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# The allele-specific synthetic lethality of *prlA-prlG* double mutants predicts interactive domains of SecY and SecE

**Ann M.Flower[1], Robert S.Osborne[2] and Thomas J.Silhavy[3]**

Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA
[1]Present address: Du Pont Merck Pharmaceutical Co., Experimental Station, Wilmington, DE 19880, USA
[2]Present address: Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720, USA

[3]Corresponding author

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**The secretion of proteins from the cytoplasm of *Escherichia coli* requires the interaction of two integral inner membrane components, SecY and SecE. We have devised a genetic approach to probe the molecular nature of the SecY–SecE interaction. Suppressor alleles of *secY* and *secE*, termed *prlA* and *prlG*, respectively, were analyzed in pair-wise combinations for synthetic phenotypes. From a total of 115 combinations, we found only seven pairs of alleles that exhibit a synthetic defect when present in combination with one another. The phenotypes observed are not the result of additive defects caused by the *prl* alleles, nor are they the consequence of multiple suppressors functioning within the same strain. In all cases, the synthetic defect is recessive to wild-type *secY* or *secE* provided *in trans*. The recessive nature argues for a defective interaction between the Prl suppressors. The extreme allele specificity and topological coincidence of the mutations represented by these seven pairs of alleles identify domains of interaction between SecY/PrlA and SecE/PrlG.**

*Key words*: protein secretion/Sec61/suppressors/translocation

## Introduction

The translocation of polypeptides across the cytoplasmic membrane of *Escherichia coli* requires the sequential co-operation of several different proteins that have been defined through the convergence of various genetic and biochemical analyses. Two main genetic approaches have been used. The first method sought conditional-lethal mutations that conferred a generalized protein secretion defect. The genes isolated by this strategy included *secA*, *secE* and *secY*. The second genetic approach employed a selection for suppressors of secretion-defective signal sequence mutations. Alleles of *secA*, *secE* and *secY* were identified by this screen and were termed *prlD*, *prlG* and *prlA*, respectively (for protein localization) (for reviews see Bieker *et al.*, 1990; Schatz and Beckwith, 1990). *In vitro* analysis of protein translocation also identified SecA/PrlD, SecE/PrlG and SecY/PrlA as essential components of the translocation reaction (Cabelli *et al.*, 1988; Brundage *et al.*, 1990; Akimaru *et al.*, 1991; Nishiyama *et al.*, 1991; for an alternative interpretation see Watanabe and Blobel, 1993; for a review see Wickner *et al.*, 1991). Clearly, the functions of these three proteins are crucial to the proper localization of periplasmic and outer membrane proteins. Several additional proteins have been identified by either genetic or biochemical analysis, but the roles of these auxiliary proteins are less clear (Brundage *et al.*, 1990, 1992; Matsuyama *et al.*, 1993; Nishiyama *et al.*, 1993; Arkowitz and Wickner, 1994; Douville *et al.*, 1994; J.Pogliano and Beckwith, 1994; K.Pogliano and Beckwith, 1994; Sagara *et al.*, 1994).

There is ample evidence to demonstrate that SecE/PrlG and SecY/PrlA interact during the translocation process. The two proteins can be chromatographically purified as a complex from detergent extracts (Brundage *et al.*, 1990) and can be co-immunoprecipitated by antibodies directed against the N-terminal portion of SecY or epitope-tagged SecE or SecY (Brundage *et al.*, 1990, 1992; Joly *et al.*, 1994). Further evidence has been obtained from studies which showed that overproduced SecY is extremely labile. The co-overproduction of SecE relieves this instability, suggesting a SecY–SecE interaction that stabilizes SecY (Matsuyama *et al.*, 1990). Finally, evidence for the SecE–SecY interaction also comes from a series of genetic studies that have identified translocation complexes *in vivo* that contain both SecE/PrlG and SecY/PrlA. These last studies suggested further that SecE must be present in functional excess to SecY, as the only limiting component in the complex formed was SecY/PrlA (Bieker and Silhavy, 1990).

SecY/PrlA and SecE/PrlG appear to be conserved throughout evolution. SecY homologs have been identified in other eubacteria, archaebacteria, plastids, yeast (Sec61p) and mammalian cells (Sec61α) (Gorlich *et al.*, 1992; Stirling *et al.*, 1992). Proposed SecE homologs also exist in other eubacteria, archaebacteria, yeast (Sss1p) and mammals (Sec61γ) (Esnault *et al.*, 1993; Hartmann *et al.*, 1994; Murphy and Beckwith, 1994). Strikingly, the mammalian counterparts, Sec61α and Sec61γ, have been isolated as a complex by two different procedures (Gorlich and Rapoport, 1993). Furthermore, genetic evidence for interaction of the yeast homologs, *SEC61* and *SSS1*, has been obtained (Esnault *et al.*, 1993).

Despite the wealth of data demonstrating the functional association of SecE and SecY (or their homologs), little is known about the specifics of this interaction. We have undertaken an analysis of the *prl* suppressor alleles of *secE* and *secY* to address this issue. We analyzed 115 pair-wise combinations of *prlA* and *prlG* alleles for synthetic defects. Synthetic phenotypes are those phenotypes that manifest only when two specific alleles are present in the same cell; neither allele by itself confers the phenotype.

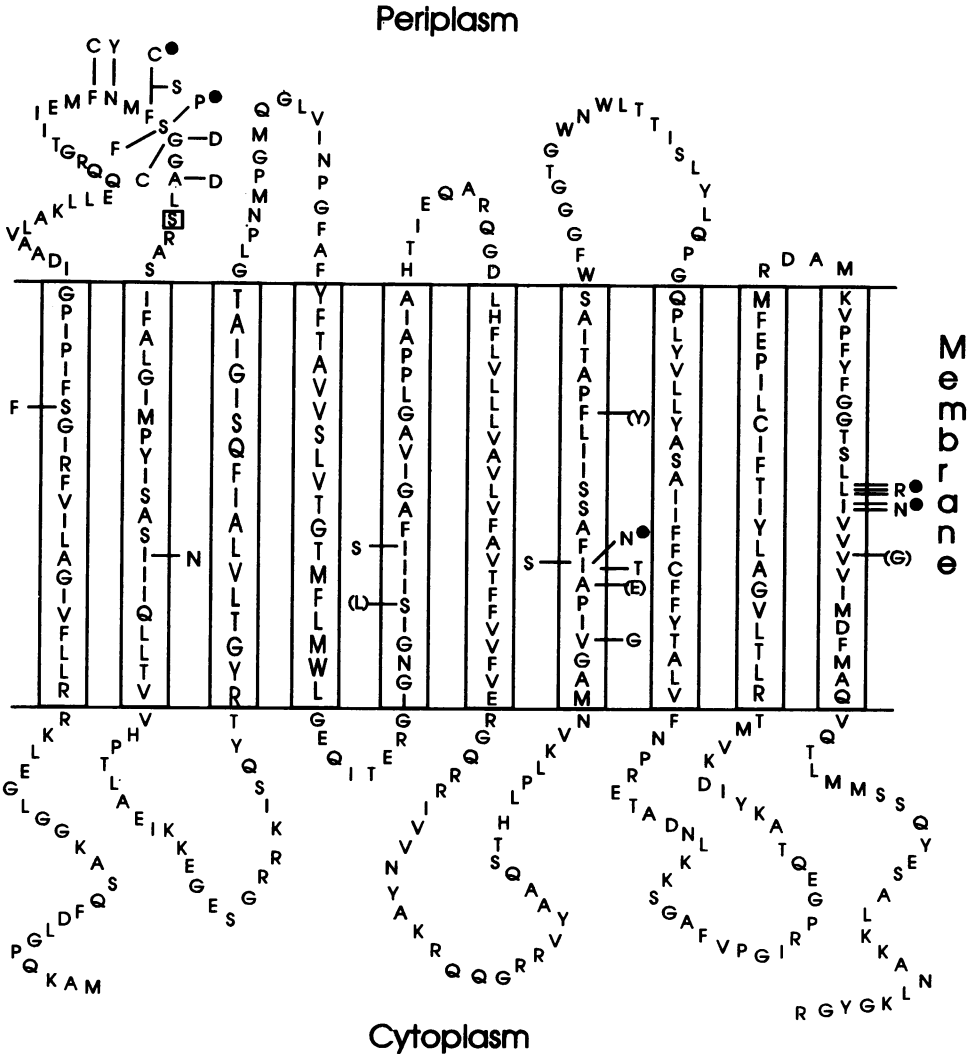


**Fig. 1.** SecY amino acid sequence showing predicted topology (Cerretti *et al.*, 1983; Akiyama and Ito, 1987). The PrlA suppressor mutations used in this study are indicated. The mutations that resulted in allele-specific synthetic lethality with certain *prlG* alleles are marked with a dot and are as follows: in periplasmic loop 1, PrlA3 = F67C and PrlA726 = S68P; in transmembrane helix 7 PrlA208 = I278N; and within transmembrane helix 10, PrlA11 and PrlA301 = L407R, PrlA4 and PrlA6 = I408N (see text for details). Mutations in parentheses are secondary mutations discussed in the text.

This type of genetic analysis has been used successfully to examine interactions between yeast proteins, for example Sec63p and Kar2p (Scidmore *et al.*, 1993) or Sec17p and Sec18p (Kaiser and Schekman, 1990). We believe that the allele-specific synthetic phenotypes observed here are caused by alterations in domains of proteins that normally interact.

## Results

Our collection of *prl* suppressors includes 23 different *prlA* alleles and five *prlG* alleles (Figures 1 and 2). Nineteen of the *prlA* alleles were described by Osborne and Silhavy (1993). We have identified another unique *prlA* allele (Flower *et al.*, 1994) and Derman and coworkers described five others (Derman *et al.*, 1993). We determined the DNA sequence of these last five alleles as well as the *prlA402* allele (Bankaitis and Bassford, 1985). Results show that three of these are identical to previously described mutations (*prlA402* = *prlA304*, Ile90 to Asn; *prlA8910* = *prlA303*, Ile208 to Thr; *prlA8912* = *prlA9*, Gly69 to Asp). The three other alleles encode novel mutations: the *prlA8911* allele encodes a mutation of Ser37 to Phe; *prlA8913* results in an alteration of Ser68 to Phe; and the *prlA8914* mutation changes Asn65 to Tyr. The *prlG* alleles have been characterized previously (Stader *et al.*, 1989; Schatz *et al.*, 1991; Flower *et al.*, 1994).

### *Complementation screen for synthetic lethal combinations*

To facilitate screening of *prlA-prlG* combinations for synthetic phenotypes, each of the chromosomal *prlG* alleles was subcloned into pBAD18 as described in Materials and methods. This vector, pBAD18, allows expression of cloned genes under control of the *araB* promoter; therefore expression is induced with arabinose. We demonstrated that each of the resultant plasmids (pAF26–pAF30) produces a functional gene product by complementation at 23°C of strains carrying the *secE15* cold-sensitive (Cs) mutation. In addition, expression of the suppressor alleles resulted in enhanced export of LamB

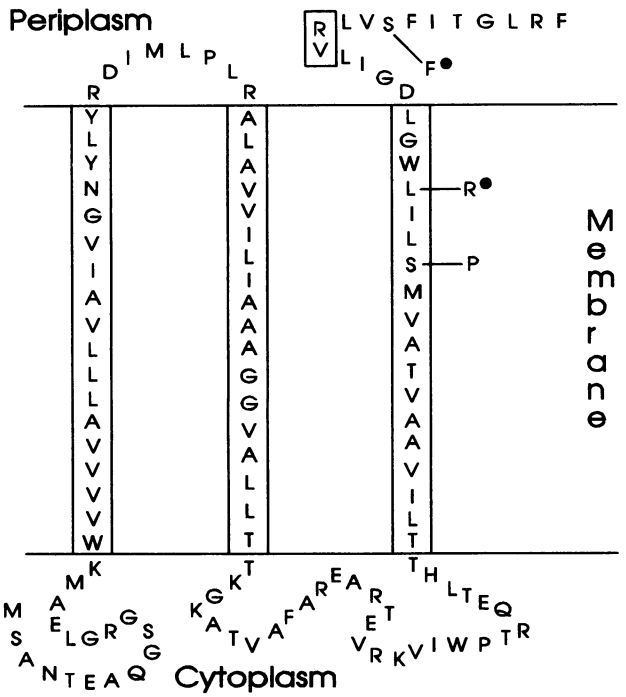


**Fig. 2.** SecE amino acid sequence and predicted topology (Schatz *et al.*, 1989; Downing *et al.*, 1990). All of the PrlG alterations are indicated, those resulting in allele-specific synthetic lethality are marked with a dot and are as follows: PrlG1 = L108R in transmembrane helix 3 and S120F in periplasmic loop 2 (see text for details).

with a defective signal sequence. Both complementation and suppression were completely dependent on the presence of arabinose.

We used a variation of the assay described by Osborne and Silhavy (1993) to test *prlA-prlG* combinations for synthetic defects. A series of isogenic strains was constructed that carried one of the *prlA* alleles (*prlAx*), the *secE15*(Cs) mutation and *recA*::*cat* (AF276–298). Each of these strains was transformed with the parent vector, pBAD18, or one of the *secE/prlG*-encoding plasmids (pAF26, pAF27, pAF28, pAF29 or pAF30). The strains were screened at 23°C to test the ability of the plasmid-borne *secE/prlG* allele to complement the cold-sensitive phenotype caused by *secE15*. It is important to note that the *secE15* mutation causes an alteration to the ribosome binding region of *secE* (Schatz *et al.*, 1991), resulting in decreased expression of SecE, which manifests as a cold-sensitive defect. Therefore, complementation requires only that more functional SecE/PrlG be produced. Our assay is based on the supposition that complementation will only be possible if the PrlG protein provided by the plasmid is able to interact with the chromosomally encoded PrlA to form a functional translocator. The lack of complementation implies that the two proteins are unable to interact productively, resulting in a synthetic defect.

As expected, pBAD18 was unable to complement the cold sensitivity in any of the strains, while pAF26, carrying the wild-type *secE/prlG* allele, complemented the cold-sensitive defect in all *prlA* backgrounds (Table I). Furthermore, the wild-type *secY/prlA* strain was complemented by all of the *prlG* plasmids. These results demonstrated that this assay provides an accurate indication of functional translocator formation.

We then screened our entire collection of 23 *prlA* and five *prlG* alleles (including wild-type) in pair-wise combinations. Out of a total of 115 combinations, we observed only seven pairs of alleles that exhibited strong synthetic defects (Table I). As observed previously (Osborne and Silhavy, 1993), *prlG1* was unable to complement the *secE15*(Cs) defect in strains containing *prlA4*, *prlA6*, *prlA11*, *prlA208* or *prlA301*. Note that the *prlA* alleles that exhibited synthetic defects with *prlG1* are topologically clustered (Figures 1 and 3). All of these *prlA* alleles encode mutations in transmembrane domains 10 or seven of SecY/PrlA. As *prlG1* codes for a mutation in transmembrane helix 3 (Figures 2 and 3), the authors speculated that the synthetic defects observed between *prlG1* and these *prlA* alleles might reflect an altered and defective interaction between transmembrane helix 3 of SecE/PrlG and transmembrane helices 10 and 7 of SecY/PrlA.

In addition to the synthetic phenotypes of certain *prlA* alleles with *prlG1*, we found other pairs of alleles that were defective in this complementation assay. The *prlG3* allele was unable to complement the cold-sensitive *secE15* mutation in strains containing *prlA3* or *prlA726*. In addition, *prlG8* complemented poorly in the *prlA726* strain. These results indicated a non-functional interaction between *prlG3* and these *prlA* alleles, and a functional, but defective, interaction between *prlG8* and the *prlA726* allele. We noted that these pairs of synthetic defective alleles also follow a topological pattern. The *prlG3* and *prlG8* alleles encode mutations within the second periplasmic loop of SecE/PrlG (Figures 2 and 3), while *prlA3* and *prlA726* both code for alterations to amino acids within the first periplasmic loop of SecY/PrlA (Figures 1 and 3). This may indicate that these two periplasmic domains normally interact and that the interaction is perturbed by these pairs of suppressors.

### *Direct demonstration of synthetic combinations*

As stated above, we believe that the inability of a given *prlG* allele to complement *secE15* in a particular *prlA* background reflects an altered interaction between the two Prl proteins. The pairs of *prlA* and *prlG* alleles that exhibited synthetic defects based on our complementation screen are summarized in Figure 3. We predicted that strains carrying both alleles in haploid from any of these pairs would be inviable. In other words, it should not be possible to construct these double mutants by P1-mediated generalized transduction. As a control for this series of experiments, we chose the *prlA666* allele, which exhibited no synthetic defects with any *prlG* allele. Thus, we should be able to construct strains with *prlA666* and each of the *prlG* alleles. Strains containing a given *prlA* allele (*prlA3*, *prlA4*, *prlA11*, *prlA208*, *prlA301*, *prlA666* or *prlA726*) were transduced with P1 phage which was grown on cells carrying one of the *prlG* alleles and a closely linked Tn*10* (Table II). The co-transduction frequency of each *prlG* allele with this Tn*10* into a wild-type background was >90%. As expected, all of the *prlG* alleles exhibited the same co-transduction frequency when transduced into the *prlA666* strain (>90%). The co-transduction frequency into the other *prlA* strains varied greatly. Some strains

**Table I.** Complementation assay for synthetic phenotypes

| *prlA* allele[a] | Domain[b] | pBAD18[c] | *secE*$^{+}$[c] | *prlG1*[c] | *prlG2*[c] | *prlG3*[c] | *prlG8*[c] |
|---|---|---|---|---|---|---|---|
| *secY*$^{+}$ | NA | − | + | + | + | + | + |
| *prlA1* | TM7 | − | + | + | + | + | + |
| *prlA3* | P1 | − | + | + | + | − | + |
| *prlA4* | TM10,TM7 | − | + | − | + | + | + |
| *prlA6* | TM10,TM5 | − | + | − | + | + | + |
| *prlA7* | TM10,TM7 | − | + | + | + | + | + |
| *prlA9* | P1 | − | + | + | + | + | + |
| *prlA11* | TM10,TM10 | − | + | − | + | + | + |
| *prlA200* | TM5 | − | + | + | + | + | + |
| *prlA202* | TM7 | − | + | + | + | + | + |
| *prlA205* | P1 | − | + | + | + | + | + |
| *prlA208* | TM7 | − | + | − | + | + | + |
| *prlA300* | P1 | − | + | + | + | + | + |
| *prlA301* | TM10 | − | + | − | + | + | + |
| *prlA302* | P1 | − | + | + | + | + | + |
| *prlA303* | TM7 | − | + | + | + | + | + |
| *prlA304* | TM2 | − | + | + | + | + | + |
| *prlA306* | P1 | − | + | + | + | + | + |
| *prlA666* | P1 | − | + | + | + | + | + |
| *prlA726* | P1 | − | + | + | + | − | +/− |
| *prlA8911* | TM1 | − | + | + | + | + | + |
| *prlA8913* | P1 | − | + | + | + | + | + |
| *prlA8914* | P1 | − | + | + | + | + | + |

Assays were performed as described in Materials and methods. Results shown are at 23°C, in the presence of arabinose.
[a]Chromosomal *prlA* suppressor allele.
[b]Domain where the PrlA mutation is located. NA indicates not applicable.
[c]Plasmid encoded *prlG* allele that was introduced into the *prlA* strain. + indicates complementation of the cold-sensitive defect, − indicates no complementation, +/− indicates poor complementation.

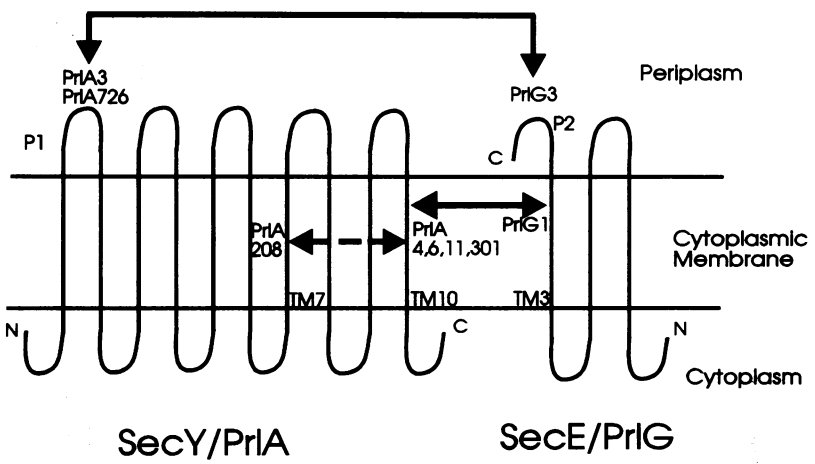


**Fig. 3.** Schematic of SecY and SecE demonstrating domains that are predicted to interact based on synthetic lethality assays. The Prl alleles that contribute to synthetic phenotypes are shown. Arrows indicate proposed domains of interaction.

containing double mutants could be constructed with approximately wild-type efficiency, while with other combinations the frequency was greatly reduced. In other cases, we were unable to construct the double mutant at all, despite repeated attempts.

With one exception, all of the double mutant combinations that we could not construct corresponded to those that we predicted to be inviable, supporting our suggestion that the two alleles are incompatible. Specifically, we could not construct strains carrying the following pairs of alleles: *prlA4-prlG1*, *prlA11-prlG1*, *prlA208-prlG1*, *prlA301-prlG1* or *prlA726-prlG3*. Obviously, the inability to construct a strain is a negative result, but given the very high frequency with which the *prlG* alleles can be moved into wild-type strains (or even other *prlA* strains) and the correlation of the strain construction results with the synthetic screen, we believe that these combinations truly represent synthetic lethal pairs. Further evidence to support this argument is presented below.

The one exception to our predictions of lethal combinations was the pair *prlA3-prlG3*. We were able to construct this strain (AF314), albeit at a much reduced co-transduction frequency. This confirms a previously reported result that *prlA3-prlG3* double mutants are viable (Bieker and Silhavy, 1990). Because this combination appeared defective in our complementation screen, we examined these results more closely. We discovered that this pair appeared defective in the complementation screen only at 23°C; when the assay is performed at 25°C or higher, complementation is observed. This implies that the inter-

**Table II.** Strain constructions demonstrate synthetic lethality

| *prlA* allele[a] | *prlG1*[b] | *prlG2*[b] | *prlG3*[b] | *prlG8*[b] |
|---|---|---|---|---|
| *secY+* | + | + | + | + |
| *prlA3* | (+) | + | (−) | + |
| *prlA4* | − | + | + | + |
| *prlA11* | − | + | + | + |
| *prlA208* | − | ND | ND | ND |
| *prlA301* | − | + | + | + |
| *prlA666* | + | + | + | + |
| *prlA726* | + | + | − | + |

[a]Chromosomal *prlA* allele.
[b]*prlG* allele that was transduced into the *prlA* strain. + indicates that the *prlG* allele could be transduced into a strain containing that *prlA* allele. − indicates that no transductants were identified that contained the given *prlG* allele.
ND indicates not determined. The combinations in parentheses are discussed in the text.

action between PrlA3 and PrlG3 is only partially functional, and can be disrupted at low temperatures. In accordance with this observation we could show that the *prlA3-prlG3* double mutant (AF314) exhibits a cold-sensitive growth defect; it fails to grow at 30°C or below.

It is important to note that the difficulties encountered in the construction of certain *prlA-prlG* double mutants were not simply the consequence of a deleterious effect caused by the presence of two strong suppressors in the same strain. If that were the case, we would not observe such strict allele specificity. In addition, the synthetic defects do not correspond to alleles that are the more potent suppressors. For example, the *prlA666* allele is an especially potent suppressor (Puziss *et al.*, 1992). However, there is very little, if any, alteration in the co-transduction frequency of any of the *prlG* alleles into a *prlA666* strain; furthermore, double mutants containing *prlA666* and any of the *prlG* alleles do not exhibit any detectable synthetic phenotypes, including cold sensitivity.

There is one final pair of alleles that merits comment, *prlA3-prlG1*. These alleles did not show a defect based on the complementation assay, and previous work suggested that the double mutant was viable (Bieker and Silhavy, 1990). We also can construct the double mutant, albeit at a low frequency (AF312). However, we were never completely confident that the strain we constructed had not acquired a secondary mutation. We have obtained no direct evidence to indicate that *prlA3-prlG1* is a synthetic lethal pair, but a firm conclusion must await further analysis.

### *Synthetic interactions are recessive and double mutants require a complementing plasmid*

There are several means by which a particular combination of *prl* alleles could exhibit a lethal phenotype: (i) if the two proteins interact in a strongly synergistic manner they could create a 'super' suppressor phenotype that results in lethal mislocalization of cytoplasmic proteins; (ii) the two proteins may interact, but in a non-productive manner or (iii) the two proteins might not interact at all. The first possibility can be distinguished from the latter two because the creation of a 'super' suppressor phenotype should be dominant. Even in the presence of wild-type Prl proteins, some doubly mutant translocators would form, leading to continued protein mislocalization. Non-productive interaction or lack of interaction would be recessive. It may also be possible to distinguish a non-productive interaction from a lack of interaction. In the former case, viability would be restored only if the gene for the component that is limiting is provided *in trans*. The defect caused by a failed interaction would be complemented by either gene (Figure 4).

To test the above predictions, diploid analysis was performed. Various *prlG* alleles were transduced into strains containing the *prlA* alleles in question and a wild-type copy of either *secE* or *secY* provided by low copy plasmids pRO13 or pJS100. Results show that all of the synthetic combinations can be rescued by either $secY^+$ or $secE^+$ *in trans*, indicating that in all cases the defect is recessive (however, see below for a discussion of *prlA726-prlG3*). This is contradictory to the explanation that invokes a 'super' suppressor phenotype.

It has been demonstrated previously that strains containing *prlA4* and *prlG1* on the chromosome along with a SecY-encoding plasmid lose the plasmid at a much reduced frequency relative to plasmid loss by wild-type strains (Bieker and Silhavy, 1990). This was interpreted as further evidence that the *prlA4* and *prlG1* alleles comprise an inviable combination: the complementing function of $secY^+$ was required. We investigated whether this was a unique property of synthetic lethal combinations or if it was generally applicable to strains that contain more than one suppressor. Strains containing *prlA301* and each of the *prlG* alleles on the chromosome and pJS100 ($secY^+$) were constructed (AF351–AF354, AF372). After 30 h of growth in non-selective media, significant plasmid loss (30–95%) was observed in all of the strains except the strain that contained *prlG1* (AF351). In this strain, 100% plasmid retention was observed. These results provide further support for our conclusion that the pairs of alleles identified as synthetic lethal combinations cannot be present in the same strain without a complementing allele *in trans*. In addition, they demonstrate that plasmid retention is not simply a consequence of multiple *prl* suppressors in the same strain.

### *PrlA726 and PrlG3 form an inactive complex*

It is difficult to distinguish between the two models proposing defective interaction (non-interaction versus non-productive interaction) owing to problems of gene dosage. Both $secY^+$ and $secE^+$ provided on low copy plasmids allowed construction of the double mutant strains. However, the *prlA726-prlG3* combination was complemented poorly by $secE^+$ provided *in trans* on the low copy plasmid pRO13 (AF345). The frequency of co-transduction was much reduced, and the colonies that contained both suppressor alleles were very small. In addition, this strain exhibited a cold-sensitive phenotype. When a higher level of $SecE^+$ was provided by the high copy plasmid pAF24 (AF374), the defect was complemented well and colony growth was similar to wild-type. We therefore believe that the defect in a *prlA726-prlG3* double mutant may be the result of the formation of an inactive complex between PrlA726 and PrlG3 that renders PrlA726 limiting in the cell (Figure 4). Because PrlA726 is the limiting factor in these inactive complexes, the low levels of $SecE^+$ supplied by pRO13 *in trans* (AF345)

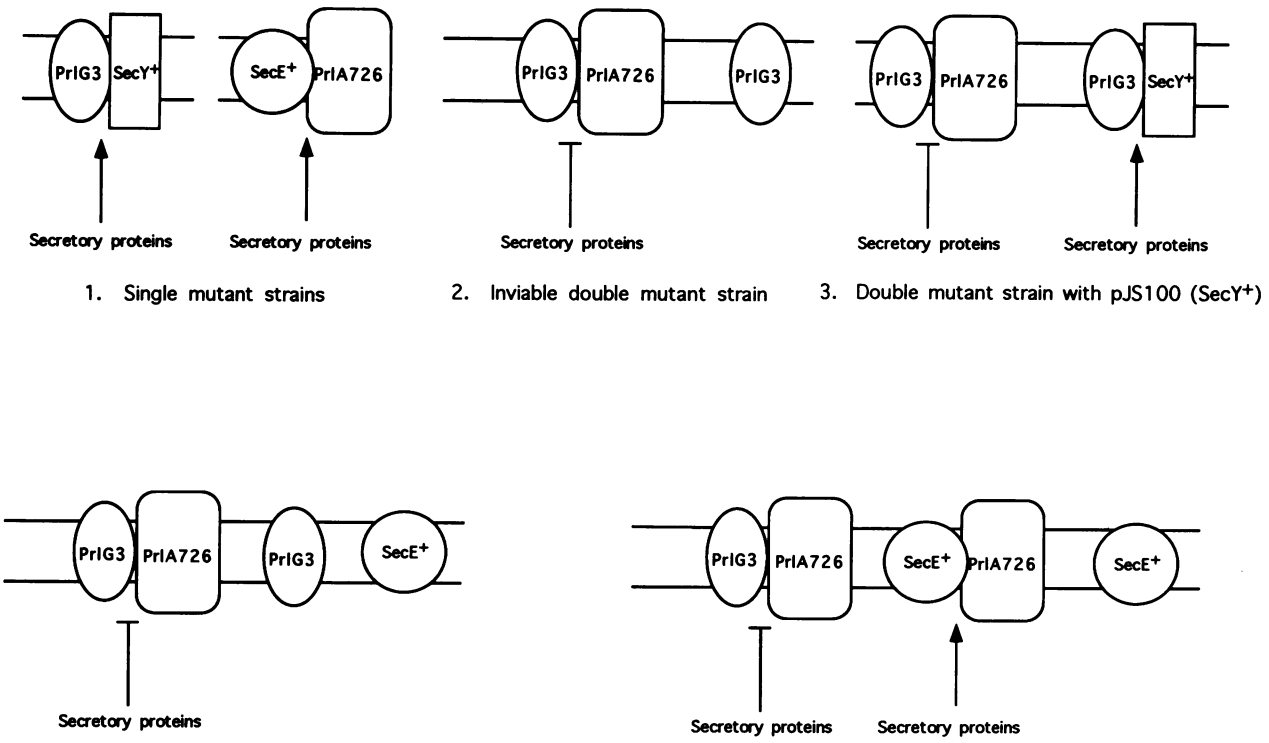


**Fig. 4.** Model of diploid analysis. (**1**) In a single mutant strain there is no detectable secretion defect. (**2**) The double mutant strain cannot be constructed due to a synthetic lethal secretion defect. Note that SecE/PrlG is in functional excess to SecY/PrlA. (**3**) The plasmid pJS100, which expresses $SecY^+$, allows construction of the double mutant strain. Complementation of the defect by SecY indicates that PrlA726 is the limiting component in the defective complex. (**4**) Low levels of $SecE^+$ provided by pRO13 complement the defect poorly because PrlG3 is not the limiting component. (**5**) High levels of $SecE^+$ (pAF24) are able to titrate the PrlA726 away from the inactive complex.

provides only marginal help. Low levels of $SecY^+$ encoded by pJS100 (AF358) provide sufficient wild-type SecY to form active complexes with PrlG3, which must be present in functional excess. The high copy plasmid, pAF24, expresses sufficient $SecE^+$ to titrate some of the PrlA726 away from the inactive complexes (AF374).

The above hypothesis was further supported by an additional observation. As a routine control for the complementation screen discussed above, all strains were grown in the presence or absence of arabinose at 37°C as well as at 23°C. We found that the strain containing *secE15* and *prlA726* on the chromosome and *prlG3* on the arabinose-inducible plasmid (pAF29) was sensitive to arabinose induction, even at 37°C (determined by growth on LB plates containing 0.2% L-arabinose). This was unusual as all of the other synthetic lethal pairs could only be detected by the lack of complementation of *secE15* at 23°C. This implies that PrlA726 and PrlG3 are able to form a complex such that the levels of wild-type SecE in the cell are not sufficient to form functional complexes with PrlA726. As the *secE15* mutation results in decreased levels of SecE even at 37°C (Schatz *et al.*, 1991), we introduced plasmid pAF29 into a strain that contained *prlA726* and wild-type *secE* on the chromosome (AF373). This strain was also sensitive to induction by arabinose at 37°C. This indicates that the PrlG3 is able to titrate PrlA726 into an inactive complex and that wild-type levels of $SecE^+$ are not sufficient to maintain cell viability.

### *Synthetic lethality is not due to two strong suppressors in the same strain*

The pairs of *prl* alleles that exhibit a synthetic defect are extremely rare and very allele-specific. Furthermore, as discussed above, the effects are not correlated with strong suppressors. Nevertheless, we wished to show that the detrimental effects were due to *specific* combinations of suppressors. To this end, we showed that the construction of chromosomally encoded synthetic double mutant strains could be rescued by another *prl* suppressor allele on a plasmid instead of the wild-type allele on a plasmid. As shown in Table III and described below, several strains were constructed in which the defect caused by the synthetic pair was complemented by a strong suppressor on the plasmid, as well as by a wild-type allele.

The *prlA11* and *prlA301* alleles both exhibit synthetic lethality with the *prlG1* allele. We built strains containing *prlA11* or *prlA301* on the chromosome with complementing plasmids carrying $secE^+$ (pAF26) or *prlG3* (pAF29). These cells were then grown in medium containing 0.2% L-arabinose to maintain expression of the plasmid encoded *secE/prlG*. We were able to introduce by P1 transduction either a non-synthetic partner allele (*prlG8*) or the synthetic lethal partner (*prlG1*) into these strains (Table III). The resultant strains (AF364–AF367) now carry three suppressor alleles, *prlG1* and either *prlA11* or *prlA301* on the chromosome, and *prlG3* on a plasmid. This demonstrates that *prlG3*, which exhibits synthetic

**Table III.** Complementation by suppressor alleles

| Strain | Chromosomal alleles[a] | Plasmid-borne allele[b] | Inducer dependent[c] |
|---|---|---|---|
| AF360 | *prlA11, prlG1* | *secE*$^+$ | Y |
| AF361 | *prlA11, prlG8* | *secE+* | N |
| AF364 | *prlA11, prlG1* | *prlG3* | Y |
| AF365 | *prlA11, prlG8* | *prlG3* | N |
| AF362 | *prlA301, prlG1* | *secE+* | Y |
| AF363 | *prlA301, prlG8* | *secE+* | N |
| AF366 | *prlA301, prlG1* | *prlG3* | Y |
| AF367 | *prlA301, prlG8* | *prlG3* | N |
| AF368 | *prlA726, prlG1* | *prlA4* | N |
| AF369 | *prlA726, prlG3* | *prlA4* | Y |

[a]Chromosomal *prlA* allele and the *prlG* allele that was transduced into the strain.
[b]Complementing allele that was carried on the plasmid.
[c]Y indicates that the strain was dependent on the presence of inducer for growth, N indicates that growth was independent of inducer.

lethality with certain other *prlA* alleles, is able to provide the complementing function necessary to build strains containing a synthetic pair, *prlA11-prlG1*, or *prlA301-prlG1*. Since in this case the complemented strain contains three *prl* suppressors, we believe this rules out explanations invoking suppressor synergy.

We also constructed a strain that contained *prlA726* on the chromosome and *prlA4* on a plasmid (Table III). This plasmid, pRLA41, expresses PrlA4 under control of the *lac* promoter; these cells were grown in media containing 2 mM IPTG to induce expression of PrlA4. We found that either *prlG1* or *prlG3* could be transduced into this strain (AF368 and AF369), demonstrating again that the synthetic defect is not due to multiple suppressors. Both of the resultant strains carry three suppressors; *prl4* encoded by the plasmid, chromosomally encoded *prlA726* and either *prlG1* or *prlG3*. These constructions were remarkable because both of the *prlG* alleles that were introduced into this strain exhibit a defect when in combination with one of the *prlA* alleles present. That is, *prlG3* and *prlA726* are a defective pair, and *prlG1* with *prlA4* is defective. However, the Prl proteins are able to assort with the appropriate partner in such a way that functional pairs are able to form translocators.

The plasmids that provided the complementing activity in these experiments expressed the Prl suppressor under control of either the *ara* or the *lac* promoter. We examined the strains that we had constructed to determine whether viability was dependent on the presence of inducer. We found that all of the control strains did not require induction of the plasmid-borne suppressor (Table III). That is, the strains that contain *prlA11* and *prlG8* (AF361 and AF365) or *prlA301* and *prlG8* (AF363 and AF367) did not require a complementing factor. In contrast, the strains that contain a synthetic lethal pair were dependent on the presence of inducer for growth. The strains carrying *prlA11* and *prlG1* (AF360 and AF364) or *prlA301* and *prlG1* (AF362 and AF366) on the chromosome were dependent on the presence of arabinose for expression of either *secE*$^+$ or *prlG3*. The strain that carried *prlA726* on the chromosome and *prlA4* on the plasmid required induction by IPTG when *prlG3* was also present in the strain (AF369), but not when *prlG1* was the allele that was introduced (AF368). Results presented in this section strengthen our conclusion that synthetic lethality is highly allele-specific and that the defect causing cell death is the consequence of an altered interaction between particular pairs of PrlA and PrlG proteins.

### *An alteration of the prlA726 allele relieves synthetic lethality*

During the course of the strain constructions described above, one experiment resulted in *prlG3* co-transduction into a *prlA726* strain with wild-type efficiency (AF371). This result was not only unexpected, it conflicted with our previous results. Therefore, we examined this strain further. Transduction of the DNA from the *secY* and *secE* loci into suitable recipients demonstrated the presence of suppressors of signal sequence mutations at both loci. We then sequenced the *secY* DNA of the parent strain. Results showed that this particular isolate had undergone a second mutation that altered the protein sequence. The original *prlA726* mutation is a Ser to Pro alteration at amino acid 68 (Flower *et al.*, 1994). The second mutation changes this Pro to a Leu. This conversion results in a *prlA* allele (now called *prlA799*) that is still a *prl* suppressor, but no longer exhibits a synthetic phenotype with *prlG3*. Further, this is a suppressor that we would not obtain by our usual selections because at this codon the Leu can only result from two mutational events. Clearly the defective interaction between PrlG3 and PrlA726 is extremely allele-specific.

## Discussion

We have exploited a genetic analysis to probe the interaction of PrlA/SecY and PrlG/SecE. We discovered that certain pairs of alleles exhibit synthetic defects. The occurrence of synthetic phenotypes is very rare; we analyzed 115 pairs of alleles and only seven demonstrated a defect. Furthermore, there is extreme allele specificity associated with these synthetic defects. An allele of *prlA* that is defective with a given *prlG* allele is defective only with that allele, not with all *prlG* alleles. It is particularly striking to note that there are 11 different *prl* suppressor mutations within periplasmic loop 1 of SecY/PrlA, yet only two of these exhibit synthetic defects with *prlG3*.

The effects that we observed were not simply due to two potent suppressors present in the same strain for the following reasons. First, none of the *prl* alleles examined in this study confers a detectable negative effect on the cell (Derman *et al.*, 1993; Osborne and Silhavy, 1993; Flower *et al.*, 1994). Therefore, the lethality caused by two alleles in combination is not due to a synergistic detrimental effect. Secondly, the alleles that have a synthetic defect with another allele do not necessarily correspond to the 'strong' suppressors. Some *prlA* suppressors that are considered quite potent (for example *prlA666*), do not exhibit synthetic defects with any *prlG* alleles. Further, if the effects were correlated with suppressor strength, we would not expect to see allele specificity. Consider the four alleles *prlA4*, *prlG1*, *prlA726* and *prlG3*. If synthetic lethality was simply due to the synergistic effect of two strong suppressors, one would expect all pairwise combinations of these alleles to exhibit the defect. Instead, there is allele specificity such that the *prlA4-prlG1* combination is lethal and the *prlA726-prlG3* com-

bination is inviable, but strains containing the other two combinations (*prlA4-prlG3*, *prlA726-prlG1*) grow well at all temperatures. Finally, we demonstrated that synthetic lethality can be complemented by another inducer-dependent *prl* suppressor allele provided *in trans*, even by a suppressor which can itself be a synthetic lethal partner of another allele.

Each of the synthetic phenotypes that we observe has characteristics that make it unique from each of the others. It is helpful to consider each pair independently and to rank them in order of decreasing severity of effect. The pair that exerts the strongest defect is the *prlA726-prlG3* combination. This duo is the only set that results in arabinose sensitivity at 37°C, in a wild-type *secE* background. This is the only combination for which we are able to hypothesize how the defect is manifested. We believe that PrlA726 and PrlG3 associate into an inactive complex that renders PrlA726 limiting in the cell. This hypothesis is supported by our finding that SecY complements this pair, while low levels of SecE complement poorly.

The first synthetic lethal pair that was discovered in this system is *prlA4-prlG1* (Bieker and Silhavy, 1990). The *prlA4* allele encodes two mutations, F286Y and I408N (Figure 1; Sako and Iino, 1988). However, unpublished work in this laboratory demonstrated that the mutation in transmembrane 10, I408N, is sufficient to cause the synthetic phenotype (S.J.Isakoff and T.J.Silhavy, unpublished observations). The *prlA6* allele also encodes two mutations, S188L and I408N (Osborne and Silhavy, 1993). As we already know that I408N causes a synthetic defect with *prlG1*, we assume that it is this alteration in *prlA6* that is responsible for the synthetic lethality that *prlA6* has with *prlG1*. Therefore, both of these alleles, *prlA4* and *prlA6*, exert their synthetic defects via the I408N mutation within transmembrane helix 10. Likewise the alleles that encode the mutation L407R (also within transmembrane helix 10) can be characterized by *prlA301*. This is the only mutation in *prlA301* (Osborne and Silhavy, 1993), therefore it must be responsible for the synthetic defect. The *prlA11* allele codes for the L407R mutation as well as a second mutation, V411G (Osborne and Silhavy, 1993). Again, we believe that the L407R mutation must lead to the synthetic lethality with *prlG1*. All of these pairs are essentially equivalent in terms of the severity of the defect. They all exhibit a defect by the complementation screen, the double mutant strains cannot be constructed unless a complementing plasmid is present and, once built, the strain cannot lose the plasmid.

There are two pairs of synthetic alleles that do not have as strong a defect as the above sets. The *prlA208-prlG1* combination is defective by the synthetic screen, but only at 23°C, not at 25°C. As defects in the protein export pathway are often manifested as cold-sensitive phenotypes (Pogliano and Beckwith, 1993), this temperature dependence may represent an increasing perturbation of the protein export pathway. However, the *prlA208-prlG1* defect is potent enough that repeated attempts to construct the double mutant strain were unsuccessful. Similarly, the *prlA3-prlG3* combination exhibits a temperature dependence in complementation analysis. In addition, it is possible to construct a strain that carries both *prlA3* and *prlG3*; however, the double mutant strain has a cold-sensitive phenotype.

One explanation for the synthetic effects is that they reflect disruptions within domains of interaction. Indeed, the pairs of alleles that demonstrate synthetic defects are highly coincident with distinct topological domains. As discussed by Osborne and Silhavy (1993), the *prlA* alleles that demonstrate synthetic lethality with *prlG1* are all localized to transmembrane domains 7 and 10 of SecY. As the *prlG1* mutation is located within transmembrane helix 3 of SecE, this led to the proposal that helices 7 and 10 of SecY may normally interact with transmembrane helix 3 of SecE. In addition, we have found that *prlG3*, which contains a mutation within periplasmic loop 2 of SecE, shows synthetic phenotypes with a subset of *prlA* alleles that cause alterations to amino acids within periplasmic loop 1 of SecY. We believe that this reflects a disruption of an interaction that normally occurs between these two periplasmic domains.

It has been proposed that there are interactive domains within cytoplasmic regions of PrlA/SecY and PrlG/SecE (Baba *et al.*, 1994). Our results are not contradictory to that proposal. No *prl* alleles of either *secE* or *secY* have been isolated that map to the cytoplasmic domains, therefore we cannot use our method to examine these regions. However, it is not unreasonable to propose that there are multiple points of interaction between these proteins. Others have suggested that the C-terminal portion of SecE (including the third transmembrane helix) interacts with SecY (Nishiyama *et al.*, 1992), an observation consistent with our current results. We do not suggest that the synthetic defects are indicative of the exact amino acids that normally interact, but rather denote domains that are involved in protein-protein contacts during the translocation process.

We have suggested previously that all of the identified PrlA and PrlG proteins exert their suppressor activity through the loss of an ability to reject proteins with a defective signal sequence from the secretion pathway. In other words, the Prl suppressors bypass a signal sequence proofreading function (Osborne and Silhavy, 1993; Flower *et al.*, 1994). In the wild-type cell, SecY and SecE would function in concert to prevent the translocation of defective precursors. Therefore, the selection of suppressors of export-defective precursors leads naturally to mutational changes that affect the interaction of SecE and SecY. We think it is not surprising that some of these altered Prl proteins are unable to interact productively with one another, leading to a lethal defect when they are present in the cell together.

Synthetic lethality is not a technique that is commonly used for the study of prokaryotes, primarily because they are haploid organisms. Nevertheless, we believe that this approach can be successfully applied to the study of multisubunit complexes in bacteria, as well as in eukaryotic organisms.

## Materials and methods

### *Bacterial strains and media*

All strains are derivatives of *E.coli* K12 strain MC4100 (Casadaban, 1976) and are listed in Table IV. Only those strains that are specifically discussed in the text are listed. Series of isogenic strains that differ only

**Table IV.** Bacterial strains and plasmids

| Strain name | Relevant genotype | Source |
|---|---|---|
| MC4100 | F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 ptsF25 deoC1 thi* | laboratory stock |
| AF111 | MC4100 *lamB14D* | this study |
| AF128 | AF111 *prlG1 zja*::Tn*10* | this study |
| AF129 | AF111 *prlG2 zja*::Tn*10* | this study |
| AF130 | AF111 *prlG3 zja*::Tn*10* | this study |
| AF131 | AF111 *prlG8 zja*::Tn*10* | this study |
| AF133 | MC4100 *secE15*(Cs) *zja*::Tn*5* | this study |
| AF230 | RO129 Ara⁺ AroE⁺ *prlA*⁺ | this study |
| AF231–AF252 | RO129 Ara⁺ AroE⁺ *prlAx* | this study |
| AF276–AF298 | AF230–AF251*secE15 zja*::Tn*5 recA*::*cat* | this study |
| AF312 | AF232 (*prlA3*) *prlG1 zja*::Tn*10* | this study |
| AF314 | AF232 (*prlA3*) *prlG3 zja*::Tn*10* | this study |
| AF345 | AF249 (*prlA726*) *prlG3 zja*::Tn*10* /pRO13 | this study |
| AF351 | AF243 (*prlA301*) *prlG1 zja*::Tn*10* /pJS100 | this study |
| AF352 | AF243 (*prlA301*) *prlG2 zja*::Tn*10* /pJS100 | this study |
| AF353 | AF243 (*prlA301*) *prlG3 zja*::Tn*10* /pJS100 | this study |
| AF354 | AF243 (*prlA301*) *prlG8 zja*::Tn*10* /pJS100 | this study |
| AF358 | AF249 (*prlA726*) *prlG3 zja*::Tn*10* /pJS100 | this study |
| AF360 | AF237 (*prlA11*) *prlG1 zja*::Tn*10* /pAF26 | this study |
| AF361 | AF237 (*prlA11*) *prlG8 zja*::Tn*10* /pAF26 | this study |
| AF362 | AF243 (*prlA301*) *prlG1 zja*::Tn*10* /pAF26 | this study |
| AF363 | AF243 (*prlA301*) *prlG8 zja*::Tn*10* /pAF26 | this study |
| AF364 | AF237 (*prlA11*) *prlG1 zja*::Tn*10* /pAF29 | this study |
| AF365 | AF237 (*prlA11*) *prlG8 zja*::Tn*10* /pAF29 | this study |
| AF366 | AF243 (*prlA301*) *prlG1 zja*::Tn*10* /pAF29 | this study |
| AF367 | AF243 (*prlA301*) *prlG8 zja*::Tn*10* /pAF29 | this study |
| AF368 | AF249 (*prlA726*) *prlG1 zja*::Tn*10* /pRLA41 | this study |
| AF369 | AF249 (*prlA726*) *prlG3 zja*::Tn*10* /pRLA41 | this study |
| AF370 | AF249 (*prlA726*) *prlG*⁺ *zja*::Tn*10* /pRLA41 | this study |
| AF371 | MC4100 Ara⁺ *prlA799 prlG3 zja*::Tn*10* | this study |
| AF372 | AF243 (*prlA301*) *prlG*⁺ *zja*::Tn*10* /pJS100 | this study |
| AF373 | AF249 (*prlA726*) /pAF29 | this study |
| AF374 | AF249 (*prlA726*) *prlG3 zja*::Tn*10* /pAF24 | this study |
| RO129 | MC4100 *lamB14D aroE*⁻ | this study |

| Plasmid name | Relevant characteristics | | Source |
|---|---|---|---|
| pAF24 | *secE*⁺ | high copy | laboratory stock |
| pAF25 | *prlG1* | high copy | laboratory stock |
| pAF26 | *secE*⁺ | arabinose inducible | this study |
| pAF27 | *prlG1* | arabinose inducible | this study |
| pAF28 | *prlG2* | arabinose inducible | this study |
| pAF29 | *prlG3* | arabinose inducible | this study |
| pAF30 | *prlG8* | arabinose inducible | this study |
| pJS100 | *secY*⁺ | low copy | laboratory stock |
| pRLA41 | *prlG1* | IPTG inducible | laboratory stock |
| pRO13 | *secE*⁺ | low copy | this study |

in the *prlA* allele are listed in groups. Most of the *prlA* and *prlG* suppressors used in this study have been described previously (Derman *et al.*, 1993; Osborne and Silhavy, 1993; Flower *et al.*, 1994). The DNA sequences of *prlA8910*, *prlA8911*, *prlA8912*, *prlA8913*, *prlA8914* and *prlA402* are described herein. Strains containing these mutations were the kind gift of Will Prinz and Jon Beckwith. Genetic manipulations were performed according to standard protocols (Silhavy *et al.*, 1984). Bacteriophage $P1_{vir}$ and $\lambda_{vir}$ stocks were prepared as described (Silhavy *et al.*, 1984). Maltodextrin (Pfansteil Laboratories, Inc.) stock solutions were prepared as described previously (Trun and Silhavy, 1987). All other media used has been described (Silhavy *et al.*, 1984).

Strains containing two chromosomal suppressor mutations were constructed by preparing P1 lysates of strains AF128, AF129, AF130 and AF131. These lysates were used to transduce appropriate strains AF230–AF252 to $Tet^R$. Marker rescue experiments were used to determine the co-transduction of *prlG* alleles by transduction of the Tn*10* marker into AF111 and screening for $\lambda^S$. The conversion of AF111 to $\lambda^S$ indicated that the *prlG* allele had co-transduced into the *prlA* strain. All synthetic double mutant strain constructions were performed a minimum of three independent times; for each experiment at least 12 transductants were analyzed. All double mutant strains were examined for cold sensitive phenotypes by growth at 37, 30, 25 and 23°C.

Diploid analyses were performed by double mutant construction in the presence of a complementing plasmid. Appropriate strains AF230–AF252 were transformed with plasmids pJS100, pAF24 or pRO13, then transduced with P1 lysates prepared from strains AF128, AF129, AF130 and AF131. Marker rescue experiments were performed as above. All double mutants constructed with a complementing plasmid were analyzed for cold sensitivity as described above.

To construct strains containing three suppressor alleles, strains AF236 (*prlA11*) and AF242 (*prlA301*) were transformed with plasmids pAF26 (*prlG*⁺) or pAF29 (*prlG3*). These transformants were then maintained in 0.2% L-arabinose and transduced with lysates prepared on strains AF128 (*prlG1*) or AF131 (*prlG8*). Marker rescues were performed as above. Strain AF248 (*prlA726*) was transformed with pRLA41 (*prlA4*), maintained in 2 mM IPTG, and transduced with lysates from AF128 (*prlG1*) or AF130 (*prlG3*). Inducer dependence was assayed by growth on LB plates containing ampicillin (125 μg/ml) in the presence or absence of 0.2% L-arabinose or 2 mM IPTG. Strains containing three suppressors were examined for cold sensitivity.

### Plasmids

Plasmids used in this study are listed in Table IV. The expression vector pBAD18 was a kind gift of J.Beckwith and coworkers. Plasmids pAF24 and pAF25 that encode *secE*⁺ and *prlG1*, respectively, under control of their native promoters, have been described (Osborne and Silhavy, 1993).

The low copy number plasmid pJS100, which encodes *secY*$^+$ and the high copy plasmid pRLA41, which expresses *prlA4* under control of the *lac* promoter have also been described previously (Bieker and Silhavy, 1989, 1990). When desired, expression of PrlA4 was maintained by growth in 2 mM IPTG.

Plasmids pAF26, pAF27, pAF28, pAF29 and pAF30 were constructed as follows. Chromosomal DNA containing the *prlG* alleles was amplified by PCR using the following oligonucleotides: 5′-ATATGAATTCCCG-AAAAGCTAATACGCGTT-3′, and 5′-ATATGGATCCTGCTCACGC-AGCGACGTTGC-3′ according to standard protocols (Russo *et al.*, 1993). The DNA was gel purified and digested with *Eco*RI (underlined sequence in primer 1) and *Eco*47III (within the chromosomal DNA) to produce a fragment of 0.5 kb. This fragment was ligated with pBAD18 that had been digested with *Eco*RI and *Sma*I. After transformation and confirmation of insert-containing clones, the DNA sequence was determined to verify that no PCR-induced errors had occurred. When appropriate, expression of the PrlG proteins was induced by 0.2% L-arabinose.

Plasmid pRO13, a low copy number plasmid which expresses wild-type *secE*, was constructed by digestion of the previously described pAF21 (Bieker-Brady and Silhavy, 1992) with *Eco*RI and *Sal*I, and filling in the ends with Sequenase 2 polymerase (United States Biochemical), followed by religation to remove the tetracycline resistance marker.

### DNA techniques

Princeton University Syn/Seq facility synthesized oligonucleotides used for PCR amplification of DNA and for DNA sequence analysis. PCR amplification of DNA from a single colony was performed as described (Russo *et al.*, 1993) and DNA sequence analysis was as described (Osborne and Silhavy, 1993). Preparation of competent cells and transformations were performed by standard protocols.

### Synthetic phenotype screen

Strains to be assayed for synthetic phenotypes (AF276–AF298) were transformed with each of the plasmids pAF26–pAF30, and single colonies were maintained on selective media. Each plasmid-containing strain was streaked on selective media with or without 0.2% L-arabinose and incubated at three different temperatures, 23, 25 and 37°C. In all cases except one (discussed in text) all strains grew equally well at 37°C in the presence or absence of arabinose. The results shown in Table I are from the 23°C incubation. Except as discussed in the text, equivalent results were obtained at 25 and 23°C.

### Plasmid loss experiments

Plasmid loss experiments were performed by growing overnight cultures of pJS100-containing strains in LB broth containing 50 μg/ml ampicillin, then subculturing at a 1:100 dilution in LB broth without ampicillin. Dilutions were repeated approximately every 12 h to maintain growing cultures. Samples were withdrawn at the indicated time points and plated onto LB plates at dilutions calculated to result in ~200–400 colonies per plate. After 8 h of growth on the non-selective media, samples were replica plated onto both LB agar and LB with ampicillin (50 μg/ml). After overnight incubation colonies were counted on each plate.

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