Alternations to the Signal Peptide of an Outer Membrane Protein (OmpA) of Escherichia coli K-12 Can Promote Either the Cotranslational or the Posttranslational Mode of Processing

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The signal sequence of the precursor of the Escherichia coli outer membrane protein OmpA was altered by oligonucleotide insertions into the corresponding gene. In one case, OmpA-S1, the hydrophobic core of the signal peptide, is reduced from 12 to 10 residues, and one positive charge is added near the NH₂-terminal. In another case, OmpA-P1, the hydrophobic core is extended from 12 to 16 residues. The pro-OmpA protein is normally processed partially co- and partially posttranslationally. Processing of the pro-OmpA-S1 protein was entirely posttranslational and that of the pro-OmpA-P1 protein strictly cotranslational. Evidence is presented which strongly suggests that posttranslational processing reflects posttranslational translocation across the plasma membrane. The generation times of cells expressing pro-OmpA-P1 or pro-OmpA-S1 were identical and the pro-OmpA-S1 polypeptide could be chased into the mature protein in the absence of protein synthesis. Hence, it does not matter which mode of processing, or rather translocation, is used. The same oligonucleotides were inserted into the ompA gene of plasmid pRD87; a plasmid which leads to overproduction of the protein and to massive accumulation of both the mature protein and the precursor. In the OmpA signal sequence encoded by pRD87-P2 the hydrophobic core is extended from 12 to 20 residues. This peptide was also rapidly removed. Therefore, regardless of whether the hydrophobic core contains 12, 16, or 20 lipophilic residues, not only does the signal sequence always function correctly to mediate export, but in each case, the cleavage site is always accessible to the signal peptidase.

Most Escherichia coli proteins to be translocated across the plasma membrane are synthesized as precursors with an NH₂-terminal signal sequence. Typically, such signal sequences are 15–25 amino acids long and possess one or more positive charges at the NH₂-terminus, followed by a stretch of about 8–12 hydrophobic residues (1). It is not known exactly how a signal peptide steers a protein to the plasma membrane (recent reviews, Refs. 2–5), but evidence has been presented suggesting that it may perform multiple functions (6). It has been shown that the time of removal of the signal peptide, i.e. processing, varies between different proteins; processing can be entirely posttranslational, strictly cotranslational, or partially co- and partially posttranslational (7, 8). It is not clear which factor(s) determines the mode of processing. There are, however, indications that the signal sequence may be involved. For example, the signal peptide of the major E. coli lipoprotein is removed largely cotranslationally (9), but the mode of processing becomes posttranslational when the positively charged NH₂-terminus of the signal peptide is replaced by a negative charge (9, 10). Also, there exist mutants, with altered signal sequences, in which the processing rate is delayed (2–4). Furthermore, evidence has been presented which shows that processing of the precursor of the E. coli alkaline phosphatase can be converted from partially cotranslational to completely posttranslational by increasing the hydrophobicity of the signal peptide (11).

The precursor of the 325-residue outer membrane protein OmpA (12) is processed partially co- (60%) and partially posttranslationally (7). We have asked if the signal peptide of this precursor can be altered in such a way that it is processed either entirely cotranslationally or entirely posttranslationally and, if so, whether one or the other type is preferred by the cell.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—Strain UH204 (13) is lac supF recA proA B rpsL / F' lacZΔM15 proA B'; strain UH204 has the same characteristics except that it is leu−:Tn10 and secA ts51 (14). Unless specified otherwise, cells were grown at 37 °C in L-broth (15) containing glucose (0.5%) or IPTG (1 mM). Ampicillin and kanamycin were used at 20 μg/ml. Minimal medium was M9 (15) supplemented with glucose (0.5%) or glycerol (1%), thiamine (1 μg/ml), and L-leucine (20 μg/ml). Cells were grown at 37 or 28 °C in case of the secA strain.

Plasmids—Plasmids pBGS19, pTUS00, pRD87, and pINT4-6 have been described (16–18). Plasmid pBGS19 possesses the multiple cloning region of pUC19 (19). The ompA Δ56-227 gene is present in pUC9 (17). The gene could therefore be placed into pBGS19 by using adjacent restriction sites. The insertion of linkers (obtained from New England Biolabs) is illustrated in Fig. 1. All operations followed standard procedures (20). The numbers of linkers (obtained from New England Biolabs) inserted and the correctness of the reading frames were determined by DNA sequencing (21) of a suitable restriction fragment cloned into phage M13 mp 19 (19).

Pulse-Chase Experiments, Immunoprecipitation, and Treatment of Cells with Trypsin—The procedures described previously were followed, and the same concentrations of reactants were used (18). Alterations are indicated in the figure legends. Since the pro-OmpA protein cannot, in our strains, be detected easily at 37 °C (22), the pulse-chase experiments were performed at the lower temperatures indicated in the figure legends. "Electrophoresis" always means sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lysis of cells was achieved by adding egg white lysozyme (0.5 mg/ml) to suspensions of cells in the sucrose/EDTA buffer (18), freezing at -25 °C, and thawing in ice. Three to four freeze-thaw cycles sufficed to lyse most of the cells. For the experiment documented in Fig. 5, the effect of chloramphenicol was measured; it blocked incorporation of [35S] methionine immediately.

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1 The abbreviation used is: IPTG, isopropylthiogalactoside.
RESULTS

Structures of Altered Signal Sequences—The signal sequence of the pro-OmpA protein was altered in two different ways by inserting oligonucleotides. The ompA gene present in the high copy number plasmid pTU 500 was used. This gene is under the control of the lac regulatory elements (17) and harbors a TAG stop codon (23). When induced, the protein is not overproduced because, in the presence of the amber suppressor supF, the concentration of the corresponding tRNA becomes limiting, and the protein is synthesized in amounts equal to that found in chromosomally ompA+ strains (24, 25).

The ompA gene contains a unique restriction site for NruI within the codon for amino acid residue -15 of the signal peptide. A SmaI linker or a PvuI linker was inserted into this site as shown in Fig. 1 to yield plasmids pTU 500-S1 and -P1, respectively. The presence of one SmaI linker in pTU 500-S1 adds a positive charge and reduces the length of the lipophilic core from 12 to 10 residues. The presence of the PvuI linker in pTU 500-P1 extends this core by 4 hydrophobic residues.

Processing and Translocation—Expression of the altered ompA genes did not have any noticeable effect on the growth rate or on the viability of the cells. The generation times of cells carrying pTU 500, pTU 500-S1, or pTU 500-P1 were identical. Electrophoretograms of cell envelopes of the ompA strain UH203 carrying plasmids pTU 500-S1 or -P1 showed the presence of wild type amounts of the OmpA protein (not shown). Also, both strains, when induced for expression of ompA, proved to be fully sensitive to the OmpA-specific phages K3 and OX2. Hence, the mature proteins are assembled correctly in the outer membrane.

Pulse-chase experiments, however, revealed that the mode of processing was altered (Fig. 2). With cells carrying pTU 500, which encodes the wild type precursor, both mature protein and complete precursor could be detected at short chase times; whereas, with cells carrying pTU 500-S1, complete precursor always appeared first and significant amounts of mature precursor could be detected only after longer chase times; i.e. processing had become essentially posttranslational. In contrast, no precursor could be detected with pTU 500-P1. Hence, in this case processing had become strictly cotranslational or was so rapid that a complete precursor could exist in parallel experiments regarding the magnitude of the synthesis block at a given time after the shift in temperature (27). The type of processing observed in the secA strain (Fig. 2) could, therefore, be severely influenced by this variable.

FIG. 2. Processing of precursors. A, pulse-chase experiment. Strain UH203 carrying pTU 500 (lanes 1 and 2), pTU 500-P1 (lanes 3 and 4), or pTU 500-S1 (lanes 5 and 6) were grown for 2 h in the presence of IPTG, labeled at 28°C for 2 min with [35S]methionine and chased with L-methionine for 30 s (lanes 1, 3, and 5) and 10 min (lanes 2, 4, and 6). B, cells of the secA strain carrying pTU 500 (lanes 1 and 2) or pTU 500-P1 (lanes 3 and 4) were grown in the presence of IPTG for 3 h at 28°C (lanes 1 and 3) or 42°C (lanes 2 and 4). They were then labeled for 2 min with [35S]methionine at 28°C. In A and B, an autoradiogram of an electrophoreogram of the immunoprecipitated proteins is shown. Note the altered electrophoretic mobilities of the mutant precursors (see also Figs. 3 and 7). p, precursor; m, mature protein.

Preceding the alteration in the signal peptides cause considerable alterations in the electrophoretic mobilities of the precursors (see also Figs. 3 and 7). The same phenomenon has been observed for other mutationally altered pro-OmpA signal peptides (26). It may be that this reflects conformational changes.

It is of some interest concerning the function of the SecA protein that even under the nonpermissive condition, processing of the mutant precursor still appears to be more efficient than processing of the wild type precursor. However, synthesis of exported proteins decreases in secA strains at the nonpermissive temperature. Moreover, substantial variability can exist in parallel experiments regarding the magnitude of this synthesis block at a given time after the shift in temperature (27). The type of processing observed in the secA strain (Fig. 2) could, therefore, be severely influenced by this variability. To exclude such potential artifacts we have used an internal control. An internally deleted ompB gene, ompB Δ86-227 (17), was placed into plasmid pBGS19 which specifies resistance to kanamycin (16). A secA strain could thus be obtained carrying both pTU 500 or pTU 500-P1 and pBGS19 ompA Δ86-227. The results of the relevant labeling experiments are shown in Fig. 3. The internally deleted OmpA proteins behaved identically in both cases while again the protein expressed from pTU 500-P1 was, at the nonpermissive temperature, processed more rapidly than the wild type precursor.

FIG. 3. Processing of two precursors in a secA strain. Cells of the secA strain carrying pBGS19 ompA Δ86-227, plus pTU 500 (lanes 1 and 2) or pTU 500-P1 (lanes 3 and 4), were grown at 28°C in minimal glycerol medium. Induction with IPTG was for 3 h at 28°C (lanes 1 and 3) or at 42°C (lanes 2 and 4). Labeling was as detailed in Fig. 2B. p, precursor; m, mature proteins. An autoradiogram of immunoprecipitated proteins is shown.

FIG. 1. Construction of altered signal sequences. A, the pro-OmpA signal sequence. The underlined 12 residues constitute the lipophilic core according to von Heijne (50). Threonine and serine residues occur quite often within lipophilic cores of signal peptides (1) and, in such an environment, these residues may be considered hydrophobic (5, 51). Hence, this core may actually extend to residue −3. The arrowhead indicates the cleavage site for NruI, the recognition sequence for the enzyme is underlined. B and C, insertion of the SmaI and the PvuI linkers, respectively.
Does posttranslational processing of the mutant precursor expressed from pTU 500-S1 reflect posttranslational translocation across the plasma membrane? In an attempt to answer this question we have performed protease accessibility experiments. In the presence of sucrose and EDTA, proteases can penetrate the outer but not the inner membrane and thus have access to the periplasmic space but not to the cytosol (28). When chromosomally ompA wild type cells are treated with trypsin in the presence of sucrose and EDTA, the ompA protein is digested in a characteristic manner, i.e., the enzyme removes most of the periplasmic part of the protein and leaves the membrane moiety (M, 22,300) which is protected from proteolytic attack (12, 29). Cells producing the mutant precursor, expressed from pTU 500-S1, or a wild type OmpA protein, encoded by pTU 500, were labeled radioactively and exposed to trypsin in the presence of sucrose and EDTA. Fig. 4 shows that the mutant precursor remained undegraded while the mature protein was degraded. To demonstrate that the mutant precursor is not itself trypsin-resistant, cells were lysed by repeated freezing and thawing in the presence of lysozyme. Fig. 4 shows that the precursor present in such lysates could be degraded completely by trypsin. However, it has been shown that with certain signal sequence mutants of the pro-maltose binding protein which are also processed posttranslationally, a fraction of the precursor polypeptides lose their export compatibility and remain cytosolic (30, 31). Therefore, to claim a posttranslational mode of export for the altered pro-OmpA protein, it was necessary to demonstrate that the trypsin-resistant species, which we find by pulse labeling, is export-compatible. Therefore, a pulse-chase experiment was performed with the chase in the presence of chloramphenicol. Fig. 5 shows that most of the mutant precursor is chased into the mature protein in the absence of protein synthesis.

For reasons detailed under “Discussion,” we have also studied the mode of processing and trypsin sensitivity of two large and abnormal precursors. We have reported recently on hybrid proteins consisting of the OmpA protein and a fragment of a polypeptide constituent of the long tail fibers of phage T4 (18). In hybrids T4-5 and T4-10, 253 amino acids of the tail fiber protein are present once and twice in tandem, respectively, between residues 153 and 154 of the OmpA protein. In both cases, the hybrid polypeptides were translated without problems and apparently cotranslationally, as the precursors could only be detected in a secA background (18). To retard processing, the SmaI linker was inserted into the NruI site of both hybrid genes, as shown in Fig. 1 for the wild type ompA gene. As expected, processing of the T4-5 hybrid became essentially posttranslational (Fig. 6A). However, processing of the T4-10 hybrid remained cotranslational and the precursor of this hybrid could still only be detected in a secA background (not shown, cf. Ref. 18). As with the wild type precursor, this posttranslationally processed precursor of the T4-5 hybrid was resistant to trypsin in plasmolyzed cells and was digested completely by the enzyme after gently lysing the cells (Fig. 6B).

Overproduction of Mutant Precursors—As stated above, the failure to detect the pro-OmpA-P1 protein in pulse-chase experiments does not prove completely that processing in this case is totally cotranslational. A completed precursor may be processed too rapidly to be detectable. We have therefore also studied processing under conditions in which the pro-OmpA protein is drastically overproduced. In plasmid pRD87 an ompA gene is present which does not possess the TAG stop codon mentioned above (17). As a consequence, induction of ompA gene expression from this plasmid leads to overproduction of the pro-OmpA protein and both the mature and

![Fig. 4. Trypsin resistance of the posttranslationally processed pro-OmpA protein in permeabilized cells. Lanes 1–4, wild type OmpA protein expressed from pTU 500; lanes 5–8, the protein expressed from pTU 500-S1. Cells induced with IPTG for 3 h were labeled with [35S]methionine for 2 min. Lanes 1, 2, 5, and 6, cells in the presence of sucrose/EDTA were incubated with (lanes 2 and 6) or without trypsin (lanes 1 and 5). Lysed cells (lanes 3, 4, 7, and 8) were also incubated with (lanes 4 and 8) or without trypsin (lanes 3 and 7). An autoradiogram of an electrophoretogram of the immunoprecipitates is shown. p, precursor; m, mature protein.](image1)

![Fig. 5. Pulse-chase in the presence of chloramphenicol. Cells carrying pTU 500-S1 were induced with IPTG for 2 h. They were labeled with [35S]methionine for 1 min (lane 1) and L-methionine was added immediately followed by chloramphenicol (150 μg/ml). Aliquots were removed after 30 s, 1 min, 2 min, 5 min, 10 min, 60 min, and 120 min (lanes 2–7, respectively). An autoradiogram of an electrophoretogram of the immunoprecipitates is shown. p, precursor; m, mature protein.](image2)

![Fig. 6. Posttranslational processing and trypsin resistance of the precursor of the OmpA-T4 hybrid in permeabilized cells. A, pulse-chase experiment. Following 30 min induction with IPTG at 37 °C, cells harboring plasmid pTU T4-5-S were labeled with [35S]methionine for 30 s (lane 1) at 24 °C. L-Methionine was then added and aliquots were removed after 30 s, 1 min, 10 min, 30 min, and 60 min (lanes 2–6, respectively). B, cells induced as in A were labeled with [35S]methionine for 2 min at 24 °C and incubated for a further 10 min in the presence of L-methionine (lane 1). They were then permeabilized by adding sucrose/EDTA and incubated without (lane 2) or with trypsin (lane 3). Lysed cells were also incubated without (lane 4) or with trypsin (lane 5). Autoradiograms of electrophoretograms of the immunoprecipitate proteins are shown. p, precursor; m, mature protein.](image3)
the pro-OmpA protein accumulate in massive amounts (31, Fig. 7). Smal or Poul linkers were also inserted into the NruI site of the ompA gene encoded by this plasmid (cf. Fig. 1). Plasmids pRD87-P1 and -P2, containing one and two Poul linkers, respectively, were recovered as well as pRD87-S1 and -S3 which harbor one and three Smal linkers, respectively. Fig. 7 shows the relevant electrophoretogram. Interestingly, considerably more total protein is produced with the posttranslationally processed species than with those processed cotranslationally. About one-half of the wild type ompA gene product is present as precursor, while hardly any pro-OmpA-P1 and no pro-OmpA-P2 proteins could be detected. As expected, the pro-OmpA-S1 and pro-OmpA-S3 proteins accumulated in much larger amounts than the wild type precursor. These results together with the pulse-chase experiments leave little doubt that the pro-OmpA-P1 and -P2 proteins are processed (and translocated) cotranslationally.

**Discussion**

The results reported show that the mode of processing can be dictated by the signal peptide. There was no effect on cell growth when this process was converted to either the cotranslational or to the posttranslational mode. Also, and in contrast to several signal sequence mutants in which processing is delayed (30, 31), most of the completed precursor could be chased into the mature form. Loss of competence for translocation of a signal sequence mutant of the E. coli maltose binding protein has been attributed to premature folding of the precursor (31). Neither the OmpA protein nor its precursor appear to form a stable conformation before the polypeptide reaches the outer membrane. Such a conformation is presumably only formed when the OmpA protein interacts with the lipopolysaccharide of this membrane (32, 33). Thus, even when processing is retarded very considerably, most of the pro-OmpA protein remains export-compatible (cf. Fig. 5), although it cannot be excluded that a very small fraction may have become incompatible for export and was lost by degradation in the cytoplasm. Therefore, it appears to be basically irrelevant how the pro-OmpA protein is processed.

We have not shown directly that the observed posttranslational processing reflects posttranslational translocation across the plasma membrane. We believe, however, that this is very likely the case. The question is whether trypsin resistance of the precursor is clear evidence that the polypeptide has not crossed the plasma membrane. Such an interpretation has been questioned recently. Lee and Beckwith (34) argued that resistance to a protease could be due to the presence of the signal sequence, as a precursor which has been translocated across the plasma membrane may be held, by the signal peptide, in such close association with the periplasmic face of the membrane that it is inaccessible to proteases. They further argued that such a protease-resistant precursor may become protease-sensitive upon lysing cells, either with a detergent or by sonication, due to disruption of the precursor-membrane complex. One possible explanation for such a hypothetical protease-resistant precursor would be the existence of a protease-resistant conformation specific for this membrane-bound form of the polypeptide. The studies with the precursor of the OmpA-tail fiber hybrid practically exclude this possibility. If a trypsin-resistant conformation specific for a pro-OmpA polypeptide held at the periplasmic face of the membrane existed, it would almost certainly be disrupted in the precursor of the hybrid protein. Yet, in plasmolyzed cells this pro-OmpA-tail fiber hybrid was completely resistant to proteolytic degradation. Furthermore, aside from using a rather mild procedure to lyse cells, we have shown that the accumulated, posttranslationally processed pro-OmpA-Si protein can be chased into the mature protein in the absence of protease synthesis (Fig. 5). Assuming then that posttranslational processing means posttranslational translocation, as it certainly appears to, it evidently also does not matter to the cell whether the pro-OmpA protein is translocated post- or cotranslationally.

Under conditions of overproduction, however, the mode of processing of the precursor does matter. Overproduction of the wild type pro-OmpA protein causes accumulation of the precursor and the mature protein at a ratio of about 1:1. We have shown before that this accumulated precursor is located in the cytoplasm in the form of large aggregates, i.e. it is lost for translocation (32). There was hardly any or no precursor detectable in cells overproducing the cotranslationally processed precursors, but high levels of the precursor of the posttranslationally processed species, much higher than the wild type precursor, were accumulated during overproduction. As evidenced by immunoelectron microscopy (32), the precursors of the posttranslationally processed species were present in large clumps in the cytoplasm (not shown). It thus appears that the posttranslationally processed precursor, accumulating in overproducing strains, rapidly loses export compatibility by forming insoluble aggregates in the cytoplasm. It was noted that considerably more posttranslationally processed protein was synthesized than that processed cotranslationally (Fig. 7). The reason for this is unknown. It may be, however, that the amount of protein synthesized, in addition to the altered signal peptides, influences the mode of processing; if less precursor is produced, the chance of its interaction with the export apparatus may increase.

We do not know exactly how the alterations of the signal peptide cause the observed effects. The data shown in Table I tend to indicate that one factor ruling the mode of processing is the number of lipophilic residues present in a single stretch uninterrupted by glycine or proline residues. Experiments using a synthetic pro-LamB signal peptide have indicated that this peptide may adopt a β-structure when approaching a lipid surface, followed by conversion to an α-helix during insertion into the membrane (35). Proline or glycine residues, both strong α-helix breakers, could partially inhibit the latter step and thus cause posttranslational processing when present within the lipophilic core of a signal sequence. In apparent agreement with this are studies with an "idealized" signal peptide of the PhoA protein (PhoA-L, Table I). In this peptide

![Fig. 7. Stained electrophoretogram of cell envelopes. Cells (strain UH203) carrying pTU500, pRD87, pRD87-P1, pRD87-P2, pRD87-S1, or pRD87-S3 (lanes 1–6, respectively) were grown for 3 h in the presence of IPTG. p, precursor; m, mature protein. Note, as in Figs. 2 and 3, the altered electrophoretic mobilities of the mutant precursors.](image-url)
Protein Export in E. coli

**TABLE 1**

**Hydrophobic areas and mode of processing**

<table>
<thead>
<tr>
<th>Precursor of</th>
<th>Relevant structure of hydrophobic area* (Ref.)</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM β-lactamase (plasmid-encoded)</td>
<td>4-P-7-P (42)</td>
<td>Posttranslational</td>
</tr>
<tr>
<td>LamB protein</td>
<td>1-P-7-G (43)</td>
<td>Posttranslational</td>
</tr>
<tr>
<td>OmpA-S1</td>
<td>7-G This paper</td>
<td>Posttranslational</td>
</tr>
<tr>
<td>AmpC β-lactamase</td>
<td>14 (44)</td>
<td>Posttranslational</td>
</tr>
<tr>
<td>OmpA P-1</td>
<td>13-G This paper</td>
<td>Posttranslational</td>
</tr>
<tr>
<td>Alkaline phosphatase PhoA-L</td>
<td>11-P (11)</td>
<td>Posttranslational</td>
</tr>
<tr>
<td>Arabinose binding protein</td>
<td>5-G (45)</td>
<td>Partially co- and partially posttranslational</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>6-P-4-P (46)</td>
<td>Partially co- and partially posttranslational</td>
</tr>
<tr>
<td>Maltose binding protein</td>
<td>14 (47)</td>
<td>Partially co- and partially posttranslational</td>
</tr>
<tr>
<td>OmpA</td>
<td>9-G (48, 49)</td>
<td>Partially co- and partially posttranslational</td>
</tr>
</tbody>
</table>

*The numbers indicate numbers of continuous lipophilic residues; serine or threonine surrounded by lipophilic residues are considered hydrophilic (see legend to Fig. 1). PhoA-L, the precursor possessing 9 consecutive leucine residues in the signal peptide (11). For the type of processing see Refs. 7 and 8. P, proline; G, glycine.

For the precursors already referred to, the processing of the altered precursor was much more rapid and, therefore, probably entirely cotranslational. The data in Table I suggest that the only change which would be required to achieve this rapid processing is the substitution of the proline residue by leucine. However, generalizations are not warranted since there are some very obvious exceptions. For example, one would expect the precursor of the maltose binding protein to be processed cotranslationally. Also, there exists an altered pro-PhoA protein which possesses a signal peptide with a lipophilic core of 16 residues (almost exclusively leucines) instead of 10 such residues present in the wild type precursor. In contrast to the relevant pro-OmpA cases and to PhoA-L (Table I), processing of this precursor was essentially posttranslational (11). These differences can be understood if one assumes that at least part of the mature protein is also involved in the processing/translocation reactions. We have demonstrated clearly that the mature part of OmpA contains no information which is required for translocation and processing (14, 18). However, it has been shown recently that the mature part of an exported protein has an effect on these processes. When the signal sequence of pro-OmpA was attached to the mature parts of the E. coli β-lactamase and Staphylococcus aureus nuclease A, the effects of mutational alterations in the signal peptide differed significantly depending on the protein to be exported (25). Other comparable cases are known (36-38), although the mechanism by which the mature parts of the precursors influence processing/translocation are probably not the same in each case. Finally, alterations within the signal peptide could lead to changes in its secondary structure. For example, the elongated pro-PhoA signal peptide just mentioned may assume a structure unfavorable for, and the pro-OmpA P1 and pro-OmpA P2 signals structures favorable for, cotranslational export.

The hydrophobic core in the signal peptide of the precursor encoded by prD87-P2 is increased from 12 to 20 residues and in pTU 500-P1 from 12 to 16 residues. It has been shown that 16 lipophilic residues suffice to anchor a protein in a membrane (39) and we know that a stretch of 16 lipophilic residues within the mature part of the OmpA protein is extremely toxic, probably because the protein becomes anchored in the plasma membrane. Expression of the ompA gene from pTU 500-P1, however, is not toxic and clearly neither this signal peptide nor that encoded by prD87-P2 functions as an anchor. This can be explained readily, in both cases, by the rapid and efficient removal of the long stretch of hydrophobic amino acids by signal peptidase (Fig. 7). A more surprising fact is that, regardless of whether the hydrophobic core contains 12, 16, or 20 hydrophobic amino acids, the cleavage site is evidently in every case correctly presented to signal peptide. The "loop model" of membrane insertion of a signal peptide suggests that following association of the positively charged NH2-terminus with the inner surface of the plasma membrane, the hydrophobic core of the peptide partitions into the membrane and thus correctly localizes the cleavage site at the periplasmic surface (40, 41). Our results could be in agreement with this model with respect to the orientation of the signal peptide, but it is somewhat difficult to reconcile with this model how 12, 16, or 20 hydrophobic amino acids can all interact with the lipid bilayer in such a way that the cleavage site is localized correctly. One possibility would be differences in secondary structure, but one should also keep in mind that there is no direct evidence to support the widely held view that the hydrophobic area of a signal peptide becomes embedded in the lipid phase of the plasma membrane.

It was found that the mutant precursor, expressed from pTU 500-P1 in the secA strain at the nonpermissive temperature, was much more efficiently processed than the wild type precursor whereas the internal control (ompA Δ86-227) behaved in the same way in both cases. The similar behavior of the internal controls indicates that the SecA protein was depleted to the same extent in both strains and that both strains were in an identical physical state. One attractive explanation for these results is that the signal peptide interacts with the SecA protein and that this interaction represents the rate-limiting step in the early stage of the export process. If this is the case, then these results also suggest that the efficiency of the SecA-signal sequence interaction is at least partially responsible for the mode of translocation of a given exported protein. However, an interaction of the signal sequence with another cellular component which itself is influenced by the SecA protein or other indirect effects cannot be excluded.

Another interesting point is the observations made with the OmpA-phage T4 hybrids. Processing of both hybrids is apparently cotranslational when the precursors possess the wild type signal sequence. Processing of the pro-OmpA protein, which is partially co- and partially posttranslational, begins when the polypeptide has reached a molecular weight of about 30,000 (7). If the hybrid precursors behave in the same way, completed precursors may not exist because of the large size of these polypeptides (M, 66,650 and 94,500).
the OmpA protein and of the smaller hybrid (T4-5) but not of the larger hybrid (T4-10) could be converted to the post-translational mode by inserting several residues, including a positively charged one, into the hydrophobic core of the signal sequence. The tail fiber amino acids in the two hybrids do not differ. The same fiber fragment is present tandemly twice in T4-10. It is therefore likely that the inability of this altered signal sequence to convert processing of the larger hybrid to the posttranslational mode is again a reflection of the size of the polypeptide. In other words, for a given length of the mature protein, the mode of processing was probably the same for both hybrids: posttranslational. This case then underlines what we stated above, namely, that the distinction between co- and posttranslational processing is unimportant. It is probably also mechanistically irrelevant.

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REFERENCES