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Membrane protein insertion: mixing eukaryotic and prokaryotic concepts

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Proteins are translocated across or inserted into membranes by machines that are composed of soluble and membrane-anchored subunits. The molecular action of these machines and their evolutionary origin are at present the focus of intense research. For instance, our understanding of the mode of insertion of β-barrel membrane proteins into the outer membrane of endosymbiotically derived organelles has increased rapidly during the past few years. In particular, the identification of the Omp85/YaeT-involving pathways in *Neisseria meningitidis*, *Escherichia coli* and cyanobacteria, and homologues of Omp85/YaeT in chloroplasts and mitochondria, has provided new clues about the ancestral β-barrel protein insertion pathway. This review focuses on recent advances in the elucidation of the evolutionarily conserved concepts that underlie the translocation and insertion of β-barrel membrane proteins.

Keywords: β-barrel proteins; membrane insertion; Omp85; Sam50/Tob55; Toc75; transport evolution

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Introduction

Protein translocation across and into membranes is a fundamental process: up to 50% of all proteins synthesized in the cytosol need to traverse at least one membrane to reach their place of function (Schatz & Dobberstein, 1996). The basic principles of protein translocation have been established, but the specific molecular mechanisms of each membrane system have been determined to varying degrees. However, some generalizations about the state of the substrate or the translocon can be made. Proteins translocated across the nuclear (Xu & Massague, 2004) and the peroxisomal membranes (Erdmann & Schliebs, 2005) and by the twin arginine translocation (TAT) pathway in bacteria and thylakoids (Robinson & Bolhuis, 2001) are folded. By contrast, proteins transported by the machines in the mitochondrial (Pfanner *et al*, 2004), chloroplast (Soll & Schleiff, 2004) and endoplasmatic reticulum membranes (Osborne *et al*, 2005) need to be unfolded. The systems also vary in the architecture of the translocon, as plastids, mitochondria, endoplasmic reticulum and nuclei contain pre-existing machines, whereas translocon formation in the peroxisomal or TAT pathway is substrate induced.

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β-barrel membrane proteins are composed of antiparallel transmembrane β-strands connected by soluble loop regions (Schulz, 2000) and are inserted into the outer membrane of bacteria, mitochondria and chloroplasts by pre-existing translocation machineries. For the bacterial system, it was suggested that the β-barrel membrane proteins are at least partially folded before their insertion into the outer membrane as disulphide bond formation catalysed by periplasmic proteins precedes the insertion of some proteins (Eppens *et al*, 1997). In addition, the inactivation of the genes that encode the periplasmic chaperone Skp (7 kDa protein) and the periplasmic peptidyl-prolyl *cis–trans* isomerase SurA in *Escherichia coli* results in a synthetic lethal phenotype. This suggests that Skp and SurA have a redundant chaperone function that is essential for outer membrane protein (OMP) topology (Fig 1A; Rizzitello *et al*, 2001), but it remains open whether they keep proteins in an unfolded state or whether they are involved in folding preceding insertion. In this review, we highlight the similarities and differences between the insertion of β-barrel membrane proteins in bacteria and in eukaryotes.

Prokaryotic β-barrel-shaped transporters

The biogenesis of the outer membrane of Gram-negative bacteria remained elusive for a long time. In 1998, a homologous protein of the chloroplast translocation channel Toc75 (Soll & Schleiff, 2004) was identified in *Synechocystis PCC6803* and was named slr1227 (Table 1; Bölter *et al*, 1998). This protein localizes to the outer membrane, is essential for viability of the bacteria (Reumann *et al*, 1999) and has similar electrophysiological properties to Toc75 from the pea (*ps*Toc75; Bölter *et al*, 1998). On the basis of this comparison, it was suggested that the protein had a function in the import or export of peptides. The authors also showed that the protein was related to Omp85 from *Neisseria meningitidis*, which was not functionally characterized at that time. Only later was it shown that Omp85 is an essential component for outer membrane biogenesis in *N. meningitidis*. Initially, two functions were suggested for Omp85: the export of lipids (Genevrois *et al*, 2003) and the assembly of OMPs (Voulhoux *et al*, 2003). The latter study showed that the depletion of Omp85 impaired the formation of complexes containing OMPs such as PorA, PorB, PilQ and FrpB. Moreover, the integration and subsequent folding of monomeric OMPs such as OmpLA or IgA1 was decreased. It was also shown that Omp85 interacts with unfolded OMPs such as PorA (Voulhoux *et al*, 2003). Therefore, the deleterious effect of Omp85 depletion on lipid export might be indirect because of the defective

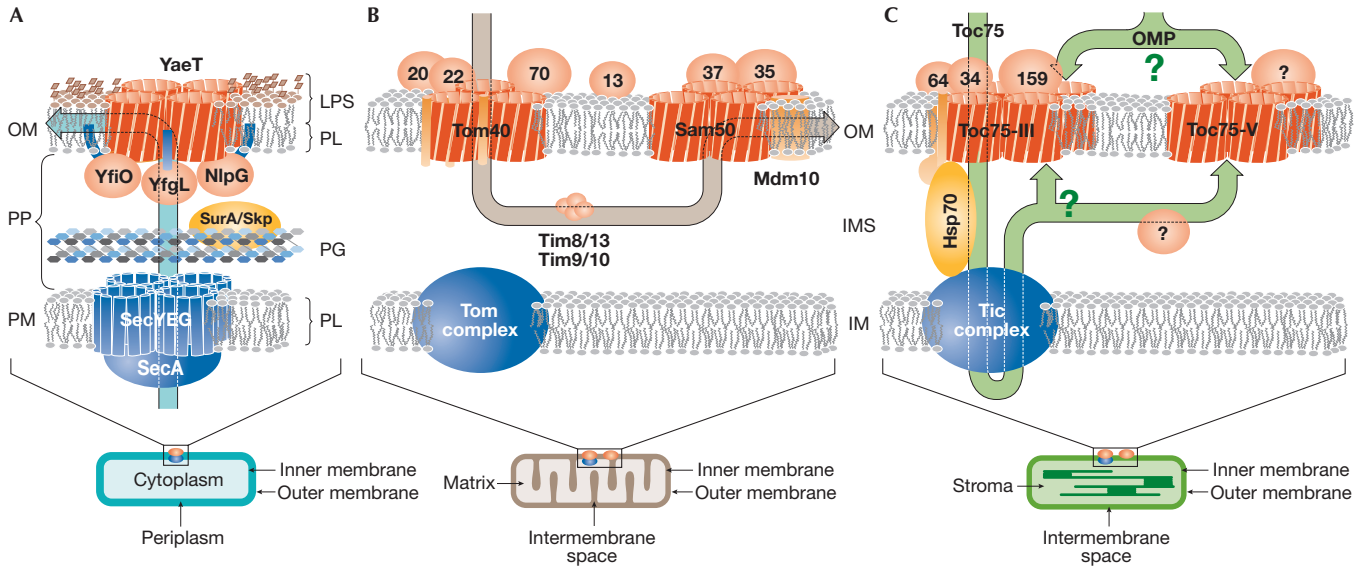

Fig 1 | β-barrel outer membrane protein insertion. The translocation system for β-barrel outer membrane proteins (OMPs) in (**A**) bacteria, (**B**) mitochondria and (**C**) chloroplasts are shown. Arrows indicate the translocation pathway. Please note, the stoichiometry of the bacterial system is not experimentally supported. In the case of chloroplasts, the translocation path is dissected into that established for Toc75 and possible routes for other OMPs. The question marks indicate the current state of knowledge. IM, inner membrane; IMS, intermembrane space; LPS, lipopolysaccharide; OM, outer membrane; PG, peptidoglycan layer; PL, phospholipid; PM, plasma membrane; PP, periplasm.

assembly of OMPs. This conclusion is supported by the identification of the OMP OstA/Imp (increased membrane permeability) in *E. coli* (Braun & Silhavy, 2002) and *N. meningitidis* (Bos *et al*, 2004), which is involved in the assembly of lipopolysaccharide in the outer membrane. However, the assembly state of Imp in the ΔOmp85 background is not yet known. In *E. coli*, the Omp85 homologue YaeT is also essential (Gerdes *et al*, 2003). Interestingly, the main protein of the inner membrane export system, SecA (Osborne *et al*, 2005), functions as a multicopy suppressor of the temperature-sensitive mutant of YaeT and partially restores OMP assembly (Doerrler & Raetz, 2005). This suppression may be caused by an increased secretion of the mutant YaeT or other OMPs across the inner membrane by the Sec complex. YaeT is part of a multi-component complex that also includes the outer membrane lipoproteins YfgL, YfiO and NlpB (Wu *et al*, 2005). In contrast to YfgL and YaeT, YfiO and NlpB are not essential but their depletion results in phenotypes that suggest they are important for maintaining the integrity of the cell envelope (Wu *et al*, 2005; Ruiz *et al*, 2005; Onofryk *et al*, 2005). NlpB and YfgL are genetically linked to SurA, which is a periplasmic chaperone specific for OMPs (Rouvière & Gross, 1996). It was suggested that these proteins function in a periplasmic folding pathway that parallels that of SurA (Onofryk *et al*, 2005). Mutations of YfgL and YfiO, but not of NlpB, reduce the amount of inserted OMPs such as LamB or OmpA (Wu *et al*, 2005; Ruiz *et al*, 2005). Combined with the results of structural predictions (Bölter *et al*, 1998; Voulhoux *et al*, 2003), it therefore seems that the prokaryotic machinery for OMP assembly is itself composed of a β-barrel OMP, which is associated with at least three outer membrane lipoproteins involved putatively in the perception and folding of the incoming OMPs. Although some evidence exists for pre-folding of the OMPs before membrane insertion, this issue requires further investigation.

Besides the machinery for the assembly of OMPs, several proteins exist for transporting outer-membrane polypeptides, for example, FhaC in *Bordetella pertussis* (Guedin *et al*, 1998) and ShlB in *Serratia marcescens* (Poole *et al*, 1988). These systems are known as 'two-partner secretion pathways', as they are specific to their substrates, such as adhesins and hemolysins (Jacob-Dubuisson *et al*, 2001). The proteins seem to transport their substrate in its unfolded state, as has been shown for FhaC (Guedin *et al*, 1998). In addition, the investigation of ShlB suggests a dual function for this protein. The channel formed by ShlB facilitates the translocation of ShlA (Schiebel *et al*, 1989) and also changes its conformation—thereby inducing the transfer of phosphatidylethanolamine (Hertle *et al*, 1997; Walker *et al*, 2004), which is required for the activation of ShlA. Despite the topological and functional homology between proteins of this class, FhaC and ShlB are not interchangeable and therefore have molecular specificity (Jacob-Dubuisson *et al*, 1997). This class of transporters also has structural features similar to proteins of the Omp85 class (Surana *et al*, 2004).

Eukaryotic β-barrel-shaped transporters

Endosymbiotically derived organelles import most of their proteinaceous components from the cytosol. In mitochondria and plastids, two pore-forming proteins—Tom40 and Toc75, respectively—are involved in the translocation of proteins across the outer membrane. However, the evolutionary development of the two transport channels is distinct. The mitochondrial channel Tom40 seems to share its ancestral roots with porins (Gabriel *et al*, 2001), whereas Toc75 belongs to the Omp85 class (Moslavac *et al*, 2005). Interestingly, another translocation system specialized for the insertion of β-barrel OMPs (Fig 1B) was identified recently in the mitochondrial outer membrane (Wiedemann *et al*, 2003). The main component of this complex is

Table 1 | Components involved in β-barrel protein insertion

		Prokaryotes				Eukaryotes	
Machinery components						Mitochondria	Chloroplasts
Primary translocon	Receptors	SecA				Tom20 Tom22 Tom70 Tom5/6/7	Toc34 Toc64 Toc159
	Pore	SecYEG				Tom40	Toc75-III
IMS machinery	Chaperone system	Skp SurA				Tim8 & Tim13 Tim9 & Tim10	Toc12 & imsHsp70
Secondary translocon	Species	*Ec*	*Nm*	*S*	*N*		
	Translocon	YaeT	Omp85	slr1227	alr2269	Sam50/Tob55/mtOmp85	Toc75-V/Oep80
	Assisting proteins	YfiO YfgL NlpB	NI	NI	NI	Sam37/Mas37 Sam35/Tob38/Tom38 Mdm10 Tom13/Mim1	NI

Ec, *Escherichia coli*; IMS, intermembrane space; *N*, *Nostoc* PCC 7210; NI, not yet identified; *Nm*, *Neisseria Meningitides*; *S*, *Synechocystis* PCC6803; Sam, sorting and assembly of mitochondria outer membrane proteins; Tic, tranlocase on inner membrane of chloroplast; Tim, tranlocase on inner membrane of mitochondria; Tob, topogenesis of mitochondrial outer membrane β-barrel proteins; Toc, tranlocase on outer membrane of chloroplast; Tom, tranlocase on outer membrane of mitochondria. In the case of several names, the nomenclature used in the manuscript is the first term.

Sam50 (Table 1; Paschen *et al*, 2003; Kozjak *et al*, 2003; Gentle *et al*, 2004; Humphries *et al*, 2005), which belongs to the Omp85 class and has the highest similarity with a subclass of Omp85 proteins previously annotated as Oma87 (Moslavac *et al*, 2005). The Sam50 protein is essential for the biogenesis of the mitochondrial outer membrane proteome. The evolutionary roots of this complex are also reflected in its mode of action: all β-barrel proteins first have to be translocated across the outer membrane, facilitated by the Tom40 machinery, followed by insertion into the outer membrane catalysed by the Tob/Sam complex (see, for example, Pfanner *et al*, 2004). In contrast to the bacterial system, no intermembrane space proteins that associate with Sam50 have been identified. However, Mdm10, an integral membrane protein previously identified as being involved in the maintenance of mitochondrial morphology (Sogo & Yaffe, 1994; Meisinger *et al*, 2004), and two proteins that associate with the cytosolic side of the complex, namely Sam37 (Gratzer *et al*, 1995; Kozjak *et al*, 2003; Waizenegger *et al*, 2004) and Sam35 (Waizenegger *et al*, 2004; Ishikawa *et al*, 2004; Milenkovic *et al*, 2004), have been found to be associated with Sam50. A third cytosolic-exposed component that influences β-barrel protein insertion, Tom13, seems to form part of a distinct, unidentified complex and is not a component of either Tom or Tob/Sam (Ishikawa *et al*, 2004; Waizenegger *et al*, 2005). The exact function of these extra components is yet to be established because the substrate approaches the translocon from the intermembrane space rather than the cytosol. The small mitochondrial Tim proteins, Tim8 and Tim13 (Hoppins & Nargang, 2004) or Tim9 and Tim10 (Wiedemann *et al*, 2004), might be the functional homologues of the factors acting in the periplasm of the bacterial system, as they are essential for the biogenesis of β-barrel OMPs.

The prokaryotic origin of Toc75, the central component of the translocation system in chloroplasts, was first suggested after the identification of a homologous protein in *Synechocystis* (Heins *et al*, 1998). Six homologues of Toc75 are encoded by the genome of *Arabidopsis thaliana* (Moslavac *et al*, 2005), which suggests functional differentiation, or substrate or tissue specificity of these proteins. Three isoforms are found in chloroplasts, namely Toc75-III, the main translocation pore of plastids (Soll & Schleiff, 2004), Toc75-IV (Baldwin *et al*, 2005) and Toc75-V (Eckert *et al*, 2002; Inoue & Potter, 2004). All proteins of the Toc75 family show a clear relation to proteins of the Omp85 class (Moslavac *et al*, 2005). So far, only the insertion of *ps*Toc75, which contains a bipartite targeting signal, has been investigated in detail. The bipartite signal directs the protein from the cytosol towards the stroma, where the first part of the signal is cleaved off by the stromal processing peptidase (Fig 1C; Tranel & Keegstra, 1996). The second portion of the signal functions as an intra-organellar targeting signal and is cleaved off in the intermembrane space. As all other β-barrel OMPs so far identified do not contain an amino-terminal signal, it is assumed that their mode of insertion differs from that of Toc75. Indeed, the insertion of outer envelope proteins Oep21 and Oep24 is independent of thermolysin-sensitive factors and ATP (Schleiff & Klösgen, 2001), which excludes the use of the Toc-core translocon. However, it remains to be resolved whether Toc75-III itself or Toc75-V, which has the highest similarity to its bacterial ancestor (Moslavac *et al*, 2005), functions in the assembly of the OMPs. Interestingly, as in the bacterial system, an intermembrane space chaperone is involved in the translocation of chloroplast proteins (Becker *et al*, 2004). However, it remains to be established whether this system is specialized for β-barrel OMPs that are routed through the intermembrane space.

Translocation through β-barrel-shaped transporters

As summarized above, β-barrel-shaped transporters such as the bacterial Omp85 (Genevrois *et al*, 2003; Voulhoux *et al*, 2003), slr1227 (Bölter *et al*, 1998; Reumann *et al*, 1999), YaeT (Gerdes *et al*, 2003; Wu *et al*, 2005), alr2269 (Moslavac *et al*, 2005, Ertel *et al*, 2005), the mitochondrial Sam50 (Wiedemann *et al*, 2003; Paschen *et al*, 2003; Gentle *et al*, 2004; Humphries *et al*, 2005) and the plastidic Toc75 (Tranel *et al*, 1995; Eckert *et al*, 2002) are present in their respective outer membranes (Table 1) and facilitate the insertion of the proteins into the membrane. A recent comparison of prokaryotic and eukaryotic β-barrel-shaped protein transporters revealed some explanations to support the evolutionary preference for this class of proteins as translocation channels (Ertel *et al*, 2005). It has been suggested that proteins of this family contain two distinct domains (Sanchez-Pulido *et al*, 2003): an N-terminal domain in which the β-strands are connected by long, soluble loops, and a pore-forming region at the carboxy-terminus (Schleiff *et al*, 2003; Voulhoux *et al*, 2003; Ertel *et al*, 2005). Indeed, the N-terminal region of *ps*Toc75 acts as a

specific but low-affinity receptor for proteins that contain a targeting sequence (Ertel *et al*, 2005). Analysis of the Omp85 homologue from *Nostoc PCC7120*, alr2269, has revealed that this feature already exists in the cyanobacterial protein. This leads to the question whether the pore-forming proteins themselves formed the first binding site for proteins to be re-imported after the cyanobacterium was taken up by the host cell. In addition, the extreme N-terminal region of *ps*Toc75 and alr2269 is involved in the formation of the translocon (Ertel *et al*, 2005), although the partner and the mode of interaction differ between both proteins. A cytosolic loop of Toc75 is involved in hetero-oligomerization by recognizing the receptor Toc34, whereas the N-terminus of alr2269 facilitates homo-oligomerization (Ertel *et al*, 2005). The C-terminal regions of both proteins are also involved in homo-oligomerization (E.S., unpublished data). Interestingly, the C-terminal domains of *ps*Toc75 and alr2269 have similar pore characteristics to the full-length proteins, which is in agreement with a functional dissection of the molecules (Ertel *et al*, 2005). However, the gating of the channel is altered by the presence of the N-terminus and suggests a mechanistic relationship between receptor function and pore gating (Ertel *et al*, 2005).

Concluding remarks

The insertion and assembly pathway of β-barrel OMPs in prokaryotes and endosymbiotically derived organelles seems to be evolutionarily conserved. Hence, the mechanistic concepts are also expected to be similar. Indeed, in mitochondria and plastids, an intermediate has been identified in intermembrane space translocation. Therefore, OMPs are inserted in the outer membrane in the same direction as bacteria—that is from the 'periplasmic space', which supports the idea of mechanistic conservation. The identification of components with similar functions in all three systems (Fig 1; Table 1) further supports this concept.

In the future, it will be interesting to determine whether proteins of the Omp85 class have an extra lipid transport function and whether or not proteins are pre-folded before their insertion into these membranes. Furthermore, it is unlikely that the transfer of the OMPs out of the pore into the bilayer occurs by lateral diffusion because a lateral opening of the barrel would require the destruction of several hydrogen bonds and this would be energetically unfavourable. Alternatively, the β-barrel proteins of the Omp85 class that facilitate the insertion of the OMPs might serve as a seed or 'chaperone' for insertion, as a cooperative but specific insertion of β-barrel proteins has been suggested (Li & Colombini, 2002). In the latter case, the function of the reported channel activity remains elusive, as the electrophysiological properties of the eukaryotic proteins suggest a large pore diameter. To understand fully protein insertion into the outer membrane of endosymbiotically derived organelles, it will be important to discover which of the Omp85 homologues of the outer membrane of chloroplasts is the integrase for the OMPs. Nevertheless, the identification of bacterial homologues in cellular organelles has already answered a long-standing question in the endosymbiotic process—by providing the first experimental evidence that the origin of mitochondria and chloroplasts is indeed monophyletic.

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