Transport of an Export-defective Protein by a Highly Hydrophobic Signal Peptide*

(Received for publication, April 28, 1993, and in revised form, June 28, 1993)

Sharyl L. Rusch and Debra A. Kendall‡

From the Department of Molecular & Cell Biology, The University of Connecticut, Storrs, Connecticut 06269

We have examined the sequence constraints on the amino-terminal region of the mature portion of alkaline phosphatase that are important for its efficient transport in Escherichia coli. Using a homopolymeric sequence of serines to replace 6 residues in this region, a transport-competent mutant was produced. Reintroduction of residues from the native sequence which restore charge and β-turn potential resulted in little improvement. However, by replacing the hydrophobic core of the signal peptide with a homopolymeric series of leucines, not only was transport restored but precursor processing was more efficient than for the wild type and was insensitive to disruption of the protonmotive force. Moreover, we have titrated the signal peptide with leucine to alanine substitutions (Doud, S. K., Chou, M. M., and Kendall, D. A. (1993) Biochemistry 32, 1251-1256) and determined the minimum level of hydrophobicity necessary to achieve transport of the mutant protein. The results indicate that signal peptide hydrophobicity can completely override possible requirements for negatively charged residues and strong β-turn forming potential in the mature protein and that the polypeptide containing signal peptide may act as a generic signal sequence for the transport of non-native proteins in E. coli.

In Escherichia coli, most secretory proteins are synthesized as precursors in the cytoplasm and are exported to their correct final location with the assistance of an amino-terminal leader sequence or signal peptide. Numerous studies have been done to elucidate the critical features of the three regions of the signal peptide: the basic amino terminus (Inouye et al., 1982; Vlasuk et al., 1983), the hydrophobic core (Chou and Kendall, 1990; Goldstein et al., 1990; for review, see Gennity et al., 1990), and the polar region preceding the signal peptidase cleavage site (Rosenblatt et al., 1980; Perlman and Halvorson, 1983; Vlasuk et al., 1984; Laforet and Kendall, 1991). While these studies underline the importance of the signal peptide, it is also clear that it is not always sufficient for transport of the attached protein.

The mature protein itself appears to play a role in the transport process. Statistical analysis reveals that amino-terminal regions of mature secretory proteins are acidic or neutral (von Heijne, 1986; Yamane and Mizushima, 1988) and the introduction of positively charged residues in the mature region disrupts transport. This has been found for mutants of alkaline phosphatase, a protein which is normally secreted in E. coli (Li et al., 1988). E. coli chimeric proteins (Yamane and Mizushima, 1988), and proteins foreign to E. coli such as chicken muscle triose-phosphate isomerase (Summers et al., 1989) and Staphylococcus aureus nuclease (Liss et al., 1985).

The reasons for this sensitivity to basic residues are not entirely clear. The “positive-inside rule” (von Heijne and Gavel, 1988) predicts that positively charged residues would be unlikely to cross the E. coli cytoplasmic membrane. Numerous studies indicate that charged residues are important determinants of the orientation of transmembrane segments with positively charged residues on the cytoplasmic side of the inner membrane and negatively charged residues on the periplasmic side (Daniels et al., 1981; Yamane and Mizushima, 1988; Boyd and Beckwith, 1989; Andersson et al., 1992). This orientation is compatible with a membrane potential of the opposite charge, i.e. negative in and positive out. Indeed, the protonmotive force, which includes both a membrane potential and ΔpH component, has been shown to be important in the translocation of polypeptide chains and may specifically influence the export of proteins containing charged residues (Daniels et al., 1981; Yamane and Mizushima, 1988; Schiebel et al., 1991; Driessen and Wickner, 1991).

Other studies emphasize the importance of the mature protein in activation of SecA for ATP hydrolysis (Lill et al., 1990; Bassilana et al., 1992). Andersson and von Heijne (1991) delineated a 30-residue long “export initiation domain” at the amino terminus of the mature protein which is critical for translocation, and they postulate that this domain requires a productive interaction with some factor of the secretory machinery, perhaps SecA.

Models of protein secretion often invoke a loop insertion mechanism in the membrane involving the signal sequence and early mature region (Engelman and Steitz, 1981; Inouye and Hagleoua, 1980), perhaps specifically in a β-turn conformation (Inouye et al., 1986; Duffaud and Inouye, 1988). On the other hand, Ferenci and Silhavy (1987) proposed that the mature region has a passive role in transport. They predict that this region does not have stable secondary structure and thus is incapable of competing with the signal peptide for the export machinery during protein transport. In support of this notion, recent biophysical analysis found that the mature segment does not significantly affect the conformation and membrane interactive ability of synthetic signal sequences (McKnight et al., 1991).

Although the role of the early mature protein may be passive, the sequence of this region must help differentiate transportable proteins from nonsecreted ones. For example, the cytoplasmic protein, β-galactosidase, cannot be exported simply by attaching the leader sequence of an exported protein (Moreno et al., 1980; Kadonaga et al., 1984; Tommassen et al., 1985). However, localization of β-galactosidase to the periplasm was accomplished when the first 28 residues of the LamB mature protein was included between the LamB signal peptide and the...
β-galactosidase protein (Rasmussen and Silhavy, 1987). Chicken muscle triose-phosphate isomerase, also a cyttoplasmic protein, was transported efficiently by the β-lactamase signal peptide plus the first 12 amino acids of the mature β-lactamase, but not with the signal peptide of β-lactamase alone (Summers and Knowles, 1989).

Understanding the requirements for a transport competent protein will help elucidate the transport pathway in general. Furthermore, there is interest in expressing and transporting foreign proteins of therapeutic value in E. coli. For this to become a feasible approach, the involvement of the mature protein needs to be clarified. We have begun by examining the early mature region of E. coli alkaline phosphatase. Using a homopolymeric sequence of serines to replace 6 residues in this region, we produced a mutant which was dysfunctional in transport. Reintroduction of some of the residues from the native sequence resulted in little improvement. However, by replacing the hydrophobic core of the signal peptide with a homopolymeric series of leucines, functionality of the all serine mutant was restored. A hydrophobic core composed of 10 leucine residues is more hydrophobic than the wild type core and was previously shown to function very efficiently in transport of wild type alkaline phosphatase (Chou and Kendall, 1990). Here we show that it also contributes to the export-competence of a mutant protein. Our findings indicate that a transport defect created the NtS mutant. The arginine residue maintains the wild type position in order to keep the signal peptidase I cleavage site intact and in the membrane-associated fraction (pellet) was precipitated with trichloroacetic acid. Cells were washed with ice-cold acetone and suspended in 20% sucrose, 30 mM Tris (pH 8), containing 20 µg/ml freshly made lysozyme and 1 mM EDTA. After a 20-min incubation at 25 °C, cells were centrifuged and the supernatant containing the periplasmic fraction was filtered through a 0.22-mm filter. Alkaline phosphatase was immunoprecipitated from the whole cell fraction but was not immunoprecipitated from the periplasmic fraction.

**Protease Accessibility Experiment**—To determine whether the dys- functional mutant precursors were cytoplasmically or periplasmically oriented, cells were harvested and resuspended as described above for the cell fractionation experiment. Cells were labeled with 40 µCi of L-[35S]methionine for 40 s at 37 °C, then chased with 4 mg/ml non-radioactive methionine for 30 s, 1 min, 5 min, or 20 min. Proteins were precipitated with trichloroacetic acid as for the whole cell fractions and alkaline phosphatase was immunoprecipitated.

**MATERIALS AND METHODS**

**Bacterial Strains and Media**

*E. coli* strain AW1043 (Δlac galU galK Δ(leu-ara) phoA-15 proC::Tn5) was used in all experiments. For general propagation of cells we show that it also contributes to the export-competence of a mutant protein. Our findings indicate that a transport defect created the NtS mutant. The arginine residue maintains the wild type position in order to keep the signal peptidase I cleavage site intact and in the membrane-associated fraction (pellet) was precipitated with trichloroacetic acid. Cells were washed with ice-cold acetone and suspended in 20% sucrose, 30 mM Tris (pH 8), containing 20 µg/ml freshly made lysozyme and 1 mM EDTA. After a 20-min incubation at 25 °C, cells were centrifuged and the supernatant containing the periplasmic fraction was filtered through a 0.22-mm filter. Alkaline phosphatase was immunoprecipitated from the whole cell fraction but was not immunoprecipitated from the periplasmic fraction.

**Construction of Mutants**

The NiS mutant was constructed using the vector, WT-XN, a derivative of CASS3 in which a unique Xhol site has been inserted at the +8 position of the mature-amino terminus (Lafont and Kendall, 1991). The CASS3 mutagenesis vector is a pBR322 phoA derivative in which unique SalI and BssHII sites have been inserted flanking the ends of the hydrophobic core-encoding region of the signal peptide (Kendall and Kaiser, 1988). The WT-XN was digested with the restriction enzymes, SalI and BssHII, and the DNA encoding the first 8 amino acids of the mature protein was removed, and synthetic oligonucleotides encoding an arginine residue plus 6 serines were ligated to the remaining vector, creating the NtS mutant. The arginine residue maintains the wild type amino acid in the +1 position, while the 6 serines are changes in the subsequent wild type sequence. The amino acid sequences of the constructed mutants are shown in Fig. 1. The 5S64, 4SP36, and 2ST2 mutants were constructed in a manner similar to NiS, but included fewer serine residues; these were replaced by 1, 2, or 4 wild type residues, respectively.

A series of mutants was also created to include mutations in the hydrophobic core region of the signal peptide with an all serine amino terminus in the mature protein. The NiS was digested with the restriction enzymes, SalI and BssHII, the wild type hydrophobic core-encoding region was removed, and synthetic oligonucleotides encoding various ratios of alanine/leucine were ligated with the remaining vector. In all cases, the wild type length of the hydrophobic core was maintained (10 amino acid residues) as was the length of the amino terminus of the mature protein. Correct mutant sequences were verified by restriction enzyme analysis and by direct DNA sequencing (Sanger et al., 1977).

**Transport Studies**

**Lysozyme-EDTA Cell Fractionation**—Cells were grown at 37 °C in MOPS low phosphate medium containing 50 µg/ml kanamycin and 250 µg/ml ampicillin, and harvested during the logarithmic phase of growth. Cells were washed in MOPS medium containing no phosphate and resuspended in MOPS supplemented with 20 µg/ml amino acids minus methionine. Cells were diluted 1:10 in this medium prior to labeling with 40 µCi of L-[35S]methionine for 60 s. Radiolabeled cells were removed to ice and whole cell fractions were precipitated with 5% trichloroacetic acid, washed twice with acetone, and air dried. Periplasmic fractions were washed with 30 mM Tris (pH 8), resuspended in 0.5 M sucrose, 30 mM Tris (pH 8), and incubated at 25 °C for 15 min. Cells were pelleted and resuspended in 0.5 M sucrose, 30 mM Tris (pH 8), containing 20 µg/ml freshly made lysozyme and 1 mM EDTA. After a 20-min incubation at 25 °C, cells were centrifuged and the supernatant containing the periplasmic fraction was filtered through a 0.22-mm filter. Alkaline phosphatase was immunoprecipitated from the whole cell fractions and alkaline phosphatase was immunoprecipitated.

**Pulse-Chase Analysis**—To determine the rate of precursor processing, cells were cultured, washed, and resuspended in MOPS medium as described above. Cells were radiolabeled with 40 µCi of L-[35S]methionine for 40 s at 37 °C, then chased with 4 mg/ml non-radioactive methionine for 30 s, 1 min, 5 min, or 20 min. Proteins were precipitated with trichloroacetic acid as for the whole cell fractions and alkaline phosphatase was immunoprecipitated.

**Sodium Hydroxide Cell Fractionation**—To distinguish soluble from membrane-associated proteins, the method of Russel and Modell (1982) was followed. Cells were treated initially as described for the lysozyme-EDTA cell fractionation experiment. Following the 20-min incubation with lysozyme at 25 °C, 25 µg/ml proteinase K was added, after which one aliquot also received 0.2% CHAPS, and the other received an equal volume of distilled water. Samples were incubated on ice for 10 min, then 25 °C for 5 min and precipitated with 5% trichloroacetic acid. Cells were washed with ice-cold acetone and suspended in sample buffer containing SDS and β-mercaptoethanol.

**SDS-Polyacrylamide Gel Electrophoresis**—Alkaline phosphatase samples from transport studies were run on Laemmli SDS-polyacrylamide gels (Laemmli, 1970) and subjected to autoradiography as described by Kendall and Kaiser (1988). The ratio of precursor to mature protein was determined using densitometry.

**RESULTS**

Fig. 1 shows the amino acid sequences of all of the mutants of *E. coli* alkaline phosphatase characterized in this study. Our goal was to examine the amino-terminal region of the mature protein in transport; therefore we initially replaced amino acids +2 through +7 of the wild type sequence with 6 serine residues (mutant NiS). The wild type residue, arginine, was maintained at the +1 position in order to keep the signal peptide I cleavage site intact. A set of consecutive serine residues was chosen.
because serine is a relatively unobtrusive amino acid; it is not charged; it is neither highly polar nor very non-polar; and it does not have a high propensity for forming or disrupting α-helix or β-sheet structure (Chou and Fasman, 1978). The DNA encoding the early mature protein contained restriction sites conveniently flanking the first 8 amino acids of this region which facilitated construction of these mutants. The wild type sequence of the signal peptide and the remainder of the mature protein was retained in the NtS mutant.

Contrary to our expectations, the NtS mutant was transport defective. Using pulse-chase analysis, no mutant precursor processing was observed even after a 20-min chase period (Fig. 2). In order to understand which critical residues had been eliminated in this mutant, we reintroduced some of the wild type residues in three subsequent mutants. Previous analysis of several exported proteins indicates that proline and glycine residues are common in the early mature region (von Heijne, 1984; von Heijne, 1986) and these may be involved in the formation of a β-turn important in the signal peptidase cleavage region (Inouye et al., 1986; Duffaud and Inouye, 1988). Consequently, the 4SP36 mutant was designed to retain the naturally occurring prolines at positions +3 and +6 within an otherwise all serine region. As shown in Fig. 2, the processing of this mutant precursor was marginally improved (6% mature at 30 s) over that of the NtS mutant (0% mature at 30 s). Other studies suggest that a net positive charge in this region may inhibit protein transport (Li et al., 1988; Yamane and Mizushima, 1988; MacIntyre et al., 1990). However, reintroduction of a glutamic acid into the serine amino terminus (creating the 5SE4 mutant, which maintains the wild type net charge) resulted in no improvement in processing (0% mature at 30 s) over the NtS mutant (which has a net charge of +1). Reintroduction of this glutamate residue as well as a threonine into the 4SP36 mutant (creating the 2ST2 mutant) resulted in improved precursor processing (51% mature at 30 s), but to an extent still less than that observed for the wild type (76% mature at 30 s) (Fig. 2).

We considered the possibility that the impaired precursor processing observed for these mutants was due to an inhibition of translocation of the precursor from the cytoplasmic to the periplasmic side of the membrane. A protease accessibility experiment differentiates between cytoplasmic or cytoplasmically oriented (inserted into the membrane but facing the cytoplasm) and periplasmic or periplasmically oriented proteins. Mature alkaline phosphatase which has become localized in the periplasm, and precursors which are membrane-bound but facing the periplasm, are folded into a conformation which is resistant to degradation by protease. However, precursor alkaline phosphatase which is in or facing the cytoplasm is sensitive to proteolysis following detergent solubilization of the inner membrane. This experiment revealed that the NtS and 4SP36 precursors are sensitive to protease in the presence of detergent indicating that they are not translocated (Fig. 3). The 2ST2 precursor is also largely oriented towards the cytoplasm (Fig. 3) but translocation must proceed, albeit slowly, since some conversion to mature is observed in a pulse-chase study (Fig. 2).

Previous work has indicated that the translocation step can be enhanced by increasing the hydrophobicity of the signal peptide core region (Chou and Kendall, 1990). Using this approach, we attempted to improve processing of the dysfunctional NtS mutant by altering the wild type signal peptide hydrophobic core. When the wild type core was replaced by a hydrophobic segment composed of 10 consecutive leucine residues in this mutant (10LS), it was processed very rapidly (Fig. 4). Other studies have shown that a polyleucine containing signal peptide works more efficiently than the wild type (Kendall et al., 1986; Chou and Kendall, 1990; Laforet and Kendall, 1991) and we have now found that the polyleucine mutant can also compensate for a dysfunctional amino terminus in the mature protein.

The polyleucine-containing signal peptide is one of the most highly hydrophobic signal peptides produced either naturally or by genetic engineering. In order to determine the minimum level of hydrophobicity required to successfully transport the alkaline phosphatase mutant, we examined its export competence when directed by a series of signal peptides of lower hydrophobicity. This was done using signal sequences which vary systematically in the ratio of alanine to leucine residues in

![Fig. 1. The amino acid sequences of the wild type and mutant alkaline phosphatase signal peptides. The natural signal peptide amino-terminal and cleavage regions are maintained in all mutants. The hydrophobic core regions are italicized and the altered amino-terminal residues of the mature protein are boldfaced. A caret (^) marks the site of cleavage by signal peptidase. WT-XN, wild type. WT-XN is a derivative of CAS88 (Kendall and Kaiser, 1988; Laforet and Kendall, 1991).](image)
Hydropathicity in Re-establishment of Protein Transport

Fig. 2. Pulse-chase analysis of precursor processing of mature region mutants. Cells were radiolabeled with 40 μCi of L-[^35]S]methionine for 40 s and then chased with cold methionine for 30 s, 1 min, 5 min, and 20 min as described under “Materials and Methods.” Migration of precursor is indicated by arrowhead a; mature enzyme is indicated by b.

Fig. 3. Cellular localization and orientation of precursors of mature region mutants. Radiolabeled, lysozyme-treated cells were proteolyzed with proteinase K in the presence or absence of CHAPS as described under “Materials and Methods.” An untreated whole cell fraction was also taken for comparison. Migration of precursor is indicated by arrowhead a; mature enzyme is indicated by b. The control lane for each mutant shows more mature protein than the other two lanes because it was incubated on ice prior to precipitation with trichloroacetic acid, allowing precursor to be converted to mature.

The results indicate that a sufficiently hydrophobic signal peptide core can compensate for a polar mature amino terminus. Further experiments were done to examine which step(s) along the transport pathway were problematic for those mutants with signal peptides insufficiently hydrophobic to compensate for the serine residues. A protease accessibility experiment indicates that the mutant precursors which accumulate are not translocated; they are cytoplasmically oriented and thus are degraded in the presence of protease plus detergent (Fig. 6).

An earlier step along the export pathway which may be blocked is membrane insertion. Using the method of Russel and Model (1982), a sodium hydroxide cell fractionation experiment was carried out to determine if the untranslocated mutant precursors are stably membrane associated. This experiment shows that the dysfunctional precursors are found in both the membrane and soluble fractions, although predominantly in the latter (Fig. 7). It appears that low hydrophobicity in the signal peptide limits membrane insertion and the small amount of precursor which is membrane inserted is not translocated to the periplasmic side of the membrane. As expected, the functional 4A6LS mutant and the wild type appear as mostly mature protein which is almost completely in the soluble fraction.

At the other extreme, the precursor proteins with more than 6 leucines in their hydrophobic core are processed so rapidly that the question arises as to whether these mutants require a protonotive force for translocation across the membrane. A protonotive force is normally required for the export of alkaline phosphatase and several other proteins (Enequist et al., 1981; Geller et al., 1986; Randall et al., 1987; Yamane et al.,...
Hydropathy in Re-establishment of Protein Transport

FIG. 5. Cellular localization of mature alkaline phosphatase. Cells were pulse-labeled with 40 μCi of L-[35S]methionine for 1 min and radiolabeled precursor and mature alkaline phosphatase were immunoprecipitated from both whole cells and periplasmatic fractions as described under "Materials and Methods." Migration of precursor is indicated by arrowhead a; mature enzyme is indicated by b. WC, whole cell fraction (total alkaline phosphatase including periplasmic); p, periplasmic fraction.

FIG. 6. Cellular localization and orientation of precursors of mature region plus core region mutants. Cells were treated as described in the legend for Fig. 3.

FIG. 7. Relative membrane partitioning of alkaline phosphatase by NaOH cell fractionation. Cells were radiolabeled with 40 μCi of L-[35S]methionine for 40 s, treated with NaOH, and separated into a soluble (cytoplasmic and periplasmic) component and a membrane component by microcentrifugation for 15 min at 4°C as described under "Materials and Methods." Migration of precursor is indicated by arrowhead a; mature enzyme is indicated by b. S, soluble fraction; M, membrane fraction.

1987; Kato et al., 1992). CCCP disrupts the electrochemical gradient across the inner membrane which is important for efficient transport and processing. Upon examining the effect of CCCP on cells harboring the mutant sequences, we find that precursor accumulates in the case of the 4A6LS mutant and the wild type (Fig. 8). However, in the case of the 2A8LS and 10LS mutants, no precursor accumulation occurs even with very high levels of CCCP. Daniels et al. (1981) found that as little as 8 μM CCCP, which is sufficient to dissipate the membrane potential in E. coli (Schaldiner and Kaback, 1975), caused precursor accumulations of about 40% in leucine-specific binding protein and about 60% in β-lactamase. Interestingly, for the very hydrophobic alkaline phosphatase signal peptides we find no precursor accumulation with as high as 1 mM CCCP (data not shown). Like precursor processing, the midpoint for CCCP sensitivity is seen with the 4A6LS mutant and the more hydrophobic mutants show no effect.

DISCUSSION

In this study we show that replacement of residues +2 to +7 of E. coli alkaline phosphatase with 6 serine residues completely blocks transport. Furthermore, reintroduction of the wild type residues leaving serine only in the +5 (replacing methionine) and +7 (replacing valine) positions results in limited improvement.

This sensitivity of transport to the composition of the early mature region is not predicted by visual inspection of the native sequences of exported proteins. No consensus sequence is apparent (von Heijne, 1984). While this region typically has a net negative charge, and studies have implicated the importance of an overall positive to negative transition from the amino terminus of the signal peptide into the early region of the mature (von Heijne, 1986), charge considerations alone do not explain our results. In producing the all serine amino terminus in alkaline phosphatase, a glutamic acid residue was replaced leaving a net charge of +1. Reintroduction of the negative charge did not enhance precursor processing. Other studies point to the occurrence of proline and glycine residues and suggest that a β-turn structure in the early mature protein is an important feature for signal peptidase recognition and signal peptide cleavage (Inouye et al., 1986; Dufau and Inouye, 1988). Serine residues have reasonable β-turn potential (Chou and Fasman, 1978) but neither the NtS mutant nor the variant with the prolines reintroduced exhibited noteworthy processing rates. Reintroduction of the wild type residues in four of the six serine positions, including the glutamic acid and 2 prolines, resulted in improved precursor processing, but not to wild type levels.

Far more striking is the effect of changing the signal peptide composition. We were able to completely alleviate export incompetence by increasing the hydrophobicity of the attached signal peptide in the form of a hydrophobic core containing 10 leucine residues. Titration of the signal peptide hydrophobicity further showed that a core of 3 alanines and 7 leucines was sufficiently hydrophobic to transport the mature region mutant and to be processed more readily than an all wild type precursor. In view of a membrane insertion mechanism which involves membrane partitioning of the early mature region as well as the signal peptide, these results make good sense; the overall hydrophobicity of the entire loop region is critical. Although it is difficult to make quantitative comparisons, it is interesting that the hydropathy values of the amino acids employed suggest that a small increase in hydrophobicity of the signal peptide (3A7L versus wild type) can overcome a relatively large increase in polarity of the early mature region. Signal peptide hydrophobicity can further override possible requirements for negatively charged residues or strong β-turn formers such as proline.

It is not clear whether the increased signal peptide hydrophobicity compensates for the defect in the early mature region because it enhances partitioning directly into the lipid phase of the bilayer and/or improves an interaction with a component of the protein machinery involved in transport. In addition to the signal peptide, the early mature region has been implicated in interactions with SecA and SecY/PrlA (Liss et al., 1985; Lill et al., 1990). Our finding that the more hydrophobic signal peptides not only compensate for the mature protein defect but also show insensitivity to CCCP treatment is intriguing. Proton motive force-independent translocation, in the presence of high concentrations of SecA, has been observed previously and a relationship between the requirements for energy derived from SecA ATP hydrolysis and the proton motive force has been suggested (Yamada et al., 1989; Schiebel et al., 1991). It may be that our CCCP-insensitive precursors derive sufficient energy...
from the former that translocation can proceed without the additional involvement of the protonmotive force.

Previous studies have shown that increased hydrophobicity of the signal peptide core can also compensate for decreased charge in the amino terminus of the signal peptide both in vivo (Puzias et al., 1989) and in vitro (Hikita and Mizushima, 1992; Sung et al., 1992). MacIntyre et al. (1990) found that export incompetence created by amino-terminal basic residues in a mature polypeptide can be partially compensated by a longer hydrophobic core in the signal peptide. In this study, we were able to completely alleviate export incompetence by increasing the hydrophobicity of the attached signal peptide in the form of a hydrophobic core containing 10 leucine residues. This same leucine core was previously found to make the signal peptide cleavage region less sensitive to mutations (Laforet and Kendall, 1991).

Our results further suggest that signal peptide optimization may be important for the transport of foreign proteins in E. coli for their subsequent isolation and purification. The amino terminus of the mature protein appears to be a critical region in determining transport competence. Foreign proteins which are not normally transported are likely to contain transport-incompetent sequences in this region and furthermore, can be expected to differ widely one to another. Rather than modifying each of these regions individually, the highly hydrophobic, polyleucine-containing signal peptide offers a possible generic approach for the transport of foreign proteins with retention of their native amino-terminal sequence.

REFERENCES
Andersson, H., and von Heijne, G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9751–9754
Andersson, H., Bakker, E., and von Heijne, G. (1992) J. Biol. Chem. 267, 1491–1495
Basiliano, M., Arkowitz, R. A., and Wickner, W. (1992) J. Biol. Chem. 267, 25246–25250
Boyd, D., and Beckwith, J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9446–9450
Chou, P. Y., and Fasman, G. D. (1978) Annu. Rev. Biochem. 47, 251–276
Chou, M. M., and Kendall, D. A. (1990) J. Biol. Chem. 265, 2873–2880
Daniela, C. J., Bole, D. G., Quay, S. C., and Ostender, D. L. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5396–5400
Dearl, S. K., Chou, M. M., and Kendall, D. A. (1993) Biochemistry 32, 1251–1266
Driessen, A. J. M., and Wickner, W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2471–2475
Duffaud, G., and Inouye, M. (1998) J. Biol. Chem. 263, 10224–10228
Enquist, H. G., Hirst, T. R., Harayama, S., Hardy, J. S., and Randall, L. L. (1981) Eur J. Biochem. 118, 227–233
Engelman, D. M., and Steitz, T. A. (1981) Cell 33, 411–422
Ferenci, T., and Silhavy, T. J. (1987) J. Bacteriol. 169, 5339–5342
Geller, B. L., Movva, N. R., and Wickner, W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4219–4222
Gennis, R., Goldstein, J., and Inouye, M. (1990) J. Biol. Chem. 265, 2323–2329
Goldstein, J., Lehnhardt, S., and Inouye, M. (1990) J. Bacteriol. 172, 1225–1231
Hikita, C., and Mizushima, S. (1992) J. Biol. Chem. 267, 12575–12579
Inouye, M., and Hasegawa, S. (1980) CRC Crit. Rev. Biochem. 7, 339–371
Inouye, S., Soberon, X., Franceschini, T., Nakamura, K., Itakura, K., and Inouye, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3438–3441
Inouye, S., Pfaffl, G., and Inouye, M. (1986) J. Biol. Chem. 261, 10970–10975
Kadowaka, J. T., Gautier, A. E., Strauss, D. R., Charles, A. D., Edge, M. D., and Knowles, J. R. (1984) J. Biol. Chem. 259, 2149–2154
Kato, M., Tokuda, H., and Mizushima, S. (1992) J. Biol. Chem. 267, 413–418
Kendall, D. A., and Kaiser, E. T. (1988) J. Biol. Chem. 263, 7261–7265
Kendall, D. A., Bock, S. C., and Kaiser, E. T. (1988) Nature 331, 706–708
Kuhn, A., and Wickner, W. (1985) J. Biol. Chem. 260, 15914–15918
Laemmli, U. K. (1970) Nature 227, 680–685
Laforet, G. A., and Kendall, D. A. (1991) J. Biol. Chem. 266, 1326–1334
Li, F., Beckwith, J., and Inouye, H. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7085–7089
Lill, R., Dowhan, W., and Wickner, W. (1990) Cell 60, 271–280
Liss, R., Johnson, B. L., and Oliver, D. B. (1985) J. Bacteriol. 165, 925–928
MacIntyre, S., Hochbach, M.-L., and Mutschler, B. (1990) Mol. Gen. Genet. 221, 466–474
McKnight, C. J., Stradley, S. J., Jones, J. D., and Giersch, L. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5799–5803
Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Morera, F., Fowler, A. V., Hall, M., Silhavy, T. J., Zabin, L., and Schwartz, M. (1989) Nature 340, 356–359
Neidhardt, F. C., Bloch, P. L., and Smith, D. F. (1974) J. Bacteriol. 116, 736–747
Petman, D., and Halvorson, H. O. (1985) J. Mol. Biol. 187, 391–409
Puzias, J. W., Fikes, J. D., and Basford, P. J. (1989) J. Bacteriol. 171, 2303–2311
Randall, L. L., Hardy, J. S., and Thom, J. R. (1987) Annu. Rev. Microbiol. 41, 507–541
Rasmussen, B. A., and Silhavy, T. J. (1987) Genetics 119, 185–196
Rosenblatt, M., Beudet, N. V., and Fasman, G. D. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2985–2987
Russel, M., and Model, P. (1982) Cell 28, 177–184
Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
Schiebel, E., Driessen, A. J. M., Hartz, F. U., and Wickner, W. (1991) Cell 64, 927–939
Schuldiner, S., and Kaback, H. R. (1975) Biochemistry 14, 5451–5461
Schweizer, M., and Knowles, J. R. (1989) J. Biol. Chem. 264, 20074–20081
Summers, R. G., Harris, C. R., and Knowles, J. R. (1989) J. Biol. Chem. 264, 20082–20088
Sung, C. Y., Gennity, J. M., Pollitt, N. S., and Inouye, M. (1992) J. Biol. Chem. 267, 997–1000
Tummassen J., Leunissen, J., van Damme-Jongsten, M., and Overduin, P. (1985)
Vlasuk, G. P., Inouye, S., Ito, H., Itakura, K., and Inouye, M. (1983) J. Biol. Chem. 258, 7141–7145
Vlasuk, G. P., Inouye, S., and Inouye, M. (1984) J. Biol. Chem. 259, 6195–6200
von Heijne, G. (1984) J. Mol. Biol. 173, 243–251
von Heijne, G. (1986) J. Mol. Biol. 192, 237–250
van Heijne, G., and Gavel, Y. (1986) Eur J. Biochem. 174, 671–678
Yamada, H., Matayuama, S., Tokuda, H., and Mizushima, S. (1989) J. Biol. Chem. 264, 18577–18581
Yamane, K., Ichihara, S., and Mizushima, S. (1987) J. Biol. Chem. 262, 2358–2362
Yamane, K., and Mizushima, S. (1988) J. Biol. Chem. 263, 19690–19696