental conditions that ameliorate jamming.

We have observed that growth at high temperature can minimize the lethal effects of LacZ hybrid jamming. Using a reductionist approach, we have tracked this observation to the heat-shock response and specifically to the proteins GroEL and DnaK (Phillips and Silhavy, 1990). Increased expression of either of these proteins can relieve jamming. However, DnaK is more effective than GroEL in this regard. Given current thought about the functions performed by these heat-shock proteins, we suspect that the problem with LacZ relates to protein folding. According to this view, folding of LacZ would occur prior to translocation and the machinery would literally "choke" on the now bulky molecule. We do not know what level of folding this reflects but given results presented in the previous paragraph, we would argue against quaternary structure.

Signal Sequence Mutations

Most of the mutations obtained by selection for Mal^r starting with a *malE-lacZ* or a *lamB-lacZ* fusion strain alter the signal sequence of the gene to which *lacZ* is fused and block export of the hybrid protein specifically. When recombined genetically into the otherwise wild-type cognate gene, these "signal sequence" mutations block export of the resulting protein as well (Emr *et al.*, 1978; Bassford and Beckwith, 1979; Emr *et al.*, 1980; Bedouelle *et al.*, 1981). These results provided direct evidence for the functional role of the signal sequence in protein export and insights into the nature of this important export signal (see the articles by Gierasch and Inouye in this issue). In addition, these mutations, when present in an otherwise wild-type gene, form the basis for genetic selections that yield *prl* (*pr*otein *l*ocalization) mutations as described below.

Although it is not our purpose to describe signal sequences in detail, a brief summary is necessary in order to focus subsequent discussions of the cellular export machinery. In general, signal sequences can be divided into three contiguous parts. The amino-terminal hydrophilic segment is quite variable with respect to length and amino acid composition but contains one or more positively charged residues that are thought to facilitate binding of this sequence to the membrane. Mutations that replace positive charge with negative charge can result in an export defect (Pollitt *et al.*, 1986; Puziss

et al., 1989). In addition, these changes may also cause decreased synthesis (Hall *et al.*, 1983). The central hydrophobic core (8–12 residues) is the site of most export-defective signal sequence mutations (Benson *et al.*, 1985). These may either be deletions that reduce the length of this segment or substitutions that introduce a charged residue. The carboxy-terminal segment contains the processing site that has been described by the consensus Ala-X-Ala (von Heijne, 1983). Mutations in this region usually have little effect on export even if they block subsequent processing (Fikes and Bassfore, 1987).

prlF. Selection for Mal^r with a *lamB-lacZ* fusion strain yields mutations at *prlF*, a gene that lies near 69 min on the current *E. coli* map (Kiino and Silhavy, 1984). Because these mutations are unlinked to the fusion, they are correctly termed suppressors of Mals^s (overproduction lethality). Indeed, they suppress the Mal^s conferred by *malE-lacZ* fusions as well. In contrast to signal sequence mutations, which block hybrid protein export, *prlF* mutations relieve Mal^s by facilitating hybrid protein export. This is evidenced not only biochemically, but also by the LacZ phenotype. Signal sequence mutations localize the LamB-LacZ hybrid in the cytoplasm and thus confer a Lac$^+$ phenotype. In *prlF* strains, the hybrid protein is localized efficiently in the membrane, and these strains are Lac$^-$.

Initial characterization of *prlF1* was perplexing because it behaves in recessive (loss of function) fashion in diploid analysis. It seemed odd that loss of particular function could facilitate hybrid protein export without also causing some detectable effect on the export of other proteins. This apparent anomaly is explained by the discovery that *prlF* encodes a bifunctional protein that is autoregulatory (Kiino *et al.*, 1990). Thus, the *prlF1* mutation caused derepression of *prlF* transcription by inactivating the regulatory domain of the protein. The function of the other PrlF domain is not known and DNA sequence analysis provides no obvious clues. What is clear is that PrlF works by a mechanism different from the heat-shock proteins. In any event, *prlF1* shares certain similarities with other *prl* mutations as described below.

The *sec* Genes

Mutations That Render malE-lacZ Fusion Strains Lac$^+$

How do you isolate an export-defective mutant? This is not a trivial question because protein export is an essential function and you cannot select dead cells. It is true that you can screen collections of conditional-lethal mutants using biochemical criteria, and indeed, this has been done. However, such a method is laborious and, in the absence of additional criteria (see below, *secY*), largely unsuccessful. The Beckwith lab solved this problem

using *lacZ* fusions and the judicious application of the "leaky" mutant concept.

It may be useful, as an introduction to this problem, to consider an analogy that we will refer to for both *sec* and *prl* (see below) mutations. In the 1960's many laboratories sought to define the promoter by mutation. Scaife and Beckwith (1966) reasoned three types of promoter mutation, up, down, and off. In the case of *lac*, it was not obvious that up mutations could be found, and the search for off mutations had proven difficult since many uninteresting mutations confer this phenotype. Therefore, they sought (and found) downs, i.e., "leaky" mutations that decreased but did not abolish promoter activity. This provided a paradigm for export-defective mutations —search for those that decrease but do not abolish export activity. Because *lacZ* fusion technology allows direct coupling of Lac phenotypes to any gene, and because LacZ activity is dependent on cytoplasmic location (see above), it was logically inferred that selection for Lac^+, starting with a *malE-lacZ* fusion strain (Lac^- due to the membrane location), should yield "leaky," export-defective mutants. The rationale was that it is not necessary to internalize all of the hybrid protein to obtain a Lac^+ phenotype, only a fraction, and it seemed likely that the cell could survive a modest export block. To further enrich the mutant collection, Beckwith and colleagues imposed the additional criteria that the mutations responsible for the Lac^+ phenotype should, in and of themselves, confer a conditional-lethal phenotype that is also the result of a defect in protein export. In other words, the mutant strains should show accumulation of the precursor form of exported proteins under nonpermissive conditions. Using these criteria, they isolated mutations that define *secA* and *secB* (Oliver and Beckwith, 1981; Kumamoto and Beckwith, 1983). An analogous selection using *phoA-lacZ* or *lamB-lacZ* fusion strains yielded mutations in a third gene, *secD* (Gardel *et al.*, 1987).

secA. The original *secA* mutation is a temperature-sensitive lethal. In otherwise wild-type strains (i.e., in the absence of a *lacZ* fusion), this mutation causes a generalized export defect that grows progressively more severe as growth temperatures are elevated. Recessive null mutations in *secA* are lethal, demonstrating that SecA function is essential for cell viability at all growth temperatures (Oliver and Beckwith, 1982). The *secA* gene has been cloned and the DNA sequence determined (Schmidt *et al.*, 1988). SecA is a large (102 kDa) soluble protein that associates peripherally with the cytoplasmic membrane. Biochemical analysis demonstrates a functional role for SecA in protein export *in vitro* and shows that the protein possesses ATPase activity (Cabelli *et al.*, 1988; Cunningham *et al.*, 1989; Lill *et al.*, 1989). This raises the possibility that SecA may function in energy coupling. Details of these biochemical studies are presented elsewhere in this issue.

Synthesis of SecA is regulated in response to the protein export requirements of the cell. When the cellular export machinery is compromised, e.g., either by LacZ hybrid jamming or by shifting *secATs* strains to high temperature, synthesis of SecA increases tenfold or more (Oliver and Beckwith, 1982; Rollo and Oliver, 1988). While details of this regulatory response remain to be elucidated, recent reports suggest an autoregulatory mechanism operating at the level of translation (Schmidt and Oliver, 1989; see Oliver, this issue). Again, this observation is important to the geneticist because it suggests yet another means to search for export-defective mutants as outlined below.

secB. The *secB* gene is unique among the *sec* gene family because it is not a truly essential gene and because SecB is required for the efficient export of only a subset of exported proteins (Kumamoto and Beckwith, 1985). The *secB* gene is perhaps best described as conditionally essential. Null mutations in *secB*, such as Tn*5* insertions, fail to grow on rich media but survive on minimal media where growth is less rapid. Under these conditions, the *secB*::Tn*5* mutation causes a pronounced defect in the export of selected proteins (Kumamoto and Beckwith, 1985). The *secB* gene has been cloned and the DNA sequence determined (Kumamoto and Nault, 1989). SecB is a small (12 kDa) cytoplasmic protein and biochemical studies demonstrate antifolding activity that facilitates export by maintaining proteins in an export-competent form as described in detail elsewhere in this issue. Various studies suggest that SecB recognition specificity is determined by sequences within the mature portion of selected exported proteins (Collier *et al.*, 1988; Gannon *et al.*, 1989). However, recent work suggests recognition of the signal sequence as well (Watanabe and Blobel, 1989). Synthesis of SecB is not regulated in response to the export needs of the cell (C. Kumamoto, unpublished).

secD. The original *secD* mutations were recessive, cold-sensitive lethals that cause generalized accumulation of the precursor forms of exported proteins under nonpermissive conditions (Gardel *et al.*, 1987). Results of cloning and DNA sequence analysis indicate that this work has uncovered yet another essential *sec* gene, *secF*. These and other data (*phoA* fusions) suggest that both SecD and SecF are cytoplasmic membrane proteins (C. Gardel, K. Johnson, A. Jacq, and J. Beckwith, unpublished).

Mutations That Increase SecA *Synthesis*

Apparently the Lac^+ selection is biased in ways we do not yet understand. Starting with a *malE-lacZ* fusion strain, selection for Lac^+ yields mutations at *secA* and *secB* repeatedly. Because potential explanations are many, methods to circumvent this bias are not obvious. Accordingly, new selection procedures were devised.

As noted above, SecA synthesis is regulated in a manner that is directly proportional to the export needs of the cell. It follows, therefore, that "leaky" mutations that compromise the cellular export machinery would derepress SecA synthesis. Using a *secA-lacZ* fusion, which has been introduced into an otherwise wild-type cell, such derepression can be simply monitored by scoring the LacZ phenotype. Under these conditions, a "leaky" export-defective mutant would "up" *lac* expression. The lore of *lac* is extensive and a variety of different selections for Lac(ups) have been described. The Beckwith laboratory employed three selections (Riggs *et al.*, 1988). All worked. However, bias was again apparent. What follows is a synopsis of all three selections. To avoid mutations that increase *lac* expression for reasons unrelated to protein export, additional criteria analogous to those mentioned above for *sec* mutants were again applied: the mutations responsible for the Lac(up) phenotype should, in and of themselves, confer a conditional-lethal phenotype, and the lethality should result from a defect in protein export.

Using the Lac(up) selection, mutations in the genes *secA* and *secD* were again recovered as well as cold-sensitive mutations in the *secY* gene (Schatz *et al.*, 1989). In addition, a new gene, termed *secE*, was discovered. The overlap between the Lac^+ and the Lac(up) selections is satisfying, and it has been argued that all the essential *sec* genes have now been identified (Riggs *et al.*, 1988).

secY. The temperature-sensitive *secY24* mutation was originally isolated following localized mutagenesis of the chromosomal region surrounding *prlA*, and, in fact, *secY* is an allele of *prlA* (Ito *et al.*, 1983; see below). The *secY24* mutation causes a generalized accumulation of the precursor form of exported proteins that grows progressively more severe as growth temperatures are raised. The *secY/prlA* gene is located at the distal of the *spc* ribosomal protein operon (Schultz *et al.*, 1982; Cerretti *et al.*, 1983; Shiba *et al.*, 1984). The protein product, SecY, is an integral cytoplasmic membrane protein that is essential for protein export and cell viability. Based on the amino acid sequence (443 amino acids) and studies with *phoA* fusions, a topology model containing 10 hydrophobic, membrane-spanning segments has been proposed (see Ito, this issue). Biochemical analysis demonstrates a functional role for SecY in protein export *in vitro* (Fandl and Tai, 1987). Given its cellular location and detailed genetic studies described below, it seems that SecY is a part of the translocator, i.e., that part of the machinery that physically moves exported proteins across the membrane bilayer. Synthesis of SecY does not appear to be regulated (like SecA) in response to the export needs of the cell.

secE. The *secE* gene is located in an operon together with *nusG* that lies near the genes for the β and β' subunits of RNA polymerase, DNA sequence analysis predicts a protein of 127 amino acids with three membrane-spanning segments (Schatz *et al.*, 1989), and topology studies with *phoA* fusions

support this view. Sequence analysis also reveals some homology with SecY; the significance of this observation is not yet clear.

Summary

As outlined here, *lacZ* fusion technology was exploited to design two basic selections that yielded the *sec* mutants. Despite differences in the manner in which they were originally identified, all of the *sec* genes, except *secB*, specify a protein product that is essential for growth. If the function of this product is compromised by conditional-lethal mutation, protein export is blocked at an early step resulting in the accumulation of the precursor form of exported proteins. This block is quite general for periplasmic and outer membrane proteins. In the case of cytoplasmic membrane proteins, the situation is less clear. Apparently some of these proteins require *sec* gene function for proper insertion, while others do not. The reason for this differential Sec requirement is not yet clear but it may relate to the overall size and the bulk of amino acid sequences that must be translocated for the membrane protein in question. For example, leader peptidase, which contains a large periplasmic domain, requires Sec participation (Wolfe *et al.*, 1985, while the much smaller M13 coat protein does not (Watts *et al.*, 1981). Although SecB is not truly essential, protein export is compromised severely in its absence. However, the export defects that are observed are confined to a subset of proteins including MalE and LamB (Kumamoto and Beckwith, 1985). PhoA and ribose-binding protein are exported normally without SecB. A summary of the *sec* gene family is presented in Table 1.

Table I. The *sec* and *prl* Genes[a]

Gene	Map position Min	Initial selection	Essential?	Phenotypes
secA	2.5	Export Defect-Lac$^+$	Yes	Ts, ED
secB	81	Export Defect-Lac$^+$	Conditional	LB$^-$, ED
secD	9	Export Defect-Lac$^+$	Yes	Cs, ED
secE	89	SecA-LacZ (Up)	Yes	Cs, ED
secY	72	Localized mutagenesis	Yes	Ts, ED
prlA	72	SS Suppression-Dex$^+$	Yes	GSS
prlC	68	SS Suppression-Dex$^+$	No	GSS
prlD	2.5	SS Suppression-Mal$^+$	Yes	GSS
prlF	69	Mal^r	No	Mal$^{r b}$
prlG	89	SS Suppression-Dex$^+$	Yes	GSS

[a] Mal^r resistance to LacZ hybrid jamming; SS, signal sequence; Ts, temperature sensitive; Cs, cold sensitive; ED, general export defect; LB, no growth on rich media; GSS, general signal sequence suppressor.

[b] PrlF1 suppresses some signal sequence mutations; see text.

The *prl* Genes

Selections for mutants th "leaky" export defects (export downs) allowed identification of the *sec* genes. An additional strategy for identifying genes whose products function in protein export would be to search for mutants in which the export process is enhanced (up). In analogy with promoter mutations (see above), the problem with this approach is twofold. First, protein export in wild-type cells is very rapid and efficient, and it is not obvious that enhancement is possible. Second, enhancement is not likely to cause a detectable change in phenotype anyway and, therefore, no appropriate selection can be designed. The availability of signal sequence mutations changes this situation. These mutations hinder export of the protein in question and, in some cases, confer a negative phenotype. For example, a subset of *lamB* signal sequence mutations cause a profound (strong) export block and confer a $LamB^-$ phenotype. In these cases, enhanced export can be detected by selecting for $LamB^+$ (ability to use maltodextrins as sole carbon source, Dex^+). In other words, suppressor mutations that enhance export of the mutant LamB protein could define genes that specify components of the cellular export machinery (Emr *et al.*, 1981).

General Considerations

Suppressors offer a powerful tool for the geneticist because they provide a means to probe the functional defect caused by a particular mutation. Indeed, suppressors have furnished key insights into many different fields of biological research including the genetic code, DNA replication and mutagenesis, transcription, translation, and protein and RNA structure/function. To the nongeneticist, suppressors are often confusing because there are many different types and they can exert their effects through numerous different mechanisms. Nonetheless, history demonstrates the utility of this approach and it is arguably the most effective method for probing an unknown mechanism or pathway.

Successful application of the suppressor approach requires careful consideration of the nature of the mutation to be supressed and the establishment of criteria that allow rapid and meaningful identification of interesting suppressors. In a sense, signal sequence mutations are ideal for suppressor analysis because these mutations alter a small segment of the protein that is physically removed during the export process. Thus, signal sequence mutations do not affect the structure or function of the mature protein; their effects are strictly confined to export. The problem with signal sequence mutations, in general, is that most have modest effects on export and are so "leaky" that they do not confer a detectable phenotype (Ferenci and Silhavy,

1987). Accordingly, one is forced to choose mutations that cause substantial changes such as deletions or those that place a charge into the hydrophobic core of the signal sequence. This choice no doubt limits the nature and types of suppressors that can be obtained.

The single most important criterion for suppressors of signal sequence mutations is that they restore export of the mutationally altered precursor. For example, suppressors of *lamB* signal sequence mutation can be obtained by selecting for growth on maltodextrins (Dex^+). Mutations that "bypass" the the requirement of LamB for maltodextrin transport will answer this selection (Benson and Decloux, 1985) and they, of course, have nothing to do with export. These can be simply identified because strains carrying these bypass suppressors remain resistant to bacteriophage λ infection (λ^r). If LamB export is restored, the strain will exhibit both a Dex^+ and a λ^s phenotype. In addition, it should be possible to demonstrate enhanced export of the mutant LamB protein biochemically using a pulse-chase assay, In such a test, more rapid cleavage of the mutant signal sequence can be observed. This demonstrates directly that the suppressor is acting at the level of export.

One might expect that suppressors of signal sequence mutations act by broadening the recognition specificity of the cellular export machinery so that altered signal sequence can be recognized. If this expectation is correct, then the suppressor should be dominant in diploid analysis. It seems unlikely that a loss of function (recessive) mutation could have such an effect without dire consequences for the cell. A useful analogy for such broadened specificity is the lactose permease, product of the *lacY* gene. This permease normally transports galactosides, yet by single amino acid substitutions it can be altered so as to transport maltose and α-glucoside. This substitution does not affect normal functions of the permease noticeably, i.e., lactose is still transported with kinetics that closely resemble the wild-type (Shuman and Beckwith, 1979). Apparently it simply broadens the substrate recognition capabilities. These *lacY* mutations are, of course, dominant.

Likely candidates for suppressors of signal sequence mutations are genes whose product interacts with the signal sequence during the export process. Genetically, evidence supporting direct interaction could be provided by the demonstration of allele-specificity. In recent years the concept of "interactive suppressors" has been widely disseminated and, consequently, the technique is commonly employed. Although conceptually simple, the demonstration of clear-cut allele-specificity (one suppressor for one and only one mutation) is quite difficult even in cases where direct interaction is almost certain. It may be that allele-specificity is a "Holy Grail" to which many strive unsuccessfully. This is especially true with respect to suppressors of signal sequence mutations for three reasons. First, what is the precise definition of allele-specificity? Many who claim interaction employ only a small number of

mutations and suppressors, and the mutations in question may be widely separated genetically; indeed, they may even affect different structural domains. If this is permissible, then demonstrating allele-specificity is easy. The signal sequence is small and thus all mutations are tightly linked. Is a suppressor that effects all charge substitutions in the hydrophobic core (an 8–12 amino acid stretch) allele-specific? Is it allele-specific if it affects mutations that cause similar amino acid substitutions in adjacent codons or different changes at the same codon? Do we really want to impose the rigid criterion that it can only suppress a particular signal sequence mutation (clear-cut allele-specificity)? If so, then the quest is daunting indeed. Second, as noted above, suppressors can only be isolated with a small subset of signal sequence mutations because most are "leaky." Thus, it is quite possible that all selections for suppressors require a functionally similar compensatory mutation. Third, signal sequences are very diverse and we must conclude that the cellular components that recognize these sequences are rather promiscuous to begin with. Becuase of these problems, the lack of allele-specificity should not be construed to imply no direct interaction. Until more definitive tests can be applied, this remains an attractive possibility.

Suppressors of Signal Sequence Mutations

In general, suppressors of signal sequence mutations define the *prl* genes. The first such suppressors were obtained in a strain containing the *lamBs60* allele (a deletion mutation that removes 12 amino acids) by selecting for $LamB^+$ (Dex^+). This yielded suppressors at *prlA* and *prlC* (Emr *et al.*, 1981). A similar selection using *lamB14D* (a charge substitution in the hydrophobic core) yielded suppressors at *prlA*, *prlD*, and *prlG* (Stader *et al.*, 1989). Starting with a strain containing the *malE12–18* (a deletion that removes seven amino acids) or *malE14–1* signal sequence mutations, Bankaitis and Bassford (1985 and Ryan and Bassford (1985) identified suppressors at *prlA* and *prlD* in analogous fashion by selecting for $MalE^+$ (Mal^+) (Bankaitis and Bassford, 1985; Ryan and Bassford, 1985). All of the *prl* suppressors are dominant in diploid analysis, and pulse-chase experiments demonstrate that they restore processing of the mutant protein to some degree. In no case has clear-cut allele-specificity been demonstrated. Indeed, they suppress export-defective signal sequence mutations in several (perhaps all?) genes. However, none of these suppressors work on all types of signal sequence mutations. Also, they have no effects on mutations that alter amino acid sequences outside this export signal, nor do they adversely affect the export of proteins with wild-type signal sequences. Given the problems with interactive suppressors mentioned above, this may be as close to allele-specific as one can come.

prlA. Alleles of *prlA* such as *prlA4* or *prlA401* are the most general and the most potent suppressors of signal sequence mutations known (Stader

et al., 1986; Bankaitis and Bassford, 1985). Signal sequence mutations in several different genes are suppressed; indeed, suppresion by *prlA* has been used to prove that an unknown mutation alters the signal sequence (Michaelis *et al.*, 1983). In several instances these suppressors restore processing of a mutant precursor at rates and levels that are nearly indistinguishable from the wild-type and, in these cases, normal levels of the otherwise wild-type, mature protein are found in the correct cellular location. Most export-defective signal sequences that contain alterations within the hydrophobic core are suppressed to some degree (5–95% depending on the particular mutation) and typically the export that is observed under conditions of partial suppression is posttranslational, i.e., it occurs after synthesis is complete. In contrast, export-defective mutations that alter the amino terminus of the signal sequence are unaffected (Stader *et al.*, 1986; Puziss *et al.*, 1989).

The *prlA* gene lies at the distal end of the *spc* ribosomal protein operon (Schultz *et al.*, 1982) and, as mentioned above, *secY* mutations are alleles of this gene (see *secY*). DNA sequence analysis of three suppressors has been reported (Sako and Iino, 1988; Stader *et al.*, 1986). The two "strong" suppressors, *prlA4* and *prlA401*, alter amino acids within transmembrane segments 7 and 10, respectively. This could indicate that PrlA recognizes signal sequences after membrane insertion. However, *prlA3*, a "weak" suppressor allele, alters an amino acid that is predicted to be in a periplasmic domain, and the significance of this is far from clear.

prlC. The *prlC* suppressors are unique. "Strong" alleles, such as *prlC8*, restore rapid and complete processing of the *lamBs78* (a four amino acid deletion) mutant signal sequence by leader peptidase (Trun and Silhavy, 1989). However, the resulting wild-type, mature LamB protein is exported to the outer membrane with poor efficiency. The suppressor directs efficient membrane insertion of the mutant signal sequence, but processing occurs before the membrane-bound components of the export machinery can recognize and translocate the majority of the inserted precursor molecules. As a consequence, the bulk of the mature protein is not translocated.

The *prlC* suppressors are more discriminating than *prlA* in that effects are observed with only a subset of *lamB* signal sequence mutations: those that alter the amino-terminal region of the hydrophobic core (Trun and Silhavy, 1989). Genetic analysis suggests that *prlC* does not specify an essential function and, if so, then *prlC* is not a *sec* gene by definition (see above).

Recent work suggests that *prlC* may be allelic with a gene in *Salmonella typhimurium* termed *optA* (N. Trun, D. Dolinger, T. Silhavy, C. Conlin, and C. Miller, unpublished). The *optA* gene is thought to specify an enzyme involved in signal sequence degradation (Novak *et al.*, 1986). If true, this is both satisfying and intriguing. It is satisfying because it shows that PrlC recognizes signal sequences normally. It is intriguing because it may suggest

a mechanism for suppressor function. Perhaps rather than removing signal sequences from the membrane, the suppressor drives the reaction in reverse, causing insertion of the mutant signal sequence.

prlD. The *prlD* suppressors resemble the *prlA* suppressors in that they suppress, to some degree, a number of different signal sequence mutations. However, there are interesting differences. First, *prlD* suppressors are less potent than *prlA4* or *prlA401* for suppression of signal sequence mutations that alter the hydrophobic core (Ryan and Bassford, 1985). On the other hand, *prlD2* suppresses the export defect resulting from charge alterations in the amino-terminal portion of the signal sequence while *prlA* suppressors do not (Puziss *et al.*, 1989). Bassford and colleagues have raised the possibility that PrlD interacts more strongly with the hydrophilic, amino terminal segment while PrlA interacts preferentially with the hydrophobic core of the signal sequence (Puziss *et al.*, 1989). This hypothesis is attractive owing to the soluble nature of PrlD (see below), the membrane-bound location of PrlA, and the fact that the two suppressors are synergistic. One can easily envision how these gene products may function sequentially in the export pathway.

DNA sequence analysis reveals that the mutations *prlD2* through *5* are alleles of *secA* (Fikes and Bassford, 1989). Some confusion remains, however, because the original allele, *prlD1*, appears not be allelic with *secA* (Bankaitis and Bassford, 1985). If true, then *prlD1* is not an allele of *prlD* and should be renamed as it would define a new gene. The nature and precise location of this new gene is not known.

prlF. As described above, the *prlF* gene was defined originally by mutations that suppress overproduction lethality (Mal^s). Nonetheless, the mnemonic *prl* is merited because *prlF1* does suppress a subset of signal sequence mutations. In particular, *prlF1* suppresses the export defect associated with charge alterations in the hydrophilic, amino-terminal portion of the signal sequence (Puziss *et al.*, 1989; Iino and Sako, 1988). In this regard, *prlF1* is unique because the export that is observed under suppressing conditions occurs in a strictly cotranslational manner. The significance of this observation is not yet clear but the demonstation of two entirely different export-related functions for PrlF strengthens greatly the premise that this protein has a role in protein export. This is true despite the fact that *prlF* does not specify an essential function and therefore is not a *sec* gene by definition.

It is worth noting at this point that *prlF* provides a vivid demonstration of the limitations inherent in the search for suppressors of signal sequence mutations. As noted above, most signal sequence mutations, including those that alter the hydrophilic amino terminus, are "leaky" and therefore cannot be used for selection. The *prlF1* mutation has no effect on "strong" signal sequence mutations that alter the hydrophobic core and, therefore, *prlF* mutations will never answer the requisite selection.

Table II. Genetically Defined Structural Components of the Export Machinery

Component	Molecular weight (kD)	Cellular location	Function
PrlA/*SecY*	49	Membrane	Translocator
PrlC/	68	Cytoplasm	?
PrlD/*SecA*	*102*	*Cytoplasm/Peripheral membrane*	*ATPase*
/SecB	*12*	*Cytoplasm*	*Antifolding*
PrlG/SecE	*13.6*	*Membrane*	*?*
/SecD	*Information on* SecD *is just emerging. See text for a summary.*		

prlG. Supressors at *prlG* were not uncovered until selections were refined to allow the use of point mutations (*lamB14D*) instead of deletions (*lamBs60*). Not surprisingly, the *prlG* suppressors are "weak." Although phenotypic suppression can be observed with a variety of mutations that alter the hydrophobic core of either the MalE or the LamB signal sequence, biochemical demonstration of increased export (a much less sensitive method) is possible only with those signal sequence mutations that are quite "leaky" (Stader *et al.*, 1989). Accordingly, questions regarding the specificity of suppression become somewhat subjective. However, given this qualification, it would appear that the pattern of *prlG* suppression resembles that seen with *prlA*.

Genetic studies indicated that the *prlG* mutations were likely to be allelic with *secE* and this has since been verified by DNA sequence analysis (P. Schatz and J. Beckwith, unpublished).

Summary

The search for suppressors of signal sequence mutations has allowed identification of the *prl* genes. Results summarized in this section and Table I demonstrate the utility of this approach for studies of protein export. In at least three cases, *prl* suppressor mutations alter the same gene as export-defective *sec* mutations that were identified by a completely different selection (*prlA/secY*, *prlD/secA*, and *prlG/secE*; see Table II). This convergence is satisfying and provides compelling evidence for direct involvement of these gene products in the export process.

The Missing Letters

Astute readers have no doubt noticed a few missing letters in the lists of *sec* and *prl* genes, and some comment on these omissions seems appropriate.

secC *and the* ssa *and* ssy *Genes*

Jarvik and Botstein (1975) pioneered an approach for identifying genes whose products interact. In this method, one selects for suppressors of

conditional-lethal (e.g., temperature-sensitive) mutations and screens the suppressors for ones that confer a new conditional phenotype (e.g., cold-sensitive). Several laboratories have attempted to apply this approach with the *sec* mutations. In particular, suppressors of temperature-sensitive *secA* and *secY* mutations have been isolated. With *secA*, mutations at *secC* (an allele of *rpsO*) and several genes termed *ssa* (*s*uppressors of *secA*) were identified (Brickman *et al.*, 1984; Ferro Novick *et al.*, 1984; Oliver, 1985). In the case of *secY*, several genes termed *ssy* (*s*uppressors of *secY*) were recognized (Shiba *et al.*. 1984). However, subsequent studies showed that most, if not all, of these mutations act by slowing the rate of protein synthesis (Lee and Beckwith, 1986). Indeed, low levels of chloramphenicol can effectively suppress these temperature-sensitive mutations. Accordingly, *secC* and the *ssa* and *ssy* mutations do not identify new genes that specify components of the cellular export machinery.

The failure of the Jarvik–Bostein approach with *sec* genes does not invalidate the method. The approach is sound and has been applied successfully in other systems. Rather, the failure highlights the problems inherent in the suppressor approach when using poorly defined mutations (see above). In other words, the *secA* and *secY* alleles employed are not appropriate. When one considers the functional defects likely to be manifest by temperature-sensitive mutations, it is not surprising that many are simply not suitable. This approach offers great promise, but successful application will require a larger, well-characterized collection of *sec* mutations.

prlB. The *prlB* mutation was identified as a suppressor of *lamBs60* (Emr *et al.*, 1981). It soon became apparent, however, that all was not well. First, repeated attempts to obtain additional alleles ended in failure. Second, and more serious, the *prlB* mutation was found to be a deletion in the gene that specifies the periplasmic ribose-binding protein. Because the mutation is dominant, it is clear that the deletion creates a novel product and obviously is not a component of the export machinery. The mechanism by which the *prlB* suppressor acts is not understood.

prlE. The *prlE* mutations were identified by S. Benson in a search for "leaky" export-defective mutants. They exhibit a slight cold-sensitive growth defect and cause generalized precursor accumulation. They are not suppressors of signal sequence mutations and therefore this name is confusing. These mutations are alleles of *secD* and should be referred to as such (Gardel *et al.*, 1987).

Problems and Promising Genetic Solutions

It is often useful to describe complex biological processes in terms of a pathway. Viewed in this manner, protein translocation across the inner

membrane would be envisioned as a series of sequential steps ordered in three dimensions beginning with the precursor form of the protein in the cytoplasm and ending with the mature form of the opposite side of the membrane bilayer. Genetic analysis has revealed several cellular proteins that participate in this pathway, and we would like to express the functions performed by the Sec/Prl proteins in a sequential as well as spatial manner. Historically, geneticists have resolved order in biological pathways using tests of epistasis. In essence, this requires characterization of the intermediate that accumulates in each of the various individual mutants and in double mutants containing all possible pairwise combinations of mutations. In practice this approach fails with the *sec* (export-defective) mutations because all of these mutations cause precursor accumulation in the cytoplasm and there is, as yet, no biochemical method to distinguish various export intermediates.

We believe that the *prl* mutations offer a potential method for identifying and characterizing export intermediates. As evidenced by their dominance, these mutations alter rather than decrease or abolish function like the corresponding recessive *sec* alleles (Table II). Accordingly, they offer distinct advantages because in combination with a signal sequence mutation they provide a means to direct a particular protein to the suppressor component selectively. To clarify this point, consider the *prl4* suppressor, a potent suppressor that restores function to defective signal sequences in dominant fashion. Recall that *lacZ* fusions often confer a phenotype termed overproduction lethality; *lamB-lacZ* fusion strains are Mal^s (see above). Signal sequence mutations block hybrid protein export and relieve this lethality (Mal^r). As expected, introduction of the *prlA4* suppressor restores lethality (Mal^s reappears). However, with respect to this lethality, the *prlA4* suppressor is recessive. This seemingly anomalous result can be understood when one considers that the lethal hybrid protein is selectively targeted to PrlA4 owing to the "allele-specific" interaction between the suppressor and the signal sequence mutation. The wild-type PrlA present in diploids is unaffected and therefore continues to function, allowing cell survival. Thus, by appropriate genetic manipulation, we can selectively inactivate PrlA4, a method we term suppressor-directed inactivation (SDI) (Bieker and Silhavy, 1989). Moreover, SDI, coupled with the toxic nature of LacZ, allows us to trap and maintain a novel export intermediate in viable cells (Fig. 1).

The results of SDI at PrlA4 can be extended in several profitable ways. First, since we can show that the hybrid protein inactivates PrlA4 while in transmembrane orientation, it follows that PrlA is an important component of the translocator (Bieker and Silhavy, 1989). In this case, we have trapped or frozen export during the translocation reaction. Second, it should be possible, by appropriate strain construction, to determine if other known Sec proteins are also part of the translocator complex. If so, then these Sec

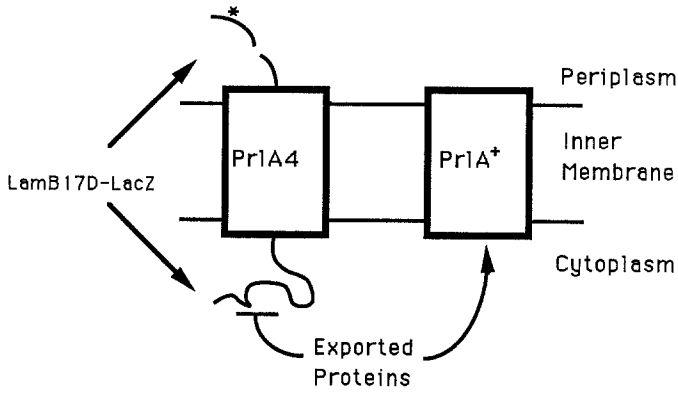

Fig. 1. Suppressor-directed inactivation (SDI) of the PrlA4 protein in a *prlA4/prlA+* diploid using a LamB-LacZ hybrid protein with a defective signal sequence. → indicates recognition by PrlA and ⊣ indicates that export is blocked as a consequence of hybrid jamming. (*) denotes a defective signal sequence.

proteins should also be trapped and thus inactivated by SDI at PrlA4. Finally, using other suppressor alleles, we should be able to trap the export reaction at different steps. Indeed, preliminary results with PrlG support this premise. Accordingly, it should be possible, using the logic of epistasis, to order events in the export pathway.

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