

Review article

The β -barrel shaped polypeptide transporter, an old concept for precursor protein transfer across membranesWolfgang Löffelhardt¹, Arndt von Haeseler², and Enrico Schleiff^{3*}¹Max F. Perutz Laboratories, University of Vienna, Department of Biochemistry, 1030 Vienna, Austria;²Center for Integrative Bioinformatics Vienna, Max F. Perutz Laboratories, University of Vienna, Medical University of Vienna, Veterinary University of Vienna, Austria;³Ludwig-Maximilians-Universität Munich, Department of Biology I, Menzinger Str. 67, 80638 Munich, Germany, Tel. +49-89-17861-182, Fax. +49-89-17861-185, Email. schleiff@lrz.uni-muenchen.de

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Abstract

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 currently the focus of intense research. Here, the relations between prokaryotic machines inserting proteins into the outer membrane and eukaryotic translocation machines are envisaged. The identification of Omp85/YaeT- in *Neisseria meningitidis*, *Escherichia coli* and cyanobacteria, and of its homologues in chloroplasts and mitochondria, has provided new clues about the ancestral β -barrel protein insertion pathway. Central to this review are recent advances in the elucidation of the evolutionary history of the polypeptide-transporting β -barrel channels.

Keywords: β -barrel membrane protein insertion, evolutionary relation of transport systems, Omp85, Sam50/Tob55, Toc75

1. Introduction

The formation of membrane surrounded intracellular compartments was one of the major evolutionary breakthroughs leading to the development of the eukaryotic kingdom (Lopez-Garcia and Moreira, 2006; Embley and Martin, 2006). Some of the compartments are derived from an endosymbiotic process, as mitochondria (e.g. Margulis, 1970; John and Whatley, 1975; Gray et al., 1999) or plastids (e.g. Mereschkowsky, 1905; Gray and Doolittle, 1982; McFadden, 1999). Others are the consequences of intracellular membrane formations as peroxisomes (e.g. Latruffe and Vamecq, 2000; Gabaldon et al., 2006). The origin of the nucleus, however, is still under debate. A viral (e.g. Sonea, 1987; Takemura, 2001) or archaeal origin (Zillig et al., 1989; Sogin, 1991) also known as fusion theory, or the development through intracellular membrane

formation (Cavalier-Smith, 1987; Lopez-Garcia and Moreira, 2006; Martin and Koonin, 2006) are discussed.

However, independent of the organelles' origins, the appearance of cellular subspaces surrounded by membranes necessitated the establishment of transport systems to ensure the exchange of solutes and proteins between the cellular compartments. Protein translocation across and into membranes is a fundamental process as up to 50% of all proteins synthesized in the cytosol need to traverse at least one membrane to reach their place of function (Schatz and Dobberstein, 1996; Wickner and Scheckman, 2005). The basic principles of protein translocation have been established, although the extent to which the molecular mechanism of each system has been determined varies. Proteins translocated across the nuclear (Xu and Massague, 2004) or the peroxisomal membranes (Erdmann and Schliebs, 2005) and by the twin arginine translocation (TAT) pathway in bacteria and thylakoids (Robinson and Bolhuis, 2001) are folded. In contrast, proteins transported into mitochondria (Rehling et al., 2004), plastids (Soll and

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Schleiff, 2004) and endoplasmic reticulum (Osborne et al., 2005) need to be unfolded. The systems also vary in the architecture of the translocon and the energetic requirements, as plastids, mitochondria, endoplasmic reticulum and nuclei contain pre-existing machines, whereas translocon formation in the peroxisomal or TAT pathway is substrate induced.

At present, the least understood process of protein translocation is the insertion of β -barrel membrane proteins composed of antiparallel transmembrane β -strands connected by soluble loop regions (Schulz, 2000) into the outer membrane of gram-negative bacteria, mitochondria and chloroplasts (Voulhoux and Tommassen, 2004; Schleiff and Soll, 2005; Paschen et al., 2005). For the bacterial system it was suggested that the β -barrel membrane proteins are first translocated across the plasma membrane via the Sec translocation machinery (e.g. Voulhoux and Tommassen, 2004). In the periplasmic space, the β -barrel membrane proteins are at least partially folded and subsequently inserted into the outer membrane (Eppens et al., 1997). Furthermore, the periplasmic chaperone *skp* (seven kDa protein) and the periplasmic peptidyl-prolyl *cis-trans* isomerase *surA* have a chaperone functions essential for the correct topology of OMPs (outer membrane proteins; Rizzitello et al., 2001). Recently it was elucidated that polypeptide-transporting β -barrel channels of the Omp85 family act as translocators or membrane inserted chaperones to facilitate the insertion of outer membrane proteins (Voulhoux and Tommassen, 2004; Schleiff and Soll, 2005; Paschen et al., 2005).

In this review we will focus on the evolution of the polypeptide-transporting β -barrel channels and explain the major impact of this analysis on the understanding of the evolution of eukaryotic transport systems after endosymbiosis.

2. The Known Polypeptide-transporting β -barrel Proteins of the Omp85 Family

Proteins investigated

The identification of a protein homologous to the chloroplast translocation channel Toc75 (Translocon on the outer chloroplast membrane of 75 kDa; Soll and Schleiff, 2004) in the cyanobacterium *Synechocystis* PCC6803 (Bölter et al., 1998; Reumann et al., 1999) marked the beginning of the generalization of the concept, that polypeptide-transporting β -barrel channels are involved in protein assembly at outer membranes (Table 1).

The authors observed similarity of the cyanobacterial protein to a sequence found in *N. meningitidis* assigned as Omp85. Subsequently, the prokaryotic polypeptide-transporting β -barrel channels of this family found in *N. meningitidis* (*nmOmp85*; Outer membrane protein of 85

kDa; Genevrois et al., 2003; Voulhoux et al., 2003), *E. coli* (YaeT; Gerdes et al., 2003; Wu et al., 2005; Malinverni et al., 2006) or *Nostoc* sp. PCC7120 (*nOmp85*; Ertel et al., 2005) were characterized. These Omp85 have a catalytic rather than a direct effect on outer membrane protein insertion. Major support for this notion comes from the observation that the SecA component of the plasma membrane translocon (Osborne et al., 2005) functions as a multicopy suppressor of the temperature-sensitive mutant of YaeT and partially restores outer membrane protein assembly (Doerrler and Raetz, 2005). The increased concentration of SecA in the suppressed YaeT knock out strains leads to an enhanced secretion of the outer membrane proteins across the plasma membrane. The subsequently higher concentration of the outer membrane proteins in the periplasmic space results in bypassing the action of the outer membrane inserted Omp85 chaperone.

Nevertheless, even though the prokaryotic Omp85 proteins are intensively investigated, the first polypeptide-transporting β -barrel protein of the Omp85 family identified was Toc75 from *Pisum sativum* (Tranel and Keegstra, 1996; Hinnah et al., 1997). At present, six homologues to Toc75 are found in the genome of *Arabidopsis thaliana* (Moslavac et al., 2005). This observation suggests a functional differentiation in terms of substrate or tissue specificity of the Omp85s in higher plants. The chloroplast localization of three isoforms was experimentally confirmed, namely of Toc75-III, the major translocation pore of plastids (Soll and Schleiff, 2004), Toc75-IV (Baldwin et al., 2005) and Toc75-V (Eckert et al., 2002; Inoue and Potter, 2004). However, the functional subdivision of the plastid localized family remains elusive.

Finally, a translocation system specialized for the insertion of β -barrel outer membrane proteins was identified in the mitochondrial outer membrane (Paschen et al., 2003; Wiedemann et al., 2003). The main component of this complex is Sam50 / Tob55 (Sorting and assembly machinery component of 50 kDa; Component of the complex for topogenesis of mitochondrial outer membrane β -barrel proteins of 55 kDa; see Table 1; Paschen et al., 2003; Kozjak et al., 2003; Gentle et al., 2004; Humphries et al., 2005), which belongs to the Omp85 family and shares the highest similarity with a subfamily of Omp85 proteins also known as Oma87 (Moslavac et al., 2005). The evolutionary roots of the Sam50 complex found in mitochondria are reflected in its mode of action. All β -barrel proteins are first translocated across the outer mitochondrial membrane, facilitated by the Tom40 machinery (Translocase at the outer mitochondrial membrane of 40 kDa). Subsequently, the β -barrel proteins are inserted into the outer membrane from the intermembrane space (originated from the periplasmic space) catalyzed by the Tob/Sam complex (e.g. Paschen et al., 2005; Pfanner et al., 2004). Interestingly, as for the plastid localized Omp85 homologous, at least two different

genes encode mitochondrial targeted Omp85 homologous (Bredemeier et al., 2006).

Therefore, polypeptide transporting β -barrel proteins are experimentally confirmed in prokaryotic outer membranes and in the outer membranes of endosymbiotically evolved organelles. A detailed overview of the functional properties of these proteins can be found elsewhere (Voulhoux and Tommassen 2004; Schleiff and Soll, 2005; Paschen et al., 2005; Steiner and Löffelhardt, 2005).

Structural features of Omp85 like proteins

All Omp85 proteins are predicted to form β -barrel structures (Hinnah et al., 1997; Bölder et al., 1998; Schleiff et al., 2003; Voulhoux et al., 2003; Ertel et al., 2005; Fitzpatrick and McNerny, 2005). However, experimental confirmation by crystallization is still missing. The predicted structure of the membrane inserted domain is consistent with the current assumption that only β -barrel structures are present in the prokaryotic outer membranes (Schulz, 2000). However, the number of membrane-inserted strands is not clear. Here, 12 to 18 strands are predicted (e.g. Voulhoux et al., 2003; Schleiff et al., 2003), although most recent predictions favor a 16-stranded β -barrel (Ertel et al., 2005). In contrast to initial predictions (Hinnah et al., 1997; Bölder et al., 1998), it is now obvious that the pore forming region is clustered in the C-terminal part of the sequence. This was confirmed by electrophysiological measurements of the entire protein and the C-terminal region (Ertel et al., 2005; Stegmeier and Andersen, 2006; Bredemeier et al., 2006).

The localization of the Omp85 pore forming domain occur also in other outer membrane polypeptide transporting proteins like FhaC or ShlB. FhaC from *Bordetella pertussis* (Guedin et al., 2000) or ShlB from *Serratia marcescens* (Könninger et al., 1999) are specific

for their substrates such as adhesins and hemolysins and are involved in "two-partner secretion" pathways (Jacob-Dubuisson et al., 2001). Similar to proteins of the Omp85 family, these transporters also contain a C-terminal pore forming region as determined by structure prediction (Guedin et al., 2000; Könninger et al., 1999) and electrophysiological experiments (Könninger et al., 1999; Meli et al., 2006). The functional splitting between N-terminal regulatory domain and C-terminal pore forming domain might be a general feature of all polypeptide-transporting β -barrel proteins (e.g. Könninger et al., 1999; Ertel et al., 2005; Meli et al., 2006). In line with this assumption, an N-terminal POTRA domain (polypeptide-transport-associated domain) was identified in Omp85 and in ShlB proteins (Sanchez-Pulido et al., 2003). This domain is postulated to represent a polypeptide-binding motif. This is supported by the association of the N-terminal domain of the *n*Omp85 (Table 1) to the transit peptide of a plastid targeted precursor protein (Ertel et al., 2005). Two further sequence motifs were identified in the pore forming C-terminal portion of the polypeptide-transporting β -barrel proteins (Eckart et al., 2002; Gentle et al., 2004). A detailed analysis of the two conserved regions revealed a conserved distance of this motif from the C-terminus (Moslavac et al., 2005). Furthermore, both motifs represent a predicted strand-hairpin-strand motif suggesting that these four strands have a particular function in that barrel (Moslavac et al., 2005). The nature of this function, however, remains to be explored.

Summarizing, Omp85 proteins from prokaryotic and eukaryotic systems were identified. They belong to the large family of the polypeptide-transporting β -barrel shaped channels localized in the outer membranes. These channels are characterized by general properties unifying the proteins of the Omp85 family and proteins that function in two-partner secretion pathways. They contain a N-terminal

Table 1. The currently investigated polypeptide transporting β -barrel proteins of the Omp85 family are listed.

	Species	Acc. number	Name	References	
Prokaryotic	Cyanobacteria	<i>Synechocystis</i> PCC6803	slr1227	synToc75	Bölder et al., 1998; Reumann et al., 1999
		<i>Nostoc</i> sp. PCC7120	alr2269	nOmp85	Ertel et al., 2005; Moslavac et al., 2005
	Proteobacteria	<i>E. coli</i>	AAC73288	YaeT	Wu et al., 2005; Doerrler and Raetz, 2005; Malinverni et al., 2006; Stegmeier and Andersen, 2006
		<i>N. meningitidis</i>	AAC17599	nmOmp85	Genevrois et al., 2003; Voulhoux et al., 2003
Eukaryotic	Chloroplasts	<i>P. sativum</i>	CAA58720	psToc75	Tranel and Keegstra, 1996; Hinnah et al., 1997; Ertel et al., 2005
		<i>A. thaliana</i>	At3g46740	atToc75-III	Baldwin et al., 2005
			At4g09080	atToc75-IV	Baldwin et al., 2005
			At5g19620	atToc75-V	Inoue and Potter, 2004
	Mitochondria	<i>S. cerevisiae</i>	YNL026w	scSam50	Paschen et al., 2003; Kozjak et al., 2003; Gentle et al., 2004
		<i>H. sapiens</i>	BC017224	hsSam50	Humphries et al., 2005

regulatory and a C-terminal pore forming domain (Könninger et al., 1999; Ertel et al., 2005; Meli et al., 2006). Further, a polypeptide-transport-associated domain was identified in the N-terminal region (Sanchez-Pulido et al., 2003), as well as two motifs found in the pore forming region (Moslavac et al., 2005) with so far unknown function.

3. Different Polypeptide-transporting β -barrel Shaped Omp85 Type Proteins

The targeting signal dependence

All Omp85 like proteins are involved in outer membrane protein assembly or translocation. It is proposed that prokaryotic Omp85 family members recognize their substrates by a terminal exposed phenylalanine (Voulhoux and Tommassen, 2004; Steiner and Löffelhardt, 2005) since this aromatic amino acid occupies a prominent position in the sequence of proteins targeted to the outer membrane (Struyve et al., 1991; Bhaya et al., 1999). This feature can still be seen in the transit sequences of plastid proteins from Glaucocystophytes, Rhodophytes, Diatoms and Cryptomonads (Steiner and Löffelhardt, 2005), where the phenylalanine is positioned near or at the N-terminus of the plastid-directing signal. The conservation of the phenylalanine is lost signals directing proteins to mitochondria or chloroplasts of higher plants. This can be explained as follows. Mitochondrial outer membrane proteins are primarily targeted toward the general insertion pore of the outer mitochondrial membrane (e.g. Pfanner et al., 2004). The essential pore component of this machinery is not related to the Omp85 proteins but to porins (Gabriel et al., 2001). In the intermembrane space, the proteins to be imported into the outer membranes are recognized by the so called tiny Tims (Translocase at the innner mitochondrial membrane), which direct the proteins to the Omp85 protein. Thus, the conservation of the phenylalanine is no longer required. In the chloroplast system, the initial binding site for phenylalanines present in Toc75 is complemented by the evolutionary addition of further receptor components assisting the recognition of transit peptides at the surface of chloroplasts in higher plants (Soll and Schleiff, 2004). This interpretation has several consequences. On one hand, suggesting Omp85 as the initial phenylalanine receptor leads to the proposal that an Omp85 type protein should occur in Cyanelles, Diatoms and Cryptomonads. On the other hand, the conservation of the phenylalanine suggests that Omp85 indeed constitutes the initial docking site for incoming precursor proteins after their genes were transferred into the nucleus of the host. Hence, the loss of the phenylalanine requirement coincides with the recruitment of additional receptor components not present in the outer membrane of "primitive plastids". However,

targeting signal of proteins imported into plastids of Glaucocystophytes, Diatoms and Cryptomonads also direct these proteins into chloroplasts of higher plants (Lang et al., 1998; Wastl and Maier, 2000; Steiner et al., 2005). Therefore, the loss of the phenylalanine residue in the targeting signal is caused by the loss of the evolutionary pressure to keep this motif rather than by an incompatibility with the new receptor components. However, it can also be argued that the phenylalanine was evolutionarily "removed" since a stringent signal required at a specific site might have withstood the integration of the plastids into the cellular networks of different tissues and the distribution of some proteins between different compartments. The remaining signal is rather undefined and only characterized by the presence of hydroxylated and positively charged amino acids (Bruce, 2001). Thereby, the signals of mitochondria and chloroplasts are rather comparable and the removal of the phenylalanine might have resulted in the feasibility of dual targeting in higher plants, not yet seen in algae.

Summarizing, the interaction between Omp85 and the phenylalanine "signal" was initially optimized for protein insertion into the outer bacterial membrane as the number of proteins exported into the periplasmic space is rather limited. This interaction was evolutionarily used after endosymbiosis to place Omp85 as the initial import complex across the outer membrane of the "newborn" organelle. However, a system recognizing a unique amino acid at a specific position might not have been necessary any longer or even inappropriate for the development of the higher plant cell. The loss of this kind of specificity likely was a consequence of the development of additional receptor components, which achieve sufficiently selective translocation by other means.

The different functions

From the preceding section one essential message can be extracted: Omp85 like proteins come in different "flavors"! On one hand, for the prokaryotic and mitochondrial Omp85 like protein a chaperoning of the membrane insertion of the outer membrane proteins is proposed (e.g. Voulhoux and Tommassen, 2004; Paschen et al., 2005). In contrast, Toc75 forms a protein conducting channel (Schleiff and Soll, 2005). On one side this leads to the question whether one protein can carry out both functions. On the other side, during evolution several components were added to the Omp85 like proteins found in the eukaryotic membranes.

Currently two models are considered to explain the function of Omp85 in assembly of outer membrane proteins (Gentle et al., 2005). The first model suggests that the outer membrane protein first enters the Omp85 pore and subsequently laterally diffuses into the membrane. However, such a model is not realistic for two reasons. For YaeT a pore diameter of 0.54 nm was estimated from its electrophysiological properties (Fig. 1; Stegmeier, and

Andersen, 2006; Bredemeier et al., 2006). This pore diameter is too small to insert an outer membrane protein. This model will only work, if an oligomerization of several Omp85 proteins is proposed. The resulting oligomer will have a larger pore, that allows for the insertion of the outer membrane proteins. This would parallel the behavior of the TAT translocon (Robinson and Bolhuis, 2001). However, β -barrel proteins are not as flexible as the helical proteins involved in the formation of the TAT translocon. Thereby, such a model appears to be less likely.

The second and more reasonable model assumes that Omp85 serves as a seed or "chaperone" for insertion. *In vitro*, a self-catalyzed insertion of β -barrel proteins has been described (Li and Colombini, 2002). This model is in line with outer membrane proteins attaining partial structural arrangement prior to translocation (Tamm et al., 2004). A so-called 'molten disc' intermediate which has partial secondary structure generated at the bilayer interface with the beta strands sitting flat on the membrane (Tamm et al., 2004) precedes the insertion of the outer membrane protein into the membrane. The properties of Sam50 resemble those of YaeT. Electrophysiological experiments confirmed that the pore characteristic of both proteins is alike (Fig. 1; Bredemeier et al., 2006). Therefore, the mode of membrane insertion of outer membrane proteins is comparable between proteobacterial and mitochondrial systems. However, the depicted mode of action may not apply to for Toc75 since it is involved in precursor transfer across the membrane.

The function of Toc75 is extensively reviewed elsewhere (e.g. Soll and Schleiff, 2004). Toc75 forms a cation selective channel, that transports precursor proteins across the outer membrane of chloroplasts (Hinnah et al., 1997). The pore diameter is estimated to about 2–3 nm, thus large enough to transport precursor proteins (Fig. 1). As for the mitochondrial and the proteobacterial proteins, similar physical properties of Toc75 and the Omp85 proteins from cyanobacteria based on the electrophysiological properties were determined (Bölter et al., 1998; Ertel et al., 2005; Bredemeier et al., 2006). This finding was somewhat unexpected since a similar function of Omp85 in cyanobacteria and proteobacteria was assumed. However, based on the current results a different function or at least a different mode of action has to be proposed for these two prokaryotic proteins. Remarkably, in *Anabaena variabilis*, *Nostoc sp. PCC7120*, and *Nostoc punctiforme* three different isoforms of Omp85 are present (Moslavac et al., 2005; Bredemeier et al., 2006). Hence, in future the properties of the different isoforms have to be compared to elucidate, whether all cyanobacterial transport proteins share similar properties or whether some of them show proteobacterial features.

Summarizing, whereas Toc75 and nOmp85 (Table 1) have a large pore diameter (Fig. 1), Sam50 and proteobacterial Omp85s have a pore size insufficient to

translocate an unfolded polypeptides (Fig. 1). Hence, the functional mode of the two families of Omp85 proteins is expected to be different.

4. The Evolutionary Concept for PTB Development

It is assumed that the first cell already contained an outer membrane (Blobel et al., 1980; Cavalier-Smith, 2001) consisting of phospholipids only since the outer membrane of Chlorobacteria and Hadobacteria (Infrakingdom Eobacteria and Subkingdom Negibacteria; Cavalier-Smith, 2002) does not contain a lipopolysaccharid layer. Cavalier-Smith (2006) argues that the development of the lipopolysaccharid layer necessarily entailed the origin of numerous outer membrane porins and that they are not as essential in Eobacteria as in Glycobacteria (Cavalier-Smith, 2002). However, this statement raises the question how these cells exchange solutes with the surroundings. A recent analysis of the fully sequenced genomes indicates that Chlorobacteria probably contain β -barrel outer membrane proteins (Schleiff, unpublished observation). In contrast, analyzing the currently available genomes an Omp85 homologue was detected in *Thermus thermophilus*, a member of Hadobacteria, but no homologues were found in Chlorobacteria. Therefore, Omp85 proteins exist at least since Hadobacteria branched off from the common root of bacteria (Fig. 2a; Bredemeier et al., 2006; Cavalier-Smith et al., 2002). Consequently, outer membrane protein insertion was not Omp85 dependent in the Chlorobacteria (Fig. 2b, grey arrow; Cavalier-Smith, 2006). Further, the Omp85 member in the genomes of Hadobacteria are most distant from the remaining sequences (Fig. 2a, b) and the next branching occurred with the development of the cyanobacteria about 2.8 Gy ago (Fig. 2b; Cavalier-Smith et al., 2006). In contrast to the scenario depicted by Cavalier-Smith (2006), we find the branch of the Eurybacteria closer to the cyanobacteria than to the proteobacteria (Fig. 2a, b). Nevertheless, the branch length indicates a large distance between the cyanobacteria and Eurybacteria (Bredemeier et al., 2006). Strikingly, analyzing the N-terminal portion of the polypeptide transporting β -barrel proteins, the Hadobacteria move closer to the Proteobacteria and Eurybacteria and are more distant to the cyanobacteria (Bredemeier et al., 2006). This suggests a different functional property of this domain in cyanobacteria when compared to the other bacteria.

Since Omp85 proteins are evolutionarily younger than outer membrane β -barrel proteins in general, we would like to propose the following scenario for the evolution of the Omp85 protein family (see Fig. 2b, marked with C) based on recent experimental and phylogenetic results. The distal C-terminal section does not only show a high sequence conservation (Eckart et al., 2002; Gentle et al., 2004; Moslavac et al., 2005) but also forms a dimeric channel as

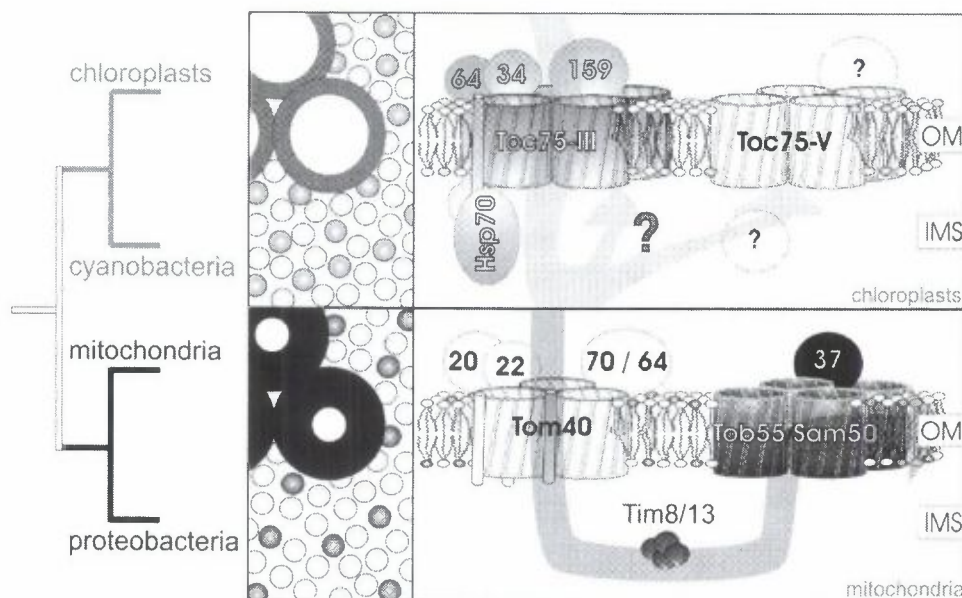


Figure 1. The physiological difference between the polypeptide transporting β -barrel proteins of the Omp85 family. The phylogenetic branching of the Omp85 sequences according to Bredemeier et al. (2006) is indicated at the left site. The branches are not in scale but indicate the phylogenetic relation of the eukaryotic Omp85 proteins to the bacterial one. The dissection of the Omp85 sequences into two different clades is well supported by the different pore size obtained by electrophysiological experiments as described in the text. The pore size difference is indicated as schematic top view of the proteins present in the membrane (middle). In addition, a complex formation of the pore forming proteins is indicated. The different pore sizes further indicate different functional categories. On the right, the established mode of action for the plastid (top, right) and mitochondrial (bottom, right) Omp85 system is depicted. Omp85 proteins of the upper clade including the Toc75-III chloroplast proteins function as protein conducting channels. The plant proteins form complexes with other components like Toc64, Toc34 and Toc159. The insertion of β -barrel proteins into the outer membrane probably occurs from the intermembrane space. The gray arrow indicates that this type of proteins first reaches the intermembrane space and is then integrated by the help of Toc75-III or Toc75-V a second protein of the Omp85 family present in the outer membrane. In contrast, Omp85 proteins of the lower clade including the mitochondrial Omp85 do not exert protein conducting channel properties. These proteins catalyze the insertion of the β -barrel type outer membrane proteins (right), which are translocated by the usher type Tom40 protein into the intermembrane space (transport route indicated by gray arrow). In the intermembrane space small Tim proteins participate in the transport or folding of the β -barrel proteins. Hence, for both, mitochondrial and chloroplast system, the insertion of β -barrel proteins occurs from the intermembrane space, which in turn parallels the finding for bacteria.

determined by biochemical and electrophysiological experiments (Ertel et al., 2005; Bredemeier et al., 2006). This dimeric state of the distal C-terminus might reflect an ancestral property of the pore forming domain having its seed in a monomeric molecule with an 8-stranded transmembrane β -barrel (Fig. 2C, step 1). Subsequent homo- or heterodimerization of two proteins containing eight transmembrane β -strands resulted in a 16 β -strand channel formation (Fig. 2C, step 2). Alternatively, the 16 β -strand protein might have evolved by subsequent strand-hairpin-strand duplication as suggested for helical transmembrane structures (e.g. Shimizu et al., 2004).

However, the combined phylogenetic analysis of the sequences representing the first 8 proposed β -strands and the 8 proposed C-terminal β -strands revealed two sub-trees representing the individual segments (Bredemeier et al., 2006). This observation supports the hypothesis of a fusion of two individual 8 β -strand channels. The evolved channel, however, exposed a high affinity for a periplasmic protein

(Bredemeier et al., 2006), which was essential to close the channel (Fig. 2C, step 3). Such scenario is found for TonB, FepB and other periplasmic proteins (e.g. Faraldo-Gomez and Sansom, 2003). This periplasmic protein subsequently evolved to a domain recognizing the incoming outer membrane proteins. Finally, the “modern” Omp85 proteins evolved by gene fusion of the gene encoding the periplasmic protein forming the N-terminal plug domain, and the gene encoding the pore, that we now call C-terminus (Fig. 2C, step 3). This assumption is in line with the proposal of Cavalier-Smith (2006), who considers the development of the Omp85 protein as a major promoter for the formation of complex outer membranes.

In the following course of evolution the bacterial proteins were adapted to respond to different environmental conditions. For instance, after endosymbiosis the Omp85s of the symbiont had to cope with new environmental conditions and a change in function. Hence, the N-terminus was remodeled to interact with newly evolved complex

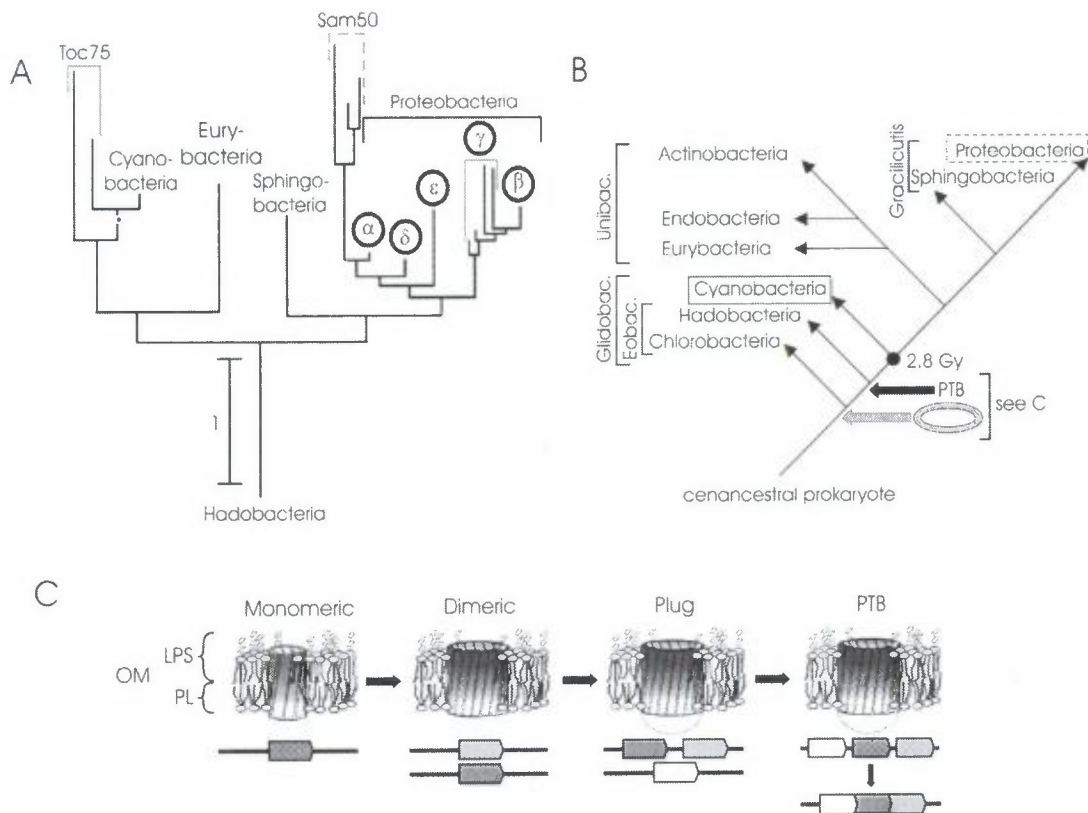


Figure 2. The evolutionary development of the polypeptide-transporting β -barrel proteins of the Omp85 family. A) The phylogenetic distribution of the polypeptide transporting β -barrel proteins of the Omp85 family according to Bredemeier et al. (2006) is shown (for detailed tree see reference). The branches end at the root of the subtree formed by the indicated bacteria class. B) The phylogeny of bacteria in the nomenclature according to Cavalier-Smith (2006) is depicted. The appearance of the outer membrane (indicated on the right side), of the polypeptide transporting β -barrel proteins of the Omp85 family (PTB) according to Bredemeier et al. (2006) and the distance between Cyanobacteria and Proteobacteria according to Cavalier-Smith (2006) is shown. The bacteria ancestral to plastids (solid lines) and mitochondria (dashed lines) are boxed. C) The model for the evolutionary development of the polypeptide transporting β -barrel proteins of the Omp85 family after appearance of the first β -barrel proteins (in Chlorobacteria) is shown.

components and the affinity of the N-terminal domain for the pore-forming region had to be reduced to accommodate to the conductance of precursor proteins (Bredemeier et al., 2006). However, the physical connection between N- and C-terminal domains still guarantees the regulation of the pore forming region.

Summarizing, Omp85 proteins evolved early in cellular development, but not as early as β -barrel proteins. The scenario for the development of this protein family suggests a membrane-inserted protein with 8 β -strands and a periplasmic protein as origin. The subsequent development of this family is determined by the early branching between Cyanobacteria and Proteobacteria about 2.8 Gy ago. During this time, two different modes of action have evolved, one (proteobacterial) without channel function and another (cyanobacterial) with a large channel. However, whether the channel is involved in the mode of action of the bacterial protein remains elusive, but the inherent channel property was the prerequisite to become the protein importer in plant chloroplasts.

5. Conclusions and Outlook

The evolutionarily "early" developed polypeptide-transporting β -barrel proteins of the Omp85 family are involved in outer membrane protein assembly or protein translocation. The protein is possibly derived from a heterodimeric assembly of an initial β -barrel protein which was gated by a periplasmically localized subunit. However, Omp85 development took different routes as the cyanobacterial proteins clearly show different features when compared to proteins found in proteobacteria. This might be the reason why Omp85 forms the central component of the polypeptide transporting machinery in the chloroplast, but not in mitochondria. The protein itself was the initial translocation complex after cyanobacteria where "enslaved" by the host cell. However, the specificity of the prokaryotic signal was not sufficient to discriminate prokaryotes to be targeted from those to be rejected. Hence, additional components evolved which recognize more specifically the incoming precursor proteins. These

components also altered some features of the Omp85 like proteins, e.g. the complex architecture. Nevertheless, it becomes obvious that the transfer of genes into the host nucleus and their subsequent import was only possible because preexisting outer membrane proteins were recycled into new import proteins. Therefore, the identification of bacterial homologues in the protein import machinery of cellular organelles solves a long-standing question in the endosymbiotic process.

Several questions have still to be addressed in future. Some cyanobacteria and plants have more than one Omp85 like protein. So far it is unknown what the different specificities of these proteins might be. Here, it has to be investigated whether some of the plant isoforms still show selectivity for the phenylalanine signal. To understand the protein insertion into the outer membranes of endosymbiotically derived organelles, it will be important to dissect, which of the Omp85 homologues of the outer membrane of chloroplasts is indeed involved in the assembly of β -barrel outer membrane proteins. Furthermore, for the chaperoning Omp85's mode of action has to be explored because it is unlikely that the transfer of the outer membrane proteins out of the pore into the bilayer occurs by lateral diffusion. A lateral opening of the barrel would require the destruction of multiple hydrogen bonds, which would be energetically unfavorable.

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