Major coat proteins of bacteriophage Pf3 and M13 as model systems for Sec-independent protein transport

ARTICLE *in* FEMS MICROBIOLOGY REVIEWS · SEPTEMBER 1995

Impact Factor: 13.24 · DOI: 10.1016/0168-6445(94)00067-0 · Source: PubMed

1 AUTHOR:

Andreas Kuhn
Hohenheim University

109 PUBLICATIONS **3,774** CITATIONS

SEE PROFILE

ELSEVIER

FEMS Microbiology Reviews 17 (1995) 185–190

FEMS MICROBIOLOGY REVIEWS

Major coat proteins of bacteriophage Pf3 and M13 as model systems for Sec-independent protein transport

Andreas Kuhn *

Department of Applied Microbiology, University of Karlsruhe, D-76128 Karlsruhe, Germany

Abstract

The membrane insertion of bacteriophage coat proteins occurs independent of the Sec-translocase of *Escherichia coli*. Detailed study of the Pf3 and M13 coat proteins has elucidated two fundamental mechanisms of how proteins invade the membrane, most likely by direct interaction with the lipid bilayer. The Sec-independent translocation of amino-terminal regions across the inner membrane is limited to a short length and a small number of charged residues. Protein regions that contain several charged residues are efficiently translocated across the membrane when these regions are flanked by two adjacent hydrophobic segments interacting synergistically. The relevance of these findings for the membrane insertion mechanism of multispanning membrane proteins is discussed.

Keywords: Bacteriophage coat protein; Membrane insertion mechanism; Sec translocase

1. Introduction

Intrinsic membrane proteins are grouped into different classes depending on whether they have a single membrane-spanning region (type I–III) or cross the membrane multiple times (Fig. 1). Type I and III encompass single-spanning proteins with their amino-terminus exposed to the external face of the membrane. Type I proteins are synthesized with a cleavable signal (or leader) peptide at their amino-terminus and are inserted into the membrane initially as double membrane-spanning proteins with two hydrophobic regions, one in the signal peptide and one in the mature region. The second hydrophobic region has been named stop-transfer segment [1]. Depending on the size of the region between the two hydrophobic parts, the translocation of this region eventually requires the translocase complex (encoded by the *sec* genes) [2,3]. This Sec-translocase is usually used by proteins that are secreted into the external medium or into the periplasmic space, or by proteins that are assembled into the outer membrane. These exported proteins contain a cleavable signal sequence like type I membrane proteins, but lack a stop-transfer segment. The signal sequence of the exported and type I membrane proteins is cleaved off by a membrane-anchored peptidase (leader peptidase), which releases the export proteins into the periplasm [4]. Type I proteins, however, are kept integrated in the membrane with the new amino-terminus at the trans side.

Type II encompasses proteins that have a single membrane-spanning region with the carboxy-terminus exposed to the trans side. They lack a stop transfer segment and their membrane-spanning re-

* Corresponding author. Fax +49 (721) 608 4889

SSDI 0168-6445(94)00067-0

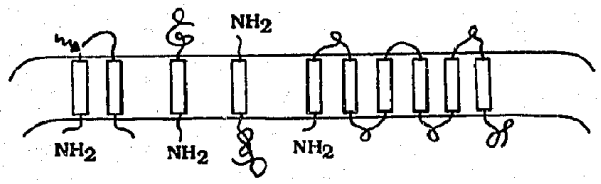

Fig. 1. Diversity of membrane protein topology. Type I proteins contain a signal (leader) sequence that poses the amino-terminus of the protein to the external. Type II and III have their amino-terminus in the cytoplasm and periplasm, respectively. Multispanning membrane proteins show a variety of topologies, depending on the number of their transmembrane segments.

gion functions similarly to the signal sequence of type I proteins. There are several examples where the membrane-spanning region has been replaced by a signal sequence [5,6]. Therefore, the type II membrane-spanning region has also been named as uncleaved signal sequence. Obviously, the membrane insertion of type II proteins requires the translocase machinery, presumably as type I proteins do. Very little is known about the insertion mechanism of type III proteins. The few cases studied in some detail do not use the Sec-translocase [8,17,18].

2. Pf3 coat protein is a model for simple single-membrane spanning proteins

The 44-amino acid coat protein of *Pseudomonas aeruginosa* phage Pf3 is synthesized with only one membrane-spanning region. When expressed from the plasmid-encoded gene in *Escherichia coli*, the protein is readily inserted into the inner membrane with its amino-terminus facing the periplasm [7]. The Pf3 coat protein is therefore one of the few type III membrane proteins. We found that the insertion process is independent of the Sec machinery as the coat protein achieves a transmembrane configuration in *E. coli* strains with a temperature-sensitive mutation in *secA* (ts51) and *secY* (ts24) leading to a non-functional translocase at the elevated temperature [8]. We assume that the protein inserts directly into the hydrophobic bilayer and translocates the amino-terminal 18 residues to the periplasmic face of the membrane.

Amino-terminal translocation seems to be limited to relatively short protein regions with only a limited number of charged residues. In the Pf3 coat protein this region encompasses 18 hydrophilic amino acids of which two are negatively charged. Extension of the amino-terminal region of a Pf3-M13 hybrid pro-

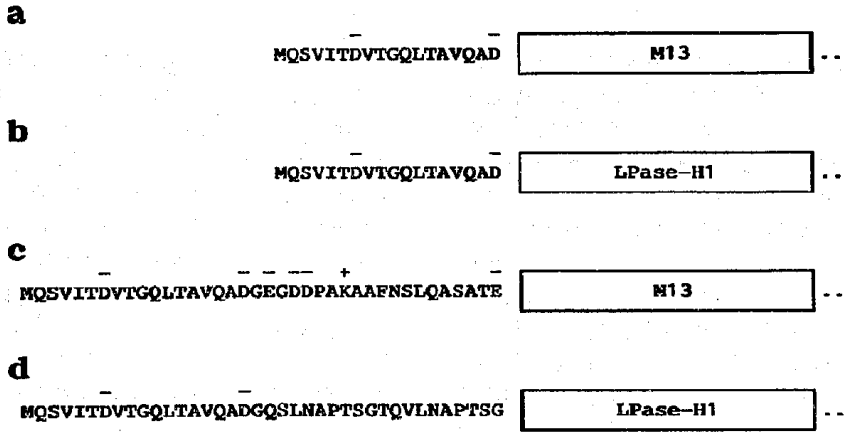

Fig. 2. Amino-terminal translocation is limited to small, uncharged regions. The 18-residue amino-terminal tail of the Pf3 coat protein is translocated by the hydrophobic region of the M13 coat protein (a) or by the first transmembrane region of leader peptidase (b). The 38-residue tail of the Pf3-M13 hybrid protein with seven charged residues (c) is not translocated, whereas the 38-residue tail with only two charged residues (d) is translocated across the membrane.

tein impaired membrane translocation (Fig. 2c). Similarly, the replacement of the 18 residue long amino-terminal region with one containing three additional charged residues prevented membrane insertion [8]. In contrast, the translocation of a highly charged and longer region at the carboxyl-terminal side of the M13 signal peptide results in efficient insertion, but then with the help of the Sec machinery [9]. These results suggest that the Sec machinery can only support carboxyl-terminal and not amino-terminal translocation.

The limitations of amino-terminal translocation were thoroughly studied with a Pf3-leader peptidase (*lep*) fusion protein. The 18-amino acid amino-terminal region of the Pf3 coat protein was fused to the amino-terminus of leader peptidase (Fig. 2b; [10]). Strikingly, the translocation of this amino-terminal region occurs independently of the Sec machinery, whereas the carboxyl-terminal region of the protein requires Sec for translocation (Fig. 3). The feature that both membrane insertion pathways are used by different regions of the same protein makes it an excellent tool to study the limitations of amino-terminal translocation. Extension of this region by an additional 20 uncharged amino acids still allows membrane insertion; however, an extension of this region by 40 or 120 residues blocked translocation. This block was removed by the addition of a signal peptide at the amino-terminus [17].

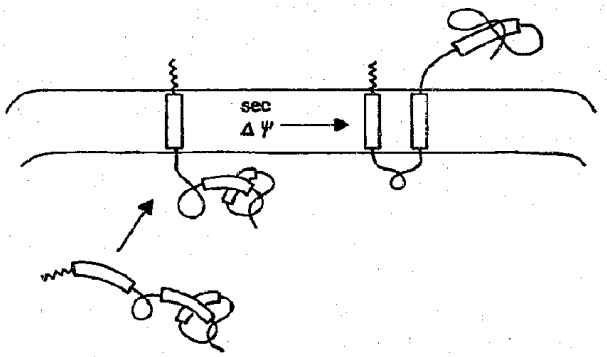

Fig. 3. Distinct regions of the Pf3–leader peptidase fusion protein are inserted into the membrane by two different mechanisms. The translocation of the amino-terminal tail is translocated in the absence of the *E. coli* translocase, whereas the carboxy-terminal region requires the translocase to cross the membrane.

3. Determinants of membrane orientation

The simplicity of the Pf3 coat protein makes it an attractive model to study the question how the orientation of membrane proteins is determined. Comparative studies of a number of membrane proteins with known topology show that the regions flanking a membrane-spanning region exhibit a bias regarding the distribution of charged amino acids [11–13]. Since the charge bias is more pronounced for positively charged amino acids it was concluded that the transmembrane orientation is determined according to the 'positive inside rule' [12]. The charge distribution found in the Pf3 coat protein is with two negatively charged residues in the amino-terminal region and two positively charged residues in the carboxyl-terminal region consistent with the positive inside rule. We have changed the charged residues in both regions to either neutral amino acids or to residues with the reverse charge (Fig. 4). Membrane insertion of each mutant protein was analyzed in vivo by pulse labelling the cells with [^{35}S]methionine for 3 min. The cells were converted to spheroplasts followed by proteinase K digestion from the periplasmic side. We found that the positively charged residues at the carboxy-terminus are not required for membrane insertion since the mutant with two asparagine residues in place of the lysine and arginine residues efficiently translocated their amino-terminus into the periplasm.

Likewise, in vitro experiments showed that the mutant protein inserted into inverted inner membrane vesicles (INV) with comparable efficiency as the wild-type protein (D. Kiefer, personal communication). However, when one positively charged residue was placed at the amino-terminal side of the transmembrane region, membrane insertion was completely blocked. We conclude from these results that the positively charged residues might actively prevent the translocation of that particular region, but they are not the only determinants for membrane orientation.

The mutation of the positively charged residues in the carboxyl-terminal region to negatively charged aspartyl residues impaired translocation across the *E. coli* membrane. We then tested whether binding to the membrane surface is affected. When the cells expressing mutant Pf3-ND, Pf3-DN or Pf3-DD pro-

	% protein in soluble fraction
Pf3 wildtype sequence	
MQSVITDVTGQLTAVQAD *ITTIGGAIIVLAAVVLGI* RWIKAQFF	0
N-terminal mutants	
RR-Pf3	
MQSVITDVTGQLTAVQRR.....RWIKAQFF	0
RS-Pf3	
MQSVITDVTGQLTAVQRS.....RWIKAQFF	0
C-terminal mutants	
Pf3-DD	
MQSVITDVTGQLTAVQAD.....DWIDAQFF	45
Pf3-ND	
MQSVITDVTGQLTAVQAD.....NWIDAQFF	41
Pf3-DN	
MQSVITDVTGQLTAVQAD.....DWINAQFF	25
Pf3-NN	
MQSVITDVTGQLTAVQAD.....NWINAQFF	0

Fig. 4. Pf3 mutants with altered charge are defective for membrane interaction. Pf3 mutant proteins were pulse-labelled for 3 min with [^{35}S]methionine and analyzed for their localization. The cells were lysed and washed with 50 mM NaOH. Membranes were sedimented by centrifugation and Pf3 protein was quantified in the supernatant and pellet fractions. Whereas the wild-type and the mutant RS-Pf3, RR-Pf3 and Pf3-NN proteins were found in the pellet, the mutants with negative charges, Pf3-DD, Pf3-ND and Pf3-DN, were present in the supernatant.

teins were fractionated after alkaline treatment, the proportion of mutant protein found in the supernatant increased with the number of negatively charged residues at the carboxy-terminus (Fig. 4). Since the *E. coli* membrane consists of negatively charged phospholipids, the negatively charged protein regions are most likely disfavored at the membrane surface.

4. Synergistic membrane insertion of two hydrophobic regions

The coat protein of *E. coli* bacteriophage M13 is synthesized as a 73-amino acid precursor, termed M13 procoat. Although the protein is synthesized with a signal (leader) sequence, its membrane insertion is independent of the Sec components of the host cell. Interestingly, several regions of the procoat protein are involved in the membrane insertion process, as evidenced by studying a number of mutations throughout the gene.

Mutations in the polar regions at the amino- and carboxy-terminus that alter the positive charge affect binding of the protein to the cellular membrane in vivo and also in vitro to artificial liposomes [14]. Similar to Pf3 coat protein, the positively charged regions are involved in the early step of protein–membrane interaction. Mutations that change the hydrophobicity of the signal sequence or of the membrane anchor in the mature part do not interfere with membrane binding, but prevent translocation of the central region of the protein. In these mutants a hydrophobic residue was substituted for an arginine, resulting in the accumulation of a membrane-bound but not transmembrane conformation (Fig. 5). Interestingly, a central position of the polar residue in either hydrophobic sequence had a more drastic effect than a peripheral position [19]. These results suggest that both hydrophobic sequences equally contribute to the translocation process.

The synergistic contribution of the two hydrophobic regions of the procoat protein was also shown by several proline substitution mutants. Since proline substitutions should not alter the hydrophobic character of a membrane-spanning region, but rather change the secondary structure of the protein backbone, the contribution of the α-helical conformation of each membrane-spanning region is tested in such

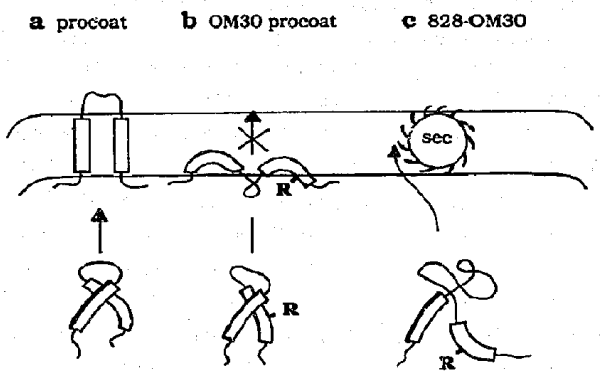

Fig. 5. Synergistic membrane insertion by two adjacent hydrophobic regions. Membrane insertion of the M13 procoat protein requires two hydrophobic regions (a). An arginine mutation in either hydrophobic region inhibits the insertion process (b). The arginine mutation has no inhibitory effect when the two hydrophobic regions are separated by 191 amino acids.

constructs. Site-directed point mutations introducing proline residues into either hydrophobic region showed similar effects whether they were located in the signal or in mature parts of the protein. Two proline residues in one of the hydrophobic regions severely affected membrane translocation. In addition, proline residues concomitantly introduced in the signal and mature part had an additive effect. Taken together, these data suggest that membrane translocation of the M13 procoat protein is driven by the partitioning and by the formation of an α-helical transmembrane loop by both hydrophobic regions.

5. The synergistic membrane insertion mechanism is limited to small transmembrane regions

The two hydrophobic regions in the M13 procoat protein are separated by a short segment of 20 hydrophilic amino acids. The hydrophilic segment was genetically extended to 191 amino acid residues [2]. This mutant protein, termed pro828coat was efficiently inserted into the membrane; however, in contrast to the wild-type, it required a functional Sec-translocase. This was also the case for a mutant that had a central region of only 118 amino acid residues. Surprisingly, the membrane insertion of these variants was not driven by both hydrophobic regions. The Sec-dependent procoat fusion proteins still inserted into the membrane when large portions of the hydrophobic region (Δ 21–39) were deleted [9]. Similarly, when pro828coat was mutated in the center of the hydrophobic region with a polar arginyl residue, membrane insertion was not affected (Fig. 5; [20]). These experiments clearly show that the second hydrophobic region is only involved in the translocation process when it is located in the vicinity of the signal sequence.

6. Conclusions

Secreted proteins and complex protein regions of integral membrane proteins are transported across the bacterial membrane via Sec-translocase. Recently, the eukaryotic counterparts of *E. coli* translocase have been identified [15]. They show a strong homology to the bacterial transport system, indicating its relevance during evolution. Simple and short protein regions have shown to translocate independently of the translocase complex, suggesting that these events might occur spontaneously involving hydrophobic and electrostatic interactions between the protein and the membrane.

Studies with fusions between Sec-dependent and independent proteins [10] have shown that both mechanisms can occur within the same protein. This suggests that the translocase does not operate linearly on the protein chain as predicted from current models [16]. Rather, the enzyme complex recognizes protein domains, possibly with the support of molecular chaperones. This might be of special relevance for multispanning membrane proteins. Since translocation of small and simple protein regions occurs spontaneously, translocase can only recognize the complex regions that are left behind at the cytoplasmic face of the membrane. Since most transmembrane loops of the multispanning membrane proteins are relatively small, the Sec-independent membrane insertion may be a very common mechanism.

References

[1] Blobel, G. (1980) Intracellular protein topogenesis. Proc. Natl. Acad. Sci. USA 77, 1496–1500.
[2] Kuhn, A. (1988) Alterations in the extracellular domain of M13 procoat make its membrane insertion dependent on *secA* and *secY*. Eur. J. Biochem. 177, 267–271.
[3] Andersson, H. and von Heijne, G. (1993) *Sec* dependent and *sec* independent assembly of *E. coli* inner membrane proteins: the topological rules depend on chain length. EMBO J. 12, 683–691.
[4] Dalbey, R.E. (1991) Leader peptidase. Mol. Microbiol. 5, 2855–2860.
[5] Dalbey, R.E., Kuhn, A. and Wickner, W. (1987) The internal signal sequence of *Escherichia coli* leader peptidase is necessary, but not sufficient, for its rapid membrane assembly. J. Biol. Chem. 262, 13241–13245.
[6] Zerial, M., Melancon, P., Schneider, C. and Garoff, H. (1986) The transmembrane segment of the human transferrin receptor functions as a signal peptide. EMBO J. 5, 1543–1550.
[7] Kuhn, A., Rohrer, J. and Gallusser, A. (1990) Bacteriophage M13 and Pf3 tell us how proteins insert into the membrane. J. Struct. Biol. 104, 38–43.
[8] Rohrer J. and Kuhn, A. (1990) The function of a leader peptide in translocating charged amino acyl residues across a membrane. Science 250, 1418–1421.
[9] Kuhn, A., Zhu, H.-Y. and Dalbey, R.E. (1990) Efficient

translocation of positively charged residues of M13 procoat protein across the membrane excludes electrophoresis as the primary force for membrane insertion. EMBO J. 9, 2385–2388, 2429.

[10] Lee J., Kuhn, A. and Dalbey, R.E. (1992) Distinct domains of an oligotopic membrane protein are Sec-dependent and Sec-independent for membrane insertion. J. Biol. Chem. 267, 938–943.

[11] Hartmann, E., Rapoport, T.A. and Lodish, H.F. (1989) Predicting the orientation of eukaryotic membrane-spanning proteins. Proc. Natl. Acad. Sci. USA 86, 5786–5790.

[12] Von Heijne, G. (1986) The distribution of positively charged residues in bacterial inner membrane proteins correlates with the trans-membrane topology. EMBO J. 5, 3021–3027.

[13] Von Heijne, G. (1989) Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues. Nature 341, 456–458.

[14] Gallusser, A. and Kuhn, A. (1990) Initial steps in protein membrane insertion. Bacteriophage M13 procoat protein binds to the membrane surface by electrostatic interaction. EMBO J. 9, 2723–2729.

[15] Hartmann, E., Sommer, T., Prehn, S., Görlich, D., Jentsch, S. and Rapoport, T. (1994) Evolutionary conservation of components of the protein translocation complex. Nature 367, 654–657.

[16] Wickner, W., Driessen, A.J.M. and Hartl, F.U. (1991) The enzymology of protein translocation across the *Escherichia coli* membrane. Annu. Rev. Biochem. 60, 101–124.

[17] Cao, G. and Dalbey, R.E. (1994) Translocation of N-terminal tails across the plasma membrane. EMBO J. 13, 4662–4669.

[18] Whitley, P., Zander, T., Ehrmann, M., Haardt, M., Bremer, E. and von Heijne, G. (1994) Sec-independent translocation of a 100-residue periplasmic N-terminal tail in the *E. coli* inner membrane protein proW. EMBO J. 13, 4653–4661.

[19] Kuhn, A., Kreil, G. and Wickner, W. (1986) Both hydrophobic domains of M13 procoat are required to initiate membrane insertion. EMBO J. 5, 3681–3685.

[20] Cao, G., Cheng, S., Whitley, P., von Heijne, G., Kuhn, A. and Dalbey, R.E. (1994) Synergistic insertion of two hydrophobic regions drives Sec-independent membrane protein assembly. J. Biol. Chem. 269, 26898–26903.