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Minireview

Sec-independent protein insertion into the inner *E. coli* membrane

A phenomenon in search of an explanation

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Abstract

Translocation of proteins through the inner membrane of *E. coli* is normally catalyzed by the so-called *sec*-machinery. Yet, many integral inner membrane proteins appear not to require a fully functional *sec*-machinery for proper insertion, in spite of the fact that sometimes quite sizable domains have to be translocated to the periplasmic side. This review will focus on recent studies of *sec*-independent translocation events in an attempt to pin-point the main differences between *sec*-dependent and *sec*-independent translocation.

Key words: Membrane protein; *Sec*-machinery; Protein secretion; *E. coli*

1. Introduction

Most studies of protein translocation through the inner membrane of *E. coli* have focused on secretory proteins, i.e. proteins that are completely extruded into the periplasm. Such proteins are translocated through the now well-characterized *sec*-machinery (see next section). On the other hand, already in 1985 it was found that the phage M13 procoat protein inserts efficiently into the inner membrane even under conditions where the *sec*-machinery is essentially blocked [1]. More recently, statistical studies of the content of positively charged amino acids in periplasmic loops in inner membrane proteins have demonstrated that short (≤ 60 residues) loops contain only few such residues, the 'positive inside' rule [2], whereas there is no reduction in the content of Arg and Lys residues in longer periplasmic loops [3,4]. This suggested that short and long periplasmic loops are translocated by different mechanisms and that only the latter can make full use of the *sec*-machinery; an idea that has received some experimental support [5,6], but that has also been questioned [7].

We have recently reconsidered the problem of what controls the degree of *sec*-dependence of a protein or a protein domain. On balance, our results suggest that the length of the translocated domain is indeed an important parameter, though the effects of an impaired *sec*-machinery on the translocation of different domains of similar lengths can vary from mild to severe. Further, it seems that the *sec*-machinery can only act on domains located on the C-terminal side of a translocation signal, whereas domains placed on the N-terminal side of such a signal can only be translocated by the *sec*-independent mechanism irrespective of their length (and must thus always follow the positive inside rule). The precise mechanistic differences between *sec*-dependent and *sec*-independent translocation remain unclear, however.

2. The *sec*-machinery

The central function of the *sec*-machinery is performed by the SecA/SecY/SecE complex [8,9]. According to present thinking, a preprotein (possibly complexed to a cytoplasmic chaperone) is delivered to SecA, a peripheral inner membrane ATPase that can interact with the signal sequence, parts of the mature chain, acidic membrane phospholipids, and the SecY/E complex in the inner membrane. Translocation proper is initiated when ATP then binds to SecA, an event that apparently releases the signal sequence from SecA and promotes its insertion into the membrane (or into the SecY/E complex). ATP hydrolysis is required to release the other parts of the chain from SecA, allowing a limited translocation driven by the protonmotive force (pmf). The chain then rebinds to SecA, and the cycle is repeated until the entire chain has been translocated. Two other inner membrane proteins, SecD and SecF, are thought to act at a late stage during the translocation process and may facilitate the release of the nascent chain from the translocase [10,11].

The *sec*-machinery can be blocked in various ways, either by using strains that carry conditionally lethal mutations in one or other of the *sec* genes, or, more

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simply, by a brief incubation in 2 mM sodium azide, a treatment that inhibits the ATPase activity of SecA [12].

3. *Sec*-dependent insertion: MalF

For integral inner membrane proteins, *sec*-dependent translocation has been demonstrated for the large periplasmic domains of leader peptidase [1], the Tsr receptor [13], and for an M13 procoat-OmpA fusion with a large periplasmic loop [5]. *Sec*-independent translocation has been observed for the short periplasmic domain of the M13 procoat protein [1], the short N-terminal tail of leader peptidase [14], and for a short loop that faces the periplasm in an engineered 'inverted' form of leader peptidase [15]. When the length of the periplasmic loop in the 'inverted' leader peptidase is progressively increased, the degree of *sec*-dependence first increases in parallel and then levels off at a length of ~ 60 residues [6].

Although these as well as other reports suggest that the length of the translocated domain is the major parameter that determines whether or not the *sec*-machinery can be used, an apparent counter-example was recently found: MalF. Here, the translocation of a large (~ 180 residues) periplasmic domain was found not to be markedly affected when the *sec*-machinery was blocked [7].

We have recently made an in-depth study of the translocation of the large periplasmic loop in MalF (Sääf, Andersson and von Heijne, in preparation). A fusion between an N-terminal part of MalF, including the large periplasmic loop, and the globular C-terminal domain of leader peptidase (Lep) was constructed (Fig. 1A). In fact, translocation of the MalF loop was found to have a significant, albeit weak, dependence on the *sec*-machinery in this construct. When the MalF loop was inserted between the two transmembrane segments of an 'inverted' Lep construct (Fig. 1B) its translocation was almost completely blocked when SecA function was inhibited by sodium azide. Likewise, when the first two transmembrane segments in MalF were removed (Fig. 1C) translocation of the large loop was again sensitive to sodium azide, whereas deletions in or replacement of the fourth transmembrane segment in MalF had little effect on the degree of *sec*-dependence (Fig. 1D). When the MalF loop was replaced by foreign loops that were known to require the *sec*-machinery for translocation when in their normal contexts a strong *sec*-dependence was still observed.

Thus, the MalF loop behaves as expected from its length when moved out of its normal context, or when certain of the surrounding transmembrane segments are removed. Neither can the first four transmembrane segments of MalF reduce the *sec*-dependence of foreign loops. It thus seems that some delicate interplay between the MalF transmembrane segments and the large periplasmic loop is responsible for its ability to be almost as efficiently translocated during conditions of impaired *sec*-function as in normal cells.

4. *Sec*-independent insertion: ProW

The periplasmic MalF loop discussed above contains as many positively charged residues as do normal, *sec*-dependent periplasmic proteins (~ 10%). An exceptional example of a large periplasmic domain with a reduced content of positively charged residues is provided by the ProW protein, an inner membrane protein involved in osmoregulation that has a 100-residues long periplasmic N-terminal tail followed by seven transmembrane segments (M. Haardt and E. Bremer, personal communication). The N-tail contains only three positively charged residues, but has a normal content (~ 12%) of negatively charged residues.

The reduced content of positively charged residues in the ProW N-tail suggested to us that, despite its length,

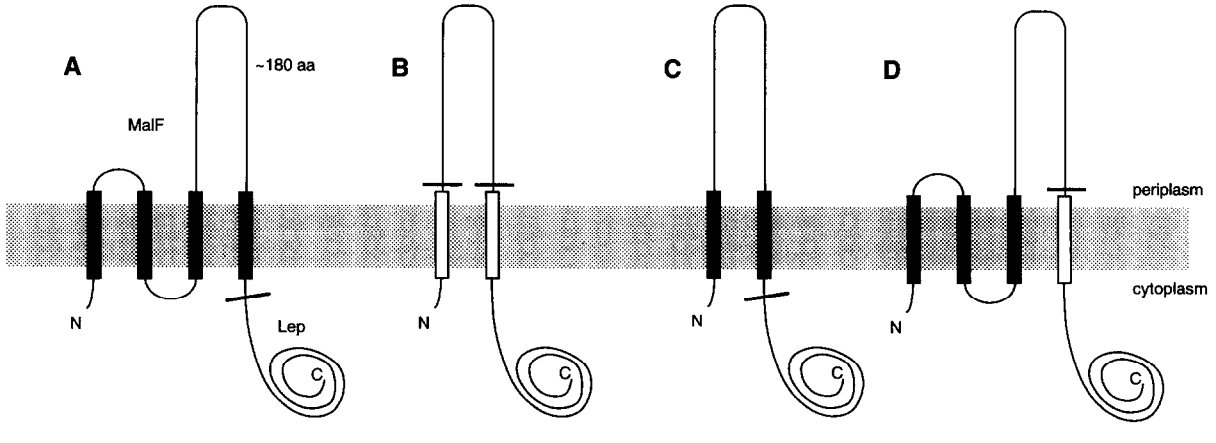

Fig. 1. MalF-Lep fusions. Transmembrane segments derived from MalF are in black, those derived from Lep are in white. Fusion joints are indicated by a thick line. Translocation of the large periplasmic MalF loop is only weakly *sec*-dependent in constructs A and D, but is strongly affected in constructs B and C when the SecA ATPase activity is blocked.

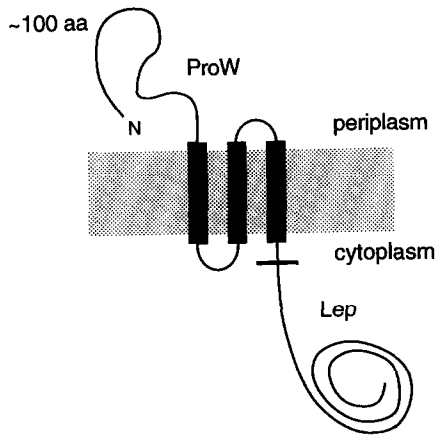

Fig. 2. ProW-Lep fusion. The fusion joint is indicated by the thick line. Translocation of the long N-terminal tail is unaffected when the SecA ATPase activity is blocked or in a *secY*^{cs} strain.

this large domain might belong to the class of *sec*-independent loops. We tested this by making a fusion between an N-terminal part of ProW (including the N-tail and the three following transmembrane segments) and the periplasmic domain of leader peptidase (Whitley, Zander, Ehrmann, Haardt, Bremer and von Heijne, in preparation) (Fig. 2). Within the accuracy of our assay, the translocation of the N-tail was found to be completely unaffected when SecA function was blocked by azide. Thus, in contrast to the MalF loop, the ProW N-tail seems to be completely *sec*-independent. When three or six extra arginines were introduced in the N-tail, however, no translocation was observed even in normal cells. Dissipation of the pmf also completely blocked translocation. These results suggest that the *sec*-machinery cannot act on domains located upstream of a translocation signal, and that such domains thus have to be designed such that they can be translocated by the *sec*-independent mechanism, i.e. they must follow the positive inside rule.

5. Role of the pmf and charged residues

As noted above, *sec*-dependent translocation can only take place if the pmf is intact. However, some *sec*-independent loops can be translocated even under conditions where the pmf is largely dissipated [16]. We have recently shown that, during *sec*-independent translocation, the pmf acts differently on positively and negatively charged residues: it facilitates the translocation of negatively charged residues but makes the translocation of positively charged ones more difficult [17]. This appears to be the major mechanistic reason behind the positive inside rule.

By replacing a block of positively charged residues with an equally long stretch of uncharged residues in a large periplasmic loop in an 'inverted' leader peptidase (Fig. 3) we have also found that, for constant length, the degree of *sec*-dependence can vary depending on the content of charged residues (Andersson and von Heijne, in preparation).

6. Does 'sec-independent' really mean sec-independent?

Against the background of the results discussed above, one might ask whether there really is a clear-cut distinction between *sec*-dependent and *sec*-independent mechanisms of translocation. Since the degree of *sec*-dependence varies between different periplasmic loops attached to the same transmembrane segments, between the same loop attached to different transmembrane segments, between closely related loops of different lengths, and between loops of the same length differing only by a few point-mutations, it is clearly possible that what we call '*sec*-independent' translocation is simply a limiting case where the degree of *sec*-dependence becomes too small to measure, rather than a mechanistically distinct process where the degree of *sec*-dependence is identically equal to zero.

For instance, the degree of *sec*-dependence measured experimentally may be a function of the number of rounds of ATP hydrolysis needed to extrude the chain through the SecA/SecY/SecE complex. If this is correct, one would expect that *sec*-dependence should increase with length for chains with similar distributions of charged, polar, and non-polar amino acids. One might

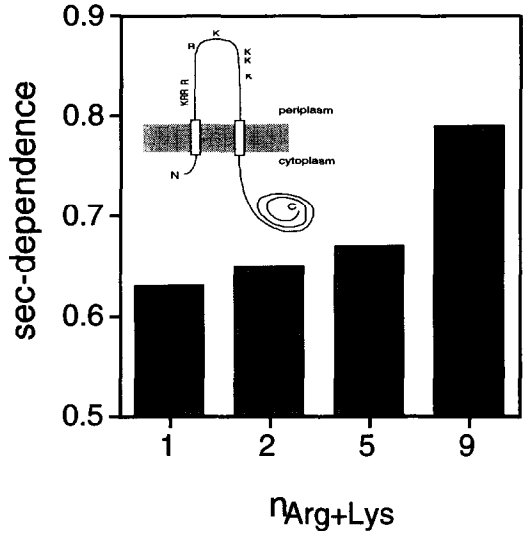

Fig. 3. The number of positively charged residues in a translocated loop can influence the degree of *sec*-dependence. Arg and Lys residues were successively removed from an 88-residues long periplasmic loop in an 'inverted' leader peptidase construct (see insert), and the degree of *sec*-dependence was calculated as $(1-f^-/f^+)$, where f^+ is the fraction of molecules where the loop was translocated in cells grown under standard conditions and f^- is the fraction translocated when they were grown in the presence of sodium azide to block SecA function.

also expect that blocks of positively charged residues, since they have to be moved against the electrical component of the pmf, would tend to increase the number of rounds of ATP hydrolysis and hence the *sec*-dependence. In the limit of very short loops or longer loops with few positively charged residues, a single round of ATP hydrolysis may be sufficient to release the chain from SecA and allow its complete insertion into the membrane in a process driven by the thermodynamically favorable partitioning of the hydrophobic flanking segment(s) into the lipid bilayer [6] and by the pmf acting on the negatively charged residues in the loop [17].

An alternative view is that *sec*-independent translocation is literally *sec*-independent; i.e. that it does not involve the *sec*-components at all. Spontaneous partitioning into the lipid bilayer would perhaps be the most likely possibility in this case. Such a model would explain weak *sec*-dependence by assuming that a fraction of the molecules inserts spontaneously, with the remaining molecules either being picked up by the *sec*-machinery before they have a chance to interact with the lipid bilayer, or failing to insert by themselves and being picked up by the *sec*-machinery at a later stage.

There seems to be no easy way to distinguish between these possibilities, since the critical distinction is whether, in vivo, a protein containing one or more potential translocation signals would be more likely to first encounter the *sec*-machinery or the lipid bilayer. It is not even clear if in vitro studies could immediately clarify the issue, since the relative concentrations of nascent protein, *sec*-components, and bilayer lipids may be hard to tune to their in vivo values. At the very least, a minimum prerequisite for in vitro studies must thus be that the general trends noted above can be reproduced; no studies of this kind have been undertaken so far.

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