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Genetics

Alteration of the amino terminus of the mature sequence of a periplasmic protein can severely affect protein export in *Escherichia coli*

(alkaline phosphatase/membrane/signal sequence)

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ABSTRACT **_Escherichia coli_ alkaline phosphatase, coded for by the *phoA* gene, is normally translocated across the cytoplasmic membrane into the periplasm with high efficiency. We have constructed a series of derivatives of the *phoA* gene that code for a wild-type signal sequence but result in altered amino acid sequences at the amino terminus of the mature alkaline phosphatase. Our results suggest that the presence of two positively charged amino acids very early in the mature sequence interferes significantly with protein export. In one case, *phoA2AB*, the presence of the sequence Arg-Ile-Arg at the amino terminus of alkaline phosphatase results in a 50-times reduction in the export of the protein. By using oligonucleotide-directed mutagenesis, we have constructed mutant derivatives of *phoA2AB* that are greatly enhanced for export. In all cases, these derivatives reduce the net positive charge in the region. Our results may explain the failure of *E. coli* to export a number of proteins coded for by artificial constructs and suggest a way to improve export in these cases.**

Proteins that are destined to be exported from the cell's cytoplasm contain information within them that is essential for the localization process. In *Escherichia coli*, proteins that are transported across the cytoplasmic membrane into the periplasmic space or to the outer membrane are made with amino-terminal signal sequences. The study of mutations that alter the signal sequence has provided strong evidence for the *in vivo* role of the signal peptide. Mutations that most strongly interfere with the export of a protein are those that alter amino acids within the hydrophobic core of the signal sequence. Such changes, particularly those that introduce charged amino acids into this region, can result in as much as a 100-times reduction in the export of the protein (1–3).

Mutations that reduce the positive charge at the amino terminus of the signal peptide have also been characterized (4–6). Some of these have no effect on the export process but reduce the rate of synthesis of the protein whereas others reduce the efficiency of export. However, even elimination of the positively charged amino acids does not lead to as severe effects on translocation of the protein as do alterations of the hydrophobic core. In a few cases, alterations of the region preceding or following the processing site have been obtained. These mutations prevent processing but do not interfere with export (7–10).

There are reasons to believe that the mature part of the exported protein also plays a role in its translocation. (*i*) Merely attaching a signal sequence to a cytoplasmic protein does not ensure its transfer across the membrane. For example, several attempts to export β-galactosidase in this way have failed (11–13). (*ii*) For the *E. coli* outer membrane protein LamB, deletion of early portions of the mature protein result in a clear effect on the kinetics or processing of the signal sequence (14). (*iii*) Evidence that protein folding can interfere with export suggests that structural features of exported proteins may be important in the translocation process (15, 16). Yet, in no case has a mutation been characterized in the mature portion of any secreted protein that has severe effects on the export process.

We have studied in some detail the transfer of alkaline phosphatase (PhoA) into the periplasmic space. One feature of this protein that makes it an attractive system for studying export is that it is only enzymatically active when it is transferred across the cytoplasmic membrane (3, 17, 18). Thus, export can be assayed and, in certain cases, genetic selections can be devised on the basis of PhoA activity. In this paper we describe derivatives of PhoA that are severely affected in their transfer across the membrane. In contrast to previous cases, these alterations are in the mature portion of the protein, not in the signal sequence. Our results suggest that the nature of the charged amino acids in the early part of the mature protein is quite important to the export process.

MATERIALS AND METHODS

Bacterial Strains. The following strains were used in this study: MZ13B F⁻ *lacX74 ΔbrnQphoB tsx trpam str*^{r}, MZ20 (F⁻ *ΔlacX74 ΔphoA20 phoR trpam malB str*^{r}, Xph62 (*ΔlacU169 ΔbrnQphoA phoR trpoc str*^{r}. Strain MZ5000 is a derivative of MZ20 that had the *supFts* introduced through P1 transduction. Strain Xph62(*prlA4*) is a derivative of Xph62 that had the *prlA4* mutation (19) introduced through P1 transduction.

Media and Chemicals. TYE, M63 minimal medium, and LB broth have been described (20). 5-Bromo-4-chloro-3-indolyl phosphate was from Bachem (Torrance, CA) and was used at 40 μg/ml. Antibiotics were used at following concentration: kanamycin at 40 μg/ml and ampicillin at 200 μg/ml. The proteinase inhibitor phenylmethylsulfonyl fluoride was purchased from Sigma.

Enzymes. Restriction enzymes, the Klenow fragment of DNA polymerase I, and ligase were purchased from New England Biolabs and were used as recommended. Reverse transcriptase (from avian myeloblastosis virus) was from International Biotechnologies (New Haven, CT).

Recombinant Phage Construction. The λ*phoA2AB* and its derivatives were recombined onto a λ transducing phage as follows. The phage λ*phoA61* (3) carries a *phoA* signal sequence mutation. Therefore, it makes white plaques on a lawn of a *phoA* deletion strain on 5-bromo-4-chloro-3-indolyl phosphate-containing medium. This phage was used to infect cells carrying the plasmids, and plate lysates were prepared.

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‡Deceased, July 24, 1986.

The lysates were streaked on a lawn of strains Xph62 or Xph62(*prlA4*) on a TYE plate containing 5-bromo-4-chloro-3-indolyl phosphate. The *prlA4* mutation suppresses *phoA61* as well as *phoA2AB*, *-X4*, and *-X5*, to a certain degree, but only the phages carrying the latter *phoA* mutations express sufficient PhoA activity on this strain so that blue plaques were generated. The Xph62 strain was used for sequences encoding PhoA active proteins, PhoAX2, -X3, and -X6. For PhoA2AB, -X4, and -X5, Xph62(*prlA4*) was used. Blue plaques were purified and were used to prepare liquid lysates by infection of Xph62 cells. Phage DNA was isolated (see below) and correct recombinant phage were distinguished from wild-type *phoA* recombinant phage through restriction analysis.

Isolation of Phage DNA. λ phage DNA was obtained as described in ref. 21 except that the phage particles were precipitated from a liquid lysate with an equal volume of SM solution containing 20% (wt/vol) polyethylene glycol and 2 M NaCl. Phage DNA was obtained by extraction with phenol followed by chloroform then precipitated with ethanol (J. Barondess, personal communication).

Oligonucleotide-Directed Site-Specific Mutagenesis and DNA Sequencing. Oligonucleotides (27- to 42-mers) were synthesized on an Applied Biosystems DNA synthesizer by Steve Lin (Harvard Medical School). The oligonucleotide-directed-double-strand-break-repair method (22) was used to obtain mutants *phoAX2*, *-X3*, *-X4*, and *-X6*. The double-strand break was made through a *Bam*HI digestion, which linearizes plasmid pPL-2AB at the site near the sequence to be mutagenized. Identification of correct mutations was facilitated by a screen for plasmids no longer sensitive to *Bam*HI digestion, since the oligonucleotides were designed to destroy the *Bam*HI site. Mutations were verified by DNA sequence analysis (23, 24).

PhoA Activity Assay. Cells carrying the pPL plasmids were grown in LB broth containing kanamycin. Lysogens were grown in maltose (0.4%)/M63 minimal medium supplemented with 19 amino acids (without methionine) at a concentration of 50 μg/ml. The assay for PhoA activity is as described (3).

Cell Fractionation and Proteolysis. Cell fractionation was carried out as described (25). Proteins from each fraction were precipitated with trichloroacetic acid at 5% (wt/vol) final concentration.

For proteolysis studies, the spheroplast-buffer-treated cells were resuspended in ice-cold water containing protease K at 25 μg/ml. $MgCl_2$ and Tris·HCl (pH 7.4) were added to 10 mM for each. To expose cytoplasmic proteins, Triton X-100 was added to 1% to disrupt the cytoplasmic membrane. After incubation on ice for 10 min, proteins were precipitated with trichloroacetic acid.

Protein Labeling, Immunoprecipitation, and Sodium Dodecyl Sulfate (SDS)/Gel Electrophoresis. Cells were grown at 37°C in maltose (0.4%)/M63 minimal medium supplemented with 19 amino acids to a concentration of 50 μg/ml (without methionine). An exponential-phase culture was pulse labeled at 37°C with [^{35}S]methionine at 20–60 μCi/ml (1 Ci = 37 GBq) for 1 min and the chase with unlabeled methionine was done for various periods. The chase was terminated by the addition of trichloroacetic acid to a final concentration of 5% (wt/vol).

Immunoprecipitation with antiserum against maltose-binding protein and PhoA was done as described (26). The Laemmli gel electrophoresis system (27) was used to separate the immunoprecipitated proteins, which are visualized after autoradiography. To quantitate the radioactivities precipitated by antibodies against PhoA and maltose-binding protein, autoradiograms were scanned with an LKB UltroScan XL laser densitometer. The areas under the peaks were integrated.

RESULTS

We have constructed derivatives of plasmids carrying the *phoA* gene in which the DNA sequence coding for the amino terminus of the mature PhoA protein has been altered by the insertion of linkers (P.L. and H.I., unpublished results). These linkers contain convenient restriction enzyme sites. In all of these constructs and the ones to be described below, the DNA coding for the signal sequence has not been altered. Thus, the signal sequence and the bulk of the mature sequence of PhoA in all of our constructs are identical to the wild-type enzyme.

The insertion of the linker in one of these plasmids, pPL2, did not alter the reading frame of *phoA*. However, it did introduce a chain-terminating amber codon in the altered sequence, so that, for expression of PhoA, pPL2 had to be transformed into an amber suppressor strain. In such strains, high levels of PhoA activity were observed; the protein was efficiently exported to the periplasm. To eliminate the need for the amber suppressor, we altered these constructs with restriction enzymes so as to generate plasmids in which there was no in-frame amber codon in the *phoA* gene. These two constructs, 2SS and 2AB are shown in Fig. 1.

To our surprise, while strains carrying the parent plasmid secreted high amounts of PhoA in the presence of the amber suppressor, neither of the two constructs above allowed efficient periplasmic localization of PhoA. This was indicated first by the low level of PhoA enzymatic activity, about 10 times and 50 times lower than a *phoA*$^+$ control plasmid for 2SS and 2AB, respectively. Cell-fractionation analysis suggested that the PhoA coded for by these two plasmids was made in normal amounts but was not transferred across the membrane; the protein was found not in the periplasm but in the membrane fraction. This protein was in the form of precursor with its signal sequence still attached.

At this point in our studies, we decided to transfer all our constructs into the single-copy vector, bacteriophage λ. This was done because quantitative studies on the plasmid-carrying strains were made difficult by their instability. All subsequent results in this paper were obtained with lysogens of λ carrying the various *phoA* constructs.

To further analyze the defect in the export of PhoA in a strain containing the *2AB* variant of *phoA*, we carried out pulse–chase experiments. The results (data not presented) show that the bulk of the PhoA in the *phoA2AB* strain is unstable. We have shown (3) that although periplasmic PhoA is unusually protease-resistant, cytoplasmically localized PhoA is unstable. Therefore, the PhoA in this strain had the properties of PhoA that is retained inside the cytoplasm. Finally, we showed that in spheroplasts, the PhoA in the *phoA2AB* strain was resistant to externally added protease. In contrast, when cells were lysed, the PhoA became susceptible to protease degradation (Fig. 2). These results indicate that the bulk of the PhoA coded for by *phoA2AB* is cytoplasmic.

The only differences between wild-type PhoA and the two altered PhoAs studied here are the short amino acid sequences at the beginning of the mature protein. Therefore, we compared these sequences for the three proteins (Fig. 3) to see if there were any features that might explain the export defect. One obvious difference between these sequences is the nature of charged amino acids. For wild-type PhoA, in the first five amino acids there is no net charge, the initial arginine being compensated for by the later glutamic acid. For 2SS and 2AB, the first five amino acids contain two arginines and no negatively charged amino acids. Furthermore, when we examined the amino-terminal portions of a number of other *E. coli* exported proteins, we found that none exhibited a net positive charge. At just this time, von Heijne (28) published

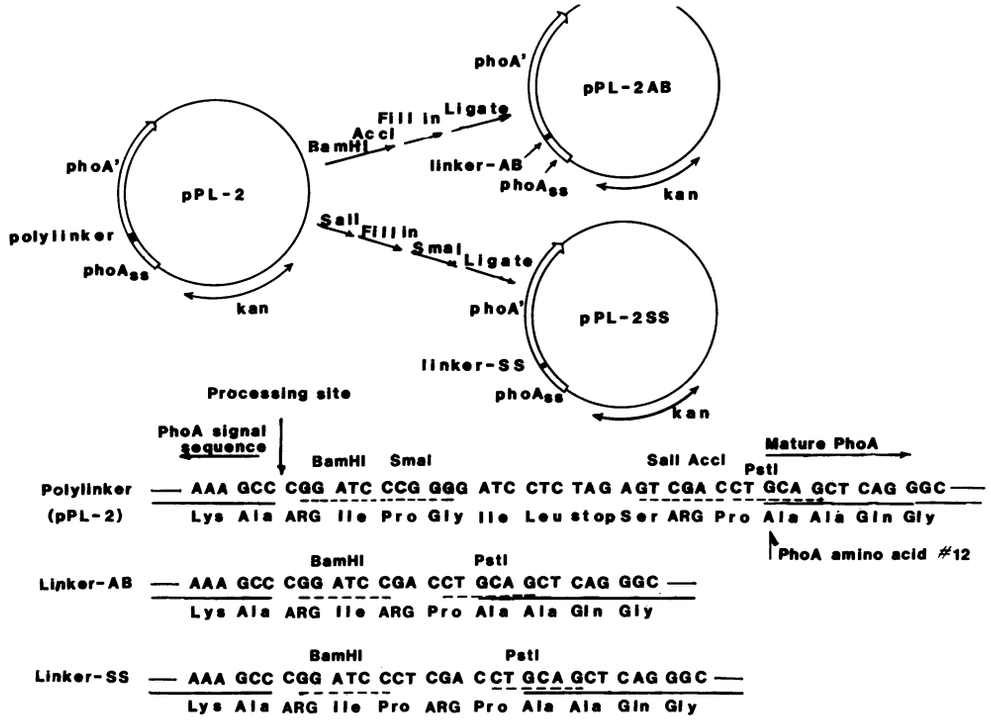

FIG. 1. Construction of plasmid pPL-2 derivatives, pPL-2AB and pPL-2SS.

a more extensive analysis of these sequences and came to the same conclusion.

As a result of this analysis, we suspected that it was the net positive charge at the amino terminus of PhoA in these two constructs that was responsible for the export defect. To test this hypothesis, we have constructed, by using oligonucleotide-directed mutagenesis, a series of mutations that alter the amino-terminal region of the mature PhoA encoded by pPL-2AB, the plasmid exhibiting the greatest defect in PhoA export. Four alterations of this region of the protein were obtained (Fig. 3). In three of these the net positive charge was reduced: (*i*) mutant *phoAX2* replaced the second arginine with a glutamic acid; (*ii*) mutant *phoAX3* replaced the same arginine with a threonine; and (*iii*) mutant *phoAX6* introduced a glutamic acid between the two arginines. All three mutations had very strong effects, restoring significant amounts of export of PhoA (Fig. 4). We also replaced the second arginine with a lysine, which resulted in a modest increase in export of (*phoAX4*). This result is consistent with a lower pK_a exhibited by lysine compared to arginine. The location of the various forms of PhoA found in these strains was verified by fractionation and proteolysis studies (Fig. 2). With *phoA2AB* and all the derivatives we have constructed, the total amount of material that cross-reacts with antibody to PhoA after pulse-labeling the protein is quite similar.

In addition to measuring the amount of PhoA exported under steady-state conditions (by determining enzyme activity), we also used pulse–chase experiments to study the kinetics of export of the protein. The derivatives of *phoA2AB* that had shown effective export of PhoA to the periplasm also showed a very rapid kinetics of export (Fig. 4). The variant with intermediate levels of export showed slower kinetics, and those from *phoA2AB* and *-X4*, both of which have two positively charged amino acids in this region, exhibited very slow kinetics. One explanation for the effect of the positively charged amino acids is that they interfere with passage of the protein through the membrane or interaction with a secretion machinery. Another explanation is that it is the charge distribution around the signal sequence that is important for the process. For instance, in the case of membrane-spanning

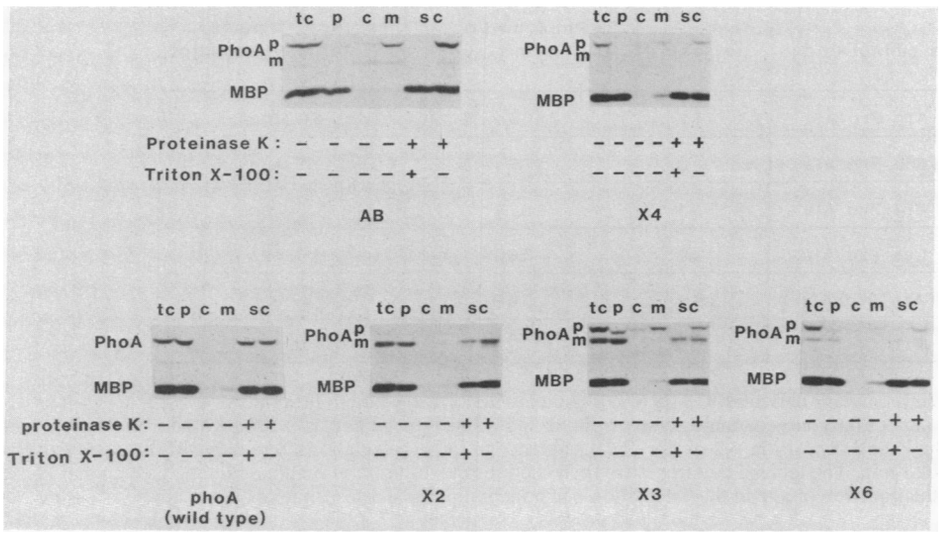

FIG. 2. Cellular location and proteolysis of wild-type and mutant PhoAs. Cells were labeled with [^{35}S]methionine for 1 min and chased with nonradioactive methionine for 5 min. The position of maltose-binding protein (MBP) and mature and precursor PhoAs are marked. tc, Total cells; p, periplasmic fraction; c, cytoplasmic fraction; m, membrane fraction; sc, osmotically shocked cells.

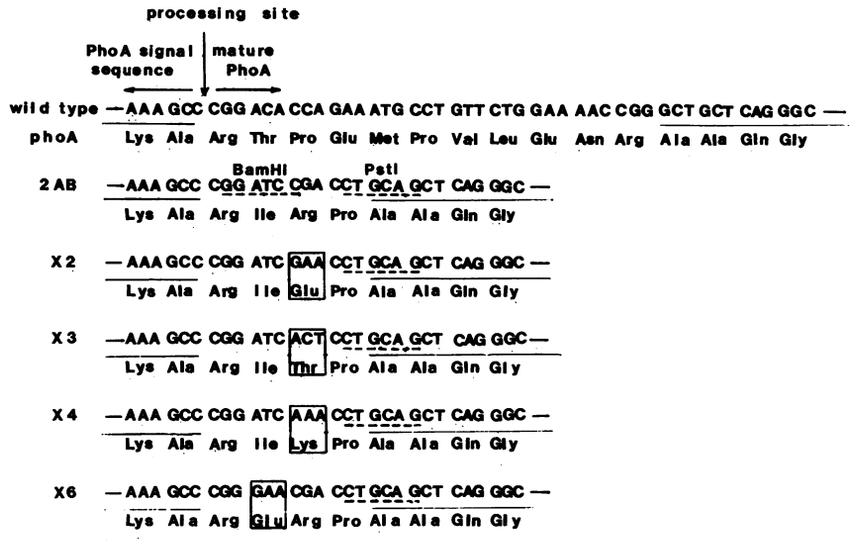

FIG. 3. Sequences of the amino terminus of the mature protein specified by *phoA2AB* and its mutant derivatives. The common sequences are underlined. The boxes mark the altered amino acids. The wild-type *phoA* sequence is included for comparison.

segments of integral membrane proteins, it has been suggested that the charge distribution around the hydrophobic sequence determines how it will orient itself in the membrane (29, 30). Given the charge polarity across the bacterial cytoplasmic membrane, it might be expected that positively charged amino acids would remain inside the cell and negatively charged amino acids would be found on the outside surface of the membrane.

It may be then that the net positive charge we have introduced in our constructs 2SS and 2AB at the amino terminus of mature PhoA has generated a problem for proper orientation of the signal sequence in the membrane. If this is the case, we might be able to compensate for the defect by introducing an additional positive charge at the amino terminus of the signal sequence in the 2AB plasmid. By using oligonucleotide-directed mutagenesis, we have constructed such a *phoA* variant, *phoAX5*. However, from *phoAX5*, there was no increase in PhoA activity or export (data not shown).

DISCUSSION

We describe here a case in which alteration of the mature portion of an exported protein has a severe effect on the transfer of that protein across the cytoplasmic membrane. When the amino terminus of the mature PhoA sequence is altered by introducing two positively charged amino acids (arginines) very close to the beginning, the export of PhoA is reduced by a factor of 50. Our results suggest that it is these amino acids that are interfering with the translocation process. Elimination of one of the positively charged amino acids by replacing it with a negatively charged or neutral amino acid restores high levels of export. Partial neutralization of the positive charges by introduction of a glutamic acid in this region also improves export. In contrast, replacement of one of the arginines by a lysine leads to only a small increase in export and no detectable effect on the kinetics of export.

We recognize that other explanations are not ruled out by these studies. It is possible that it is other structural features of the altered amino terminus that lead to the export block. (The alterations we have made, which were intended to change the charged nature of the region, may also be altering the structure in such a way as to relieve the block.) However, we favor the explanation based on the charge of the region both because all of our findings are consistent with it and because of the finding by von Heijne (28) that the absence of net positive charge in this region is a conserved feature of bacterial exported proteins.

We have considered several explanations for the effect of the positively charged amino acids. One is that it is simply the charge in this region of the protein that blocks the export process after it has begun. We can imagine, for instance, that transfer of the signal sequence is initiated through the

phoA sequence	first five amino acids	net charge	PhoA activity unit	earliest time for detectable processing
AB	Arg Ile Arg Pro Ala	+2	91	30 min
X2	Arg Ile Glu Pro Ala	0	1761	1 min
X3	Arg Ile Thr Pro Ala	+1	1679	5 min
X4	Arg Ile Lys Pro Ala	+2	390	30 min
X6	Arg Glu Arg Pro Ala	+1	720	5 min
wild type	Arg Thr Pro Glu Met	0	1772	0 min

FIG. 4. Characteristics of PhoA2AB and its mutant derivatives. The first five amino acids at the amino terminus of the mature protein are shown. The net charge is calculated as the sum of the charged amino acids. Arginine and lysine are counted as +1 and glutamic acid is counted as −1. We have not taken into account the lysine at position −2 of the signal sequence in these calculations. Fractions of the same cultures were pulse-labeled for 1 min and chased for 1 min. The labeled cultures were then used for quantitation of maltose-binding protein and PhoA. Corrections for the variation in PhoA production were made in the calculation of PhoA units. The earliest time for detectable processing was determined by the pulse–chase analysis.

membrane but when the positive charges are encountered, their interaction with the negatively charged phospholipid halts the process (Fig. 5*A*). Alternatively, the charge distribution around the hydrophobic core of the signal sequence may determine how it orients itself in the membrane. With a higher positive charge at the carboxyl end of the signal sequence, it may insert in the membrane in such a way that the rest of the protein cannot be transferred (Fig. 5*B*). To test this latter hypothesis, we have introduced an additional positive charge at the amino terminus of the signal sequence to see whether there was any effect on export. We saw no improvement in the translocation of PhoA across the membrane. This result suggests that it may not be the charge distribution around the signal sequence that is responsible for the export block and would lend more support to our first explanation. However, it may be that the position in which the charged amino acid is introduced is important or that additional substitutions would have to be made to observe an effect. In this discussion, we have not considered the role of the lysine at the −2 position of the signal sequence. This residue may contribute to the problems in export in some of our constructs.

The block in export may occur at an earlier step. Since there is evidence that soluble factors are involved in the early steps in recognition of the signal sequence (31, 32), it may be that the early part of the mature protein can interfere with the interaction. Alternatively, the sequence at the beginning of the mature protein may affect its folding, and, in this way, impede translocation.

These findings not only add information on the components of exported proteins that are important for their translocation across the membrane. They also may explain the failure to export certain cytoplasmic proteins or other proteins in artificial constructs. A precise fusion of staphylococcal nuclease to the PhoA signal sequence permitted efficient export of the enzyme in *E. coli*. However, export was abolished when an arginine residue was inserted at the beginning of the mature protein (33). Interestingly, in this case, beginning at residue 5, there were several positively charged amino acids in the first construct. Yet, it was only when an additional positively charged amino acid was inserted to replace the first amino acid that an effect was seen. These results suggest that it may be only excess positive charges in the first five amino acids that can have significant effects on export (see, e.g., our 2SS construct.)

When chicken triose phosphate isomerase was fused directly to the β-lactamase signal sequence, no export occurred (34). In this case, there were two positively charged amino acids close to the amino terminus of the isomerase. When a fusion was constructed with the amino terminus of triose phosphate isomerase attached to a portion of the mature β-lactamase, so that the positive charges did not directly follow the signal sequence, export took place (35). Finally, we have found (unpublished results) that the export of a fragment of β-galactosidase is not promoted by the PhoA signal sequence unless one of the positively charged amino acids at the amino terminus of β-galactosidase is eliminated.

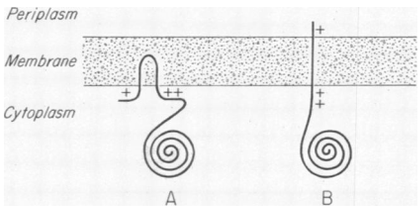

FIG. 5. Models for the interference of positive charge with PhoA export.

All these results suggest that care must be taken in the devising of artificial constructs for secretion to ensure that the charge distribution abutting the signal sequence is appropriate.

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1. Bassford, P. & Beckwith, J. (1979) *Nature (London)* **277,** 538–541.
2. Emr, S. D. & Silhavy, T. J. (1980) *J. Mol. Biol.* **141,** 63–90.
3. Michaelis, S., Inouye, H., Oliver, D. & Beckwith, J. (1983) *J. Bacteriol.* **154,** 366–374.
4. Hall, M. N., Gabay, J. & Schwartz, M. (1983) *EMBO J.* **2,** 15–19.
5. Inouye, S., Soberon, X., Franceschini, T., Nakamura, K., Itakura, K. & Inouye, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3438–3441.
6. Vlasuk, G. P., Inouye, S., Ito, H., Itakura, K. & Inouye, M. (1983) *J. Biol. Chem.* **258,** 7141–7148.
7. Koshland, D., Sauer, R. T. & Botstein, D. (1982) *Cell* **30,** 903–914.
8. Kuhn, A. & Wickner, W. (1985) *J. Biol. Chem.* **260,** 15914–15918.
9. Lin, J. J. C., Kanazawa, H., Ozols, J. & Wu, H. C. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4891–4895.
10. Russel, M. & Model, P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1717–1721.
11. Bassford, P., Silhavy, T. J. & Beckwith, J. (1979) *J. Bacteriol.* **139,** 19–31.
12. Michaelis, S., Guarente, L. & Beckwith, J. (1983) *J. Bacteriol.* **154,** 356–365.
13. Tommassen, J., Leunissen, J., vanDammen-Jongsten, M. & Overduin, P. (1985) *EMBO J.* **4,** 1041–1047.
14. Rasmussen, B. A. & Silhavy, T. J. (1987) *Genes Dev.* **1,** 185–196.
15. Randall, L. L. & Hardy, S. J. S. (1986) *Cell* **46,** 921–928.
16. Collier, D. N., Bankaitis, V. A., Weiss, J. B. & Bassford, P. J., Jr. (1988) *Cell* **53,** 273–283.
17. Hoffman, C. & Wright, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5107–5111.
18. Manoil, C. & Beckwith, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8129–8133.
19. Emr, S. D., Hanley-Way, S. & Silhavy, T. J. (1981) *Cell* **23,** 79–88.
20. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
22. Mandecki, W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7177–7181.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Zagursky, R. J., Baumeisler, N., Lomax, N. & Berman, M. (1985) *Gene Anal. Technol.* **2,** 89–94.
25. Manoil, C. & Beckwith, J. (1986) *Science* **233,** 1403–1408.
26. Ito, K., Bassford, P., Jr., & Beckwith, J. (1981) *Cell* **24,** 707–714.
27. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
28. von Heijne, G. (1986) *J. Mol. Biol.* **192,** 287–290.
29. von Heijne, G. (1986) *J. Mol. Biol.* **189,** 239–242.
30. von Heijne, G. (1986) *EMBO J.* **5,** 3021–3027.
31. Muller, M. & Blobel, G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7737–7741.
32. Weng, Q. P., Chen, L. L. & Tai, P. L. (1988) *J. Bacteriol.* **170,** 126–131.
33. Liss, L. R., Johnson, B. L. & Oliver, D. (1985) *J. Bacteriol.* **164,** 925–928.
34. Kadonaga, J. T., Gautier, A. E., Straus, D. R., Charles, M. D. E. & Knowles, J. R. (1984) *J. Biol. Chem.* **259,** 2149–2154.
35. Pluchthun, A. & Knowles, J. R. (1987) *J. Biol. Chem.* **262,** 3951–3957.