Review

Two evolutionarily conserved essential β-barrel proteins in the chloroplast outer envelope membrane

Shih-Chi Hsu, Kentaro Inoue*

Department of Plant Sciences, University of California, Davis, CA, USA.

Summary **Chloroplasts are organelles specific to photosynthetic eukaryotes that support the lives of most organisms on earth. Chloroplasts were derived from an ancient cyanobacterium by endosymbiosis, and one characteristic shared between them and extant cyanobacteria is the presence of β-barrel proteins in the outer membrane. These integral membrane proteins are also found in the outer membranes of proteobacteria and mitochondria. In particular, a group of homologous β-barrel proteins called BamA homologs are present in all Gram-negative bacteria and the endosymbiotic organelles, *i.e.*, chloroplasts and mitochondria. It was recently revealed that, in both proteobacteria and mitochondria, there is a single essential BamA homolog that mediates β-barrel protein assembly. In a chloroplast, there are two distinct BamA homologs, Toc75 and OEP80, which diverged early in the evolution of chloroplasts from their common ancestor with extant cyanobacteria. Recent genetic studies demonstrated that each of these proteins is indispensable for viability of plants although neither has been shown to be involved in β-barrel protein assembly. Toc75 catalyzes import of nuclear-encoded precursor proteins, a process that is not required for bacteria, whereas the molecular function of OEP80 remains elusive. Establishment of a protein import apparatus was required to facilitate the transfer of genes from the endosymbiont to the host cell nucleus. Hence, we propose that the gene duplication giving rise to the two essential BamA homologs was a prerequisite for the successful conversion of the cyanobacterial endosymbiont into the chloroplast. Consequently, continued study of these two chloroplast proteins should advance our understanding of endosymbiosis and evolutionarily conserved proteins in general.**

Keywords: β-barrel membrane proteins, chloroplast outer envelope membrane, endosymbiosis, OEP80, Toc75

1. Introduction

Oxidative photosynthesis supports the lives of virtually all organisms on earth. In eukaryotes, this reaction takes place in the chloroplast, the organelle specific to photosynthetic protists (eukaryotic algae) and plants. In higher plants, chloroplasts are further integrated into the development of organisms by differentiating into various inter-convertible non-photosynthetic plastid types, such as chromoplasts in red and orange fruit and floral petals, and amyloplasts in root tips (*1*). In addition to photosynthesis, plastids perform many functions essential for normal growth and development of plants. These include assimilation of nitrogen and sulfur, biosynthesis of amino acids, fatty acids, carotenoids, tocopherols, and precursors of plant growth regulators such as abscisic acid and gibberellins (*2-5*), and gravity sensing (*6*). A number of genes encoding enzymes responsible for chloroplast metabolism have been

**Address correspondence to:*
Dr. Kentaro Inoue, Department of Plant Sciences, 131 Asmundson Hall, University of California, One Shields Avenue, Davis, CA 95616, USA.
e-mail: kinoue@ucdavis.edu

identified and characterized. These achievements have made possible genetic engineering of crop plants that produce high amounts of compounds beneficial for human nutrition (*2*). In addition, the knowledge of biochemical processes in chloroplasts has been used to develop and test a computational model for a metabolic network (*7*).

Chloroplasts originated from an ancestral cyanobacterium, which was engulfed by a primitive mitochondriate eukaryotic cell about a billion years ago (*8*). This event, called primary endosymbiosis, gave rise to three major autotrophic lineages, Glaucophyta, Rhodophyta (red algae) and Viridiplantae (green algae and land plants), all of which contain chloroplasts surrounded by an envelope consisting of two distinct membranes (*9-11*). The outer membrane of the chloroplast envelope used to be considered as a remnant of the phagosomal membrane of the eukaryotic host (*12*). However, the presence of galactolipids and β-barrel proteins, a feature shared with membranes of extant cyanobacteria but not with the eukaryotic endomembrane systems, supports the prokaryotic origin of the chloroplast outer membrane (*13,14*). Chloroplasts spread into other protist lineages, too, such as diatoms, which play a major role in net primary oxygen production in the ocean, through multiple eukaryote-eukaryote (secondary and tertiary) endosymbioses (*15*). A secondary endosymbiotic event also gave rise to apicoplasts, organelles that lost photosynthetic capacity but still play important roles in viability of protozoan parasites (*16*). Hence, the chloroplast provides footprints of evolutionary events relevant to global energy production and human disease.

The presence of two distinct membranes, the outer and inner membranes, is a feature shared not only by cyanobacteria and chloroplasts, but also by all the other Gram-negative bacteria and mitochondria of eukaryotic cells. Furthermore, β-barrel membrane proteins are found exclusively in the outer membranes of these bacteria and the endosymbiotically derived organelles. Consequently, addressing questions about the chloroplast outer envelope should provide insights into the functions and evolution of various membrane systems. In this review, we intend to highlight a limited but interesting feature of the outer envelope of higher plant chloroplasts, which has generally been under-examined compared to its counterparts in bacteria and mitochondria. We propose that two chloroplast β-barrel membrane proteins, which belong to an evolutionarily conserved group of proteins in Gram-negative bacteria and the endosymbiotic organelles, played key roles in the successful conversion of the free-living prokaryote into the organelle. This review aims to emphasize the relevance of the study of these proteins for understanding endosymbiosis and the evolutionary event in general.

2. The outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts

2.1. *Conserved and divergent properties of the outer membranes*

The outer and inner membranes of Gram-negative bacteria and the endosymbiotic organelles function as a physical barrier that separates two aqueous compartments, *i.e.*, the inside (the cytoplasm for the bacteria, the matrix for mitochondria, and the stroma for chloroplasts) and the outside (extracellular space for the bacteria, and the cytoplasm for mitochondria and chloroplasts), and catalyze communications between them. These two membranes also facilitate metabolic compartmentalization by providing an aqueous space in between, *i.e.*, the periplasm in the bacteria and the intermembrane space in mitochondria and chloroplasts. A number of features are conserved among the outer membranes of Gram-negative bacteria and the endosymbiotic organelles. However, these membranes have also evolved many distinct properties (Table 1).

It has been postulated that immediately after the engulfment by the host cell, the eubacterial endosymbionts were surrounded by a phagosomal membrane in addition to their own outer and inner membranes. This host-derived outermost membrane, however, quickly disappeared (*9*), and the remaining two lipid bilayers started evolving to adapt to the new environment (*17*). One of the significant changes in these two membranes was the establishment of protein import apparatus. This was required by the endosymbiont to complete gene transfer to the host nucleus, which is essential for its successful conversion to an organelle. Endosymbiotic gene transfer consists of i) duplication and transfer of genes in the endosymbiont to the host nucleus, ii) expression of the transferred genes in the nucleus, iii) targeting of the nuclear-encoded proteins back to its original location in the endosymbiont, and iv) loss of the genes from the endosymbiont. Consequently, most proteins currently found in chloroplasts and mitochondria are encoded in the nuclear genome, although each of these organelles still carries its own genome (*18*). In addition to protein import apparatus, the endosymbiont had to develop various machineries at the surrounding membranes to exchange numerous metabolites and solutes in order to integrate their metabolic processes into that of the host cell. While bacterial outer membranes play roles in symbiotic and/or pathogenic interactions with host cells (*19,20)*, their counterparts in the endosymbiotic organelles play significant roles in other processes, such as organelle division, movement, and the biosynthesis of membrane lipids (*14,21,22*). Recent studies have also demonstrated the role of the mitochondrial outer membrane in apoptosis

Table 1. Comparisons of the outer membranes of Gram-negative bacteria and the endosymbiotic organelles*

	Gram-negative bacteria		Endosymbiotic organelles	
	Proteobacteria**	Cyanobacteria	Mitochondria	Chloroplasts
Peptidoglycan layer	~6 nm[a]	12 nm[b]	None	None (except cyanelles)
Exclusion limit	~0.6 kD[c], ~6 kD[d]	~2 kD[e]	~3.4-6.8 kD[f]	~10 kD[g]
Protein content (wt%)	50[h]	30[i]	60[j]	25-30[k]
Number of integral membrane proteins identified/predicted***	42 (β-barrel) + 1 (α-helical)[l]	17 (β-barrel)[m]	4 (β-barrel) + 28 (α-helical)[n]	8 (β-barrel) + 26 (α-helical)[o]
Major non-protein components****	Lipopolysaccharides Phospholipids	Lipopolysaccarides Phospholipids	Phospholipids	Phospholipids Galactolipids
Functions*****	• Permeability barrier • Passive and active solute transport • Defense • Symbiotic and/or pathogenic interaction with host cells	• Permeability barrier • Passive and active solute transport • Symbiotic interaction with host cells	• Permeability barrier • Protein import • Solute transport (passive) • Biosynthesis of lipids, nicotinic acid, cysteine, erythroascorbic acid • Regulation of organelle morphology (shape, fission, and fusion) • Apoptosis	• Permeability barrier • Protein import • Biosynthesis of membrane lipids • Organelle division • Anchorage to the plasma membrane • Organelle movement • Sugar signaling

*The listed data are from the following species: [a], *Escherichia coli* (*102*); [b], *Synechocystis sp*. PCC 6714 (*103*); [c], *E. coli* (*104*); [d], *Pseudomonas aeruginosa* (*105*); [e], *Anabaena variabilis* (*106*); [f], *Neurospora crassa* (*107*); [g], *Spinacia oleracea* (spinach) (*108*); [h], *Salmonella typhimurium* (*109*); [i], *Synechocystis sp*. PCC6714 (*110*); [j], *Saccharomyces cerevisiae* (*111*); [k], *S. oleracea* (*112*); [l], *E. coli* (*113,114*, the White lab website (*http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html*)]; [m], *Anabaena sp*. strain PCC 7120 (*115*); [n], *N. crassa* (*22*); [o], *A. thaliana* (*14*); the Inoue lab website (*http://www.plantsciences.ucdavis.edu/kinoue/OM.htm*)]; **This phylum includes *Alphaproteobacteria*, the closest prokaryotic homologs of mitochondria (*116*); ***These should be underestimated numbers; ****These are in *E. coli* (*109,117*) for proteobacteria; *Synechocystis sp*. PCC6714 (*110*) for cyanobaceria; *N. crassa*, rat liver (*118*), and *S. Cerevisiae* (*111*) for mitochondria; *S. oleracea* (*21*) for chloroplasts. Galactolipids have been found in the inner but not in the outer membranes of extant cyanobacteria (*110,119*), whereas they are present in both membranes of chloroplasts (*112*). Hence, galactolipids in the chloroplast outer membrane may have been recruited from the inner membrane of the cyanobacterial endosymbiont; *****See text for appropriate references.

(*23*), and that of the chloroplast outer membrane in sugar-sensing (*24*). Indeed, the composition of integral membrane proteins has diverged significantly between the outer membranes of the extant bacteria and those of the endosymbiotic organelles (Table 1). These proteins in the organelles must have evolved either from those already present in the endosymbiont, or from gene products of the host cell (see section 3.1. for protein import components as examples).

2.2. *Beta-Barrel proteins – the common constituents of the outer membranes of Gram-negative bacteria and the endosymbiotic organelles*

One of the common features shared by Gram-negative bacteria, mitochondria, and chloroplasts is the presence of β-barrel proteins in the outer membrane (Table 1). These integral membrane proteins are postulated to form pores consisting of multiple transmembrane β-strands, which are laterally hydrogen-bonded in a circular pattern (*25-27*). A number of bacterial β-barrel proteins have been shown to use these hydrophilic pores directly to transport various solutes, metabolites and proteins (*20*). Furthermore, in some cases, these transmembrane structures appear to function as a membrane anchorage to support the soluble catalytic sites located in *cis*, as seen in OmpLA (*28*) and OmpT (*29*), and may also provide physical integrity of the lipid bilayer, as seen in OmpA (*20*). Three-dimensional structures of a number of bacterial β-barrel proteins, which consists of even numbers (8 to 24) of transmembrane β-strands, have been demonstrated by X-ray crystal analyses (*30,31*). In addition, electron microscopic studies have shown pores formed by putative β-barrel membrane proteins including those from mitochondria (*32,33*). Using these data, a number of hidden Markov model prediction programs have been developed to examine the presence of transmembrane β-strands in a given protein (*34-36*). Recently, three independent groups reported the first three-dimensional structures of non-bacterial β-barrel membrane proteins, VDACs (Voltage-Dependent Anion Channels), from human and mouse mitochondria (*37-39*). Unlike bacterial proteins, these mammalian proteins consist of an odd number of transmembrane β-strands. Interestingly, however, their *N*-terminal α-helical domain was found to bind to the β-barrel, orienting both the *N*- and *C*-termini towards the space between the outer and inner membranes, similar to the topology of the bacterial β-barrel proteins (*25*). Finally, there is no report on the crystal structure of any chloroplast β-barrel membrane proteins yet, although some of them can be predicted to contain an even number (8 to 16) of transmembrane β-strands by the programs such as PRED-TMBB (*34*) and PROFtmb (*35*), which are designed for

bacterial β-barrel membrane proteins (Inoue and Hsu, unpublished).

In *Escherichia coli*, 42 proteins have been found and/or predicted to integrate into lipid bilayers with multiple β-strands, whereas only a handful of β-barrel proteins have been identified in mitochondria and chloroplasts (Table 1) (*14,40*). Quite interestingly, although the proteins in the endosymbiotic organelles may have evolved from bacterial ancestors, only BamA homologs (see below) show apparent sequence similarities to prokaryotic proteins (*41*). Furthermore, the outer membranes of both mitochondria and chloroplasts are enriched with proteins with α-helical transmembrane domains, which seem to be very rare in the bacterial outer membranes (Table 1). Nonetheless, the major integral constituents of the outer membranes of mitochondria and chloroplasts are represented by β-barrel proteins, VDAC (*42*) and Toc75 (*43*), respectively. Hence, the importance of β-barrel proteins in the outer membranes appears to be conserved between the bacteria and the organelles.

3. Two essential β-barrel proteins in the chloroplast outer envelope – Toc75 and OEP80

3.1. *Chloroplast protein import*

Currently, most nuclear-encoded proteins targeted to the interior of chloroplasts are synthesized by cytoplasmic ribosomes with an *N*-terminal extension called a transit peptide. Extensive biochemical and genetic studies have identified multiple proteinaceous components involved in the transit peptide-dependent import of these precursor proteins at the chloroplast envelope membranes, and they are designated as Toc and Tic (Translocon at the outer- and the inner-envelope-membranes of chloroplasts) proteins (*44-51*). How did the pre-organelle establish these protein import machineries? The presence of apparent homologs in extant cyanobacteria suggests that some components evolved from proteins in the eubacterial endosymbiont, whereas other proteins may have been recruited from the host eukaryote (*52*). Some non-essential components may have adopted multi-functionality during evolution (*17*). Overall, however, the mechanism by which the protein import machinery was established remains largely unexplored, mainly due to the lack of appropriate tools.

3.2. *The protein translocation channel in the chloroplast outer envelope, Toc75*

As described in section 3.4., one of the chloroplast protein import components that was derived from prokaryotic proteins is the major β-barrel outer membrane protein Toc75, originally identified in seedlings of pea (*Pisum sativum*). Chemical cross-linking assays (*53,54*) and reconstitution into liposomes (*55,56*) have established that Toc75 is a major protein translocation pore. It forms a hetero-oligomeric complex in the outer membrane with two homologous GTPases, Toc159 and Toc34, which expose their large *N*-terminal portions to the cytoplasmic surface (*57*). Toc34 has been shown to be anchored to the membrane with a transmembrane α-helical domain, whereas the exact conformation of the transmembrane domain in Toc159 is not completely understood (*46*). Toc75 also plays a role in the insertion of a signal-anchored α-helical outer membrane protein, OEP14, which does not carry a transit peptide (*58*). Because Toc75 itself is encoded in the nuclear genome, it also has to be targeted to the organelle posttranslationally and inserted into the membrane. It is intriguing that unlike other outer membrane proteins in chloroplasts and mitochondria that do not need cleavable targeting sequences, Toc75 requires a transit peptide, which consists of two parts and is removed by two steps, for its correct targeting (*59-63*). In particular, Toc75 depends for its complete maturation on a membrane-bound protein called Plsp1 (plastidic type I signal peptidase 1) (*64,65*). Interestingly, similar to Toc75, Plsp1 appears to have derived from a protein in a cyanobacterial ancestor (*66*).

3.3. *A distinct paralog of Toc75 in chloroplasts, OEP80*

There are four genes in the genome of the model plant *Arabidopsis thaliana* that encodes apparent Toc75 homologs (*67,68*). Among them, the one on chromosome I was shown to be a pseudo-gene (*69*). The protein encoded on chromosome III is the sole functional Toc75, which contains the unique bipartite transit peptide (*61*), and is essential for plant viability as its gene knockout disrupted embryo development as early as at the two-cell stage (*69,70*). By contrast, functions of the other two Toc75 paralogs remain largely elusive. The protein encoded on chromosome IV is a truncated form of Toc75 without the N-terminus. Its gene knockout caused slight abnormalities in the structure of non-photosynthetic plastids, but did not significantly disrupt normal plant growth (*69*). The paralog encoded on chromosome V of *A. thaliana* was annotated to encode an 80 kDa protein, which shows only 22% sequence identity to Toc75. A pea ortholog of this protein, which was named Toc75-V, appears to be 66 kDa and was not found in Toc complexes prepared by sucrose-gradient centrifugation (*67*). Later immunoblotting results using an antibody against part of the deduced sequence and *in vitro* import data suggested that the size of this Toc75 paralog may be similar to that of the precursor, 80 kDa (*71*). This protein from *A. thaliana*, which was named OEP80 for outer envelope protein 80, does not require an *N*-terminal cleavable bipartite transit peptide as does

Toc75 for targeting to chloroplasts (*71*). More recently, disruption of the OEP80 gene was shown to cause embryo abortion in *A. thaliana* at a stage later than that affected by *TOC75* knockout (*72*). This indicates that, while both Toc75 and OEP80 are essential for viability of plants from very early stages of development, they probably have distinct functions (*73*).

3.4. *The evolutionary origin of Toc75*

The prokaryotic origin of Toc75 was first suggested in the late 1990s, when a gene encoding an apparent Toc75 homolog was found in the cyanobacterium *Synechocystis* sp. PCC6803 (*74,75*). The encoded protein Slr1227 was localized in the bacterial outer membrane and could be reconstituted as a voltage-gated channel in artificial liposomes (*74*). A genetic study demonstrated that Slr1227 is essential for cell viability (*75*) although its exact function remains unknown.

Interestingly, proteins homologous to Toc75 are found not only in cyanobacteria, but also in a wide range of Gram-negative bacteria and mitochondria. These homologs include surface antigens named D15 and TpsB transporters of two partner secretion systems (*52*). The conserved features of these proteins include an *N*-terminal soluble portion and a *C*-terminal transmembrane region consisting of 10 to 16 β-strands in the predicted structure (Figure 1) (*76*). The soluble portion contains one to five polypeptide translocation associated (POTRA) domains, which were also found in the *N*-terminal regions of FtsQ/DivIB bacterial division protein family (*77*). The initial prediction suggested that a typical POTRA domain consists of 70 to 90 amino acids, containing three β-strands and two α-helices (*77*). Recent structural studies have revealed the core βααββ motif of a POTRA domain, in which two α-helices are packed against a three-strand mixed β-sheet (*78-81*). The number of POTRA domains varies: three each for homologs from chloroplasts and cyanobacteria, one for those from mitochondria, and one to five for the Omp85 homologs from proteobacteria including *Alphaproteobacteria*, which are believed to share the common ancestor with mitochondria (Figure 1) (*82*). In the case of a subset of proteobacterial and mitochondrial proteins, the *N*-terminal portion containing the POTRA domains is postulated to exist in the space between the outer and inner membranes. This model is supported by extensive sequence comparisons between various putative homologs (*83*), proteolytic fragmentations of the proteins in the intact cells or organelles (*32,79,84*), reconstitution assays (*85*), and *in vitro* association of the POTRA domains with lipoproteins located in

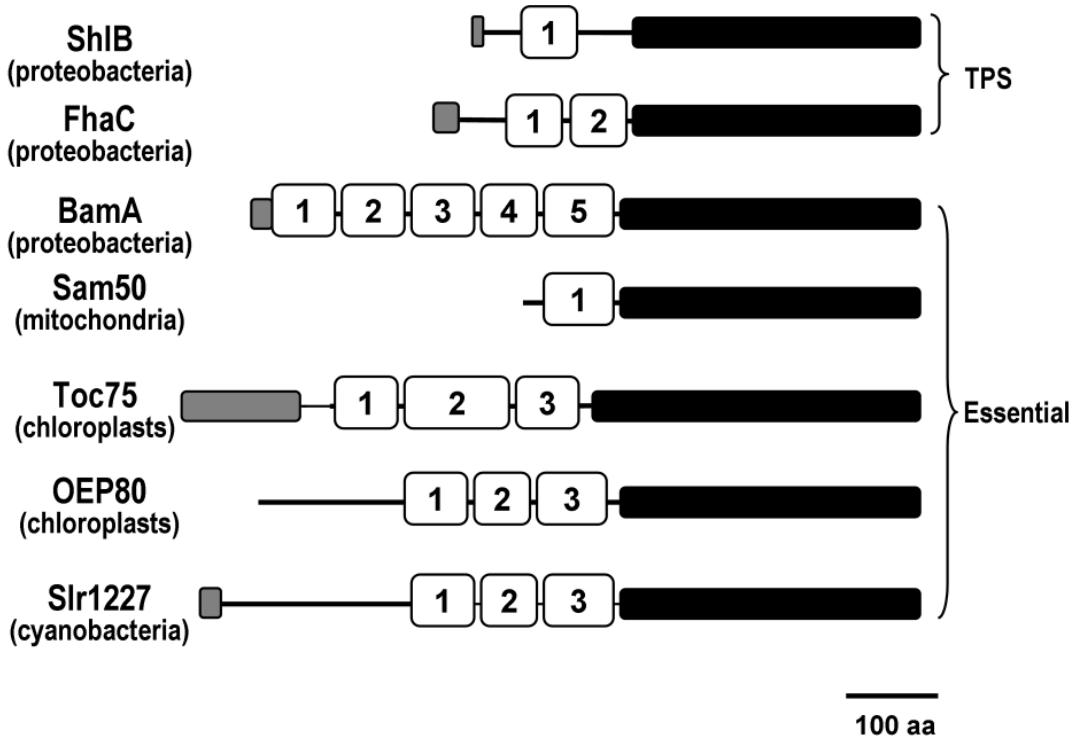

Figure 1. Schematic representation of the domain architectures of representative BamA homologs. The proteins are drawn approximately to scale. The signal peptides of four bacterial proteins (ShlB, FhaC, BamA and Slr1227) and the bipartite transit peptide of Toc75 are indicated with gray bars. POTRA and β-barrel transmembrane domains are indicated with white (with numberings) and black bars, respectively. ShlB and FhaC play a role in two-partner secretion (TPS) in proteobacteria, *Serratia marcescens* and *Bordetella bronchiseptica*, respectively (*100*), and the latter protein was shown to be dispensable for viability of the bacteria (*101*). The structures of FhaC (full sequence) and BamA (the first four POTRA domains) were confirmed by crystallography (*78,80*). Models for other structures are based on publications (*40,77*).

the periplasmic side of the bacterial outer membrane (*80*). By contrast, topologies of the homologs in chloroplasts and cyanobacteria have been less defined. Indeed, an initial model of Toc75 included the putative POTRA domains in the membrane-anchored region consisting of 16 β-strands, based on secondary structure prediction and proteolytic fragmentation patterns of proteins reconstituted into liposomes (*86*). Recently, however, a distal *C*-terminal part of Toc75 by itself was shown to form a pore *in vitro*, and a new model was presented, in which the predicted POTRA domains were included in the "*N*-terminal soluble domain" apart from newly-assigned 16 transmembrane β-strands (*87,88*).

Because protein import is not an essential process for free-living prokaryotes, it is postulated that Toc75 evolved from an ancestral cyanobacterial protein whose function was modified during endosymbiosis (*52*). A detailed phylogenetic analysis indicated that Toc75 and OEP80 diverged early in the evolution of chloroplasts from their common ancestor with extant cyanobacteria (*71*). Thus, it may be possible that both Toc75 and OEP80 evolved to take on distinct functions, which are essential for organelle development but are distinct from that of the bacterial protein. Another possibility is that OEP80 has retained the function of the ancestral protein which is essential for viability of both bacteria and chloroplasts, whereas Toc75 acquired a new role in order to facilitate the conversion of the endosymbiont to the organelle (*73*).

4. BamA and its homologs – evolutionarily conserved essential proteins in Gram-negative bacteria, mitochondria and chloroplasts

4.1. *Mechanism of β-barrel protein sorting in proteobacteria and mitochondria*

In Gram-negative bacteria, all of the β-barrel membrane proteins are synthesized in the cytoplasm, targeted first to the plasma membrane, then to the periplasm before getting sorted into the outer membrane. Although they can spontaneously be inserted and assembled in artificial liposomes (*89*), involvement of proteinaceous machinery in their targeting *in vivo* had been predicted because their insertion is unidirectional and specific to the outer membrane. Nonetheless, the mechanism of β-barrel protein insertion remained unknown until a breakthrough was made in 2003, when one of the D15-related proteins called Omp85 (Outer membrane protein 85) that has five POTRA domains was found to be responsible for accumulation of β-barrel proteins in the outer membrane of a Gram-negative bacterium, *Neisseria meningitidis* (*83*). Omp85 was shown to be part of a high molecular weight oligomeric complex of unknown composition. Later, an Omp85 homolog in *Escherichia coli* called YaeT was also shown to play an essential role in accumulation of outer membrane proteins, and to form a hetero-oligomeric complex with lipoproteins, YfgL, YfiO, NlpB, and SmpA (*90,91*). The Omp85 homolog and other four complex partners in *E. coli* were later renamed as BamA and BamB-E, respectively, for β-barrel assembly machine proteins A-E (*92*).

In contrast to their bacterial counterparts, all the β-barrel proteins in mitochondria are synthesized outside the organelle, and first traverse the outer membrane. However, they are eventually inserted into the lipid bilayers from the space between the outer and inner membranes (*93*). Following the discovery of the function of Omp85 in *N. meningitidis*, the evolutionarily conserved β-barrel protein sorting to the mitochondrial outer membrane was found to be catalyzed by novel eukaryotic BamA homologs, Tob55 (Topogenesis of mitochondrial outer membrane β-barrel proteins 55) from *Neurospora crassa* and Sam50 (Sorting and assembly machinery 50) from *Saccharomyces cerevisiae* (*32,94,95*). Similar to the bacterial homologs, both Tob55 and Sam50 are also part of a multi-subunit protein complex (*41*).

Recent studies have also revealed species-specific features of β-barrel protein sorting. First, all the essential proteobacterial BamA homologs have five, whereas mitochondrial counterparts have only one POTRA domain (Figure 1). Second, some of the subunits that form a complex with the BamA homologs appear to be unique to an individual species (*96*). Third, bacterial β-barrel precursor proteins contain species-specific *C*-terminal signal sequences recognized by bacterial BamA homologs (*97*), whereas each mitochondrial substrate contains a specific sorting signal that is recognized by one of the Sam50-interacting partners, Sam35, which is unique to this organelle (*40*). Finally, the complex containing Sam50 mediates integration of not only β-barrel, but also α-helical proteins in the outer membrane of yeast mitochondria (*98*), whereas there has been no report on such an activity in bacterial BamA homologs.

In summary, bacteria and mitochondria share a homologous mechanism of β-barrel protein assembly, but the similarity is limited to the core subunits (BamA homologs), while the sorting signal and partner proteins are distinct.

4.2. *Functions of POTRA domains in BamA homologs*

Recent genetic, biochemical and structural studies have also revealed the possible species-specific functions of the POTRA domains of BamA homologs. For the bacterial proteins, Kim *et al*. showed *in vivo* that the three *C*-terminal domains (POTRA3, 4, and 5) are indispensable for the function of BamA, and also that all but POTRA1 are necessary for

interaction of BamA with one of oligomeric partners, BamB (YfgL) (*80*). Furthermore, three-dimensional structures resolved by X-ray crystallography and NMR indicated that the POTRA functions as a scaffold for either substrate proteins or interacting partners by β-augmentation (*79-81*). Interestingly, in contrast to the case with the *E. coli* homolog, only the most *C*-terminal domain (POTRA5) was found to be indispensable for the function of the *N. meningitidis* Omp85 *in vivo* (*99*). As for the mitochondrial homologs that contain only one POTRA domain, a genetic study showed that this domain is not required for the proper function of the protein *in vivo* (*40*). Interestingly, however, deletion of part of the *N*-terminus of the POTRA domain, which left its *C*-terminal 18 residues containing the last β-strand intact, disrupted the function of Tob55/Sam50, but not its complex formation, causing a growth defect in yeast (*40,84*). In addition, Habib *et al*. showed *in vitro* that the sequence containing the entire POTRA domain could specifically bind to the mitochondrial β-barrel protein VDAC and inhibit its import into the organelle *in vitro* (*84*). This result indicates that the POTRA domain of the mitochondrial BamA homolog may also have a chaperone-like activity.

4.3. *BamA homologs in chloroplasts*

Among the two BamA homologs, Toc75 functions as a conducting channel for various cytoplasmically synthesized precursor proteins. Hence, it is tempting to speculate that another homolog, OEP80, plays an evolutionarily conserved role, which is to sort β-barrel proteins into the chloroplast outer membrane. Currently, however, there is no experimental evidence to support this idea. Indeed, we know almost nothing about the mechanism of β-barrel protein insertion into the chloroplast outer membrane. Nonetheless, several biochemical studies suggested the conserved chaperone-like functions of the POTRA domains of Toc75 and its cyanobacterial homolog (*87,88*). It is also worth mentioning that no gene product has been identified in *A. thaliana* chloroplasts that are similar to any proteins forming the complexes with BamA homologs in proteobacteria and mitochondria (Hsu and Inoue, unpublished).

5. Conclusions

Chloroplasts of higher plants and some algae play vital roles in survival of most organisms on earth. These organelles have also served as systems that allow us to address various biochemical and evolutionary questions. The presence of BamA homologs in the outer membranes of proteobacteria, cyanobacteria, mitochondria, and chloroplasts reflects the common evolutionary origin of these membranes. This idea is further supported by recent findings of the conserved β-barrel protein-sorting pathway mediated by BamA homologs in proteobacteria and mitochondria, although this has not been demonstrated in cyanobacteria and chloroplasts yet. There appears to be one single essential BamA homolog each in Gram-negative bacteria and mitochondria, whereas two distinct homologs are present in chloroplasts. One of them, Toc75, was identified as the main component of the protein import machinery in the 1990s, which is long before the proliferation of studies of the bacterial and mitochondrial homologs began in 2003. Another chloroplast BamA homolog, OEP80, which was first reported in 2002, is essential for viability of organisms and is targeted from the cytoplasm to chloroplasts by a mechanism distinct from that used by Toc75. Although the molecular function of OEP80 remains unknown, the presence of two essential BamA homologs is a feature unique to the chloroplast outer envelope among the evolutionarily conserved biological membrane systems. The obvious question is if Toc75 and/or OEP80 play a role in β-barrel protein sorting, similar to their homologs in proteobacteria and mitochondria. Another question is if Toc75 and OEP80, as they are β-barrel proteins themselves, share the same mechanism of insertion into the outer membrane. Addressing these questions should not only define the properties of evolutionarily conserved membrane systems, but also advance our understanding of mechanisms underlying the endosymbiotic event.

Acknowledgments

We thank Dr. Daniel Potter and an anonymous reviewer for their critical and useful comments on the manuscript. The work in the Inoue laboratory on evolutionarily conserved Plsp1 and its homologs has been funded by Energy Biosciences Program at the US Department of Energy DE-FG02-08ER15963.

References

1. Waters M, Pyke K. Plastid development and differentiation. Plastids. Annual Plant Reviews, ed S.G. M (Blackwell, Oxford), Vol 13, 2005; pp. 30-59.
2. DellaPenna D, Pogson BJ. Vitamin synthesis in plants: tocopherols and carotenoids. Annu Rev Plant Biol. 2006; 57:711-738.
3. Neuhaus HE, Emes MJ. Nonphotosynthetic metabolism in plastids. Annu Rev Plant Physiol Plant Mol Biol. 2000; 51:111-140.
4. Seo M, Koshiba T. Complex regulation of ABA biosynthesis in plants. Trends Plant Sci. 2002; 7:41-48.
5. Yamaguchi S, Kamiya Y. Gibberellin biosynthesis: its regulation by endogenous and environmental signals. Plant Cell Physiol. 2000; 41:251-257.
6. Morita MT, Tasaka M. Gravity sensing and signaling. Curr Opin Plant Biol. 2004; 7:712-718.

7. Poolman MG, Sebu C, Pldcock MK, Fell DA. Modular decomposition of metabolic systems *via* null-space analysis. J Theor Biol. 2007; 249:691-705.
8. McFadden GI. Endosymbiosis and evolution of the plant cell. Curr Opin Plant Biol. 1999; 2:513-519.
9. Cavalier-Smith T. Membrane heredity and early chloroplast evolution. Trends Plant Sci. 2000; 5:174-182.
10. Gould SB, Waller RF, McFadden GI. Plastid evolution. Annu Rev Plant Biol. 2008; 59:491-517.
11. Reyes-Prieto A, Weber AP, Bhattacharya D. The origin and establishment of the plastid in algae and plants. Annu Rev Genet. 2007; 41:147-168.
12. Whatley JM, Whatley RF. Chloroplast evolution. New Phytol. 1981; 87:233-247.
13. Joyard J, Block MA, Douce R. Molecular aspects of plastid envelope biochemistry. Eur J Biochem. 1991; 199:489-509.
14. Inoue K. The chloroplast outer envelope membrane: The edge of light and excitement. J Integr Plant Biol. 2007; 49:1100-1111.
15. Bowler C, Karl DM, Colwell RR. Microbial oceanography in a sea of opportunity. Nature. 2009; 459:180-184.
16. McFadden GI, Reith ME, Munholland J, Lang-Unnasch N. Plastid in human parasites. Nature. 1996; 381:482.
17. Gross J, Bhattacharya D. Revaluating the evolution of the Toc and Tic protein translocons. Trends Plant Sci. 2009; 14:13-20.
18. Martin W, Herrmann RG. Gene transfer from organelles to the nucleus: how much, what happens, and Why? Plant Physiol. 1998; 118:9-17.
19. Tseng TT, Tyler BM, Setubal JC. Protein secretion systems in bacterial-host associations, and their description in the Gene Ontology. BMC Microbiol. 2009; 9 Suppl 1:S2.
20. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003; 67:593-656.
21. Block M, Douce R, Joyard J, Rolland N. Chloroplast envelope membranes: a dynamic interface between plastids and the cytosol. Photosynth Res. 2007; 92:225-244.
22. Schmitt S, Prokisch H, Schlunck T, Camp DG 2nd, Ahting U, Waizenegger T, Scharfe C, Meitinger T, Imhof A, Neupert W, Oefner PJ, Rapaport D. Proteome analysis of mitochondrial outer membrane from *Neurospora crassa*. Proteomics. 2006; 6:72-80.
23. Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends Cell Biol. 2008; 18:157-164.
24. Huang J, Taylor JP, Chen JG, Uhrig JF, Schnell DJ, Nakagawa T, Korth KL, Jones AM. The plastid protein THYLAKOID FORMATION1 and the plasma membrane G-protein GPA1 interact in a novel sugar-signaling mechanism in Arabidopsis. Plant Cell. 2006; 18:1226-1238.
25. Schulz GE. beta-Barrel membrane proteins. Curr Opin Struct Biol. 2000; 10:443-447.
26. Tamm LK, Hong H, Liang B. Folding and assembly of beta-barrel membrane proteins. Biochim Biophys Acta. 2004; 1666:250-263.
27. Wimley WC. The versatile β-barrel membrane protein. Curr Opin Struct Biol. 2003; 13:404-411.
28. Snijder HJ, Dijkstra BW. Bacterial phospholipase A: structure and function of an integral membrane phospholipase. Biochim Biophys Acta. 2000; 1488:91-101.
29. Vandeputte-Rutten L, Kramer RA, Kroon J, Dekker N, Egmond MR, Gros P. Crystal structure of the outer membrane protease OmpT from *Escherichia coli* suggests a novel catalytic site. EMBO J. 2001; 20:5033-5039.
30. Galdiero S, Galdiero M, Pedone C. beta-Barrel membrane bacterial proteins: structure, function, assembly and interaction with lipids. Curr Protein Pept Sci. 2007; 8:63-82.
31. Remaut H, Tang C, Henderson NS, Pinkner JS, Wang T, Hultgren SJ, Thanassi DG, Waksman G, Li H. Fiber formation across the bacterial outer membrane by the chaperone/usher pathway. Cell. 2008; 133:640-652
32. Paschen SA, Waizenegger T, Stan T, Preuss M, Cyrklaff M, Hell K, Rapaport D, Neupert W. Evolutionary conservation of biogenesis of β-barrel membrane proteins. Nature. 2003; 426:862-866.
33. Mannella CA. Structure of the outer mitochondrial membrane: ordered arrays of porelike subunits in outer-membrane fractions from *Neurospora crassa* mitochondria. J Cell Biol. 1982; 94:680-687.
34. Bagos PG, Liakopoulos TD, Spyropoulos IC, Hamodrakas SJ. PRED-TMBB: a web server for predicting the topology of beta-barrel outer membrane proteins. Nucleic Acids Res. 2004; 32:W400-W404.
35. Bigelow HR, Petrey DS, Liu J, Przybylski D, Rost B. Predicting transmembrane beta-barrels in proteomes. Nucleic Acids Res. 2004; 32:2566-2577.
36. Waldispuhl J, Berger B, Clote P, Steyaert JM. transFold: a web server for predicting the structure and residue contacts of transmembrane beta-barrels. Nucleic Acids Res. 2006; 34:W189-W193.
37. Bayrhuber M, Meins T, Habeck M, Becker S, Giller K, Villinger S, Vonrhein C, Griesinger C, Zweckstetter M, Zeth K. Structure of the human voltage-dependent anion channel. Proc Natl Acad Sci U S A. 2008; 105:15370-15375.
38. Hiller S, Garces RG, Malia TJ, Orekhov VY, Colombini M, Wagner G. Solution structure of the integral human membrane protein VDAC-1 in detergent micelles. Science. 2008; 321:1206-1210.
39. Ujwal R, Cascio D, Colletier JP, Faham S, Zhang J, Toro L, Ping P, Abramson J. The crystal structure of mouse VDAC1 at 2.3 Å resolution reveals mechanistic insights into metabolite gating. Proc Natl Acad Sci U S A. 2008; 105:17742-17747.
40. Kutik S, Stojanovski D, Becker L, Becker T, Meinecke M, Krüger V, Prinz C, Meisinger C, Guiard B, Wagner R, Pfanner N, Wiedemann N. Dissecting membrane insertion of mitochondrial β-barrel proteins. Cell. 2008; 132:1011-1024.
41. Paschen SA, Neupert W, Rapaport D. Biogenesis of β-barrel membrane proteins of mitochondria. Trends Biochem Sci. 2005; 30:575-582.
42. Goncalves RP, Buzhysnskyy N, Scheuring S. Mini review on the structure and supramolecular assembly of VDAC. J Bioenerg Biomembr. 2008; 40:133-138.
43. Cline K, Andrews J, Mersey B, Newcomb EH, Keegstra K. Separation and characterization of inner and outer envelope membranes of pea chloroplasts. Proc Natl Acad Sci U S A. 1981; 78:3595-3599.
44. Cline K, Dabney-Smith C. Plastid protein import and

sorting: different paths to the same compartments. Curr Opin Plant Biol. 2008; 11:585-592.
45. Inaba T, Schnell DJ. Protein trafficking to plastids: one theme, many variations. Biochem J. 2008; 413:15-28.
46. Jarvis P. Targeting of nucleus-encoded proteins to chloroplasts in plants. New Phytol. 2008; 179:257-285.
47. Schnell DJ, Blobel G, Keegstra K, Kessler F, Ko K, Soll J. A consensus nomenclature for the protein-import components of the chloroplast envelope. Trends Cell Biol. 1997; 7:303-304.
48. Kessler F, Schnell D. Chloroplast biogenesis: diversity and regulation of the protein import apparatus. Curr Opin Cell Biol. 2009; 21:494-500.
49. Sommer MS, Schleiff E. Molecular interactions within the plant TOC complex. Biol Chem. 2009; 390:739-744.
50. Benz JP, Soll J, Bolter B. Protein transport in organelles: The composition, function and regulation of the Tic complex in chloroplast protein import. FEBS J. 2009; 276:1166-1176.
51. Agne B, Kessler F. Protein transport in organelles: The Toc complex way of preprotein import. FEBS J. 2009; 276:1156-1165.
52. Reumann S, Inoue K, Keegstra K. Evolution of the general protein import pathway of plastids (review). Mol Membr Biol. 2005; 22:73-86.
53. Ma Y, Kouranov A, LaSala SE, Schnell DJ. Two components of the chloroplast protein import apparatus, IAP86 and IAP75, interact with the transit sequence during the recognition and translocation of precursor proteins at the outer envelope. J Cell Biol. 1996; 134:315-327.
54. Perry SE, Keegstra K. Envelope membrane proteins that interact with chloroplastic precursor proteins. Plant Cell. 1994; 6:93-105.
55. Hinnah SC, Hill K, Wagner R, Schlicher T, Soll J. Reconstitution of a chloroplast protein import channel. EMBO J. 1997; 16:7351-7360.
56. Hinnah SC, Wagner R, Sveshnikova N, Harrer R, Soll J. The chloroplast protein import channel Toc75: pore properties and interaction with transit peptides. Biophys J. 2002; 83:899-911.
57. Schleiff E, Soll J, Kuchler M, Kuhlbrandt W, Harrer R. Characterization of the translocon of the outer envelope of chloroplasts. J Cell Biol. 2003; 160:541-551.
58. Tu SL, Chen LJ, Smith MD, Su YS, Schnell DJ, Li HM. Import pathways of chloroplast interior proteins and the outer-membrane protein OEP14 converge at Toc75. Plant Cell. 2004; 16:2078-2088.
59. Baldwin AJ, Inoue K. The most C-terminal tri-glycine segment within the polyglycine stretch of the pea Toc75 transit peptide plays a critical role for targeting the protein to the chloroplast outer envelope membrane. FEBS J. 2006; 273:1547-1555.
60. Inoue K, Demel R, de Kruijff B, Keegstra K. The N-terminal portion of the preToc75 transit peptide interacts with membrane lipids and inhibits binding and import of precursor proteins into isolated chloroplasts. Eur J Biochem. 2001; 268:4036-4043.
61. Inoue K, Keegstra K. A polyglycine stretch is necessary for proper targeting of the protein translocation channel precursor to the outer envelope membrane of chloroplasts. Plant J. 2003; 34:661-669.
62. Tranel PJ, Froehlich J, Goyal A, Keegstra K. A component of the chloroplastic protein import apparatus is targeted to the outer envelope membrane *via* a novel pathway. EMBO J. 1995; 14:2436-2446.
63. Tranel PJ, Keegstra K. A novel, bipartite transit peptide targets OEP75 to the outer membrane of the chloroplastic envelope. Plant Cell. 1996; 8:2093-2104.
64. Inoue K, Baldwin AJ, Shipman RL, Matsui K, Theg SM, Ohme-Takagi M. Complete maturation of the plastid protein translocation channel requires a type I signal peptidase. J Cell Biol. 2005; 171:425-430.
65. Shipman RL, Inoue K. Suborganellar localization of plastidic type I signal peptidase 1 depends on chloroplast development. FEBS Lett. 2009; 583:938-942.
66. Inoue K, Potter D, Shipman R, Perea J, Theg S. Involvement of a type I signal peptidase in biogenesis of chloroplasts – Towards identification of the enzyme for maturation of the chloroplast protein translocation channel. Photosynthesis: Fundamental Aspects to Global Perspectives, eds van der Est A, Bruce D (Allen Press, Lawrence, KS), 2005; pp. 933-935.
67. Eckart K, Eichacker L, Sohrt K, Schleiff E, Heins L, Soll J. A Toc75-like protein import channel is abundant in chloroplasts. EMBO Rep. 2002; 3:557-562.
68. Jackson-Constan D, Keegstra K. Arabidopsis genes encoding components of the chloroplastic protein import apparatus. Plant Physiol. 2001; 125:1567-1576.
69. Baldwin A, Wardle A, Patel R, Dudley P, Park SK, Twell D, Inoue K, Jarvis P. A molecular-genetic study of the Arabidopsis Toc75 gene family. Plant Physiol. 2005; 138:715-733.
70. Hust B, Gutensohn M. Deletion of core components of the plastid protein import machinery causes differential arrest of embryo development in *Arabidopsis thaliana*. Plant Biol (Stuttg). 2006; 8:18-30.
71. Inoue K, Potter D. The chloroplastic protein translocation channel Toc75 and its paralog OEP80 represent two distinct protein families and are targeted to the chloroplastic outer envelope by different mechanisms. Plant J. 2004; 39:354-365.
72. Patel R, Hsu SC, Bedard J, Inoue K, Jarvis P. The Omp85-related chloroplast outer envelope protein OEP80 is essential for viability in Arabidopsis. Plant Physiol. 2008; 148:235-245.
73. Hsu SC, Patel R, Bedard J, Jarvis P, Inoue K. Two distinct Omp85 paralogs in the chloroplast outer envelope membrane are essential for embryogenesis in *Arabidopsis thaliana*. Plant Signal Behav. 2008; 3:1134-1135.
74. Bolter B, Soll J, Schulz A, Hinnah S, Wagner R. Origin of a chloroplast protein importer. Proc Natl Acad Sci U S A. 1998; 95:15831-15836.
75. Reumann S, Davila-Aponte J, Keegstra K. The evolutionary origin of the protein-translocating channel of chloroplastic envelope membranes: identification of a cyanobacterial homolog. Proc Natl Acad Sci U S A. 1999; 96:784-789.
76. Gentle IE, Burri L, Lithgow T. Molecular architecture and function of the Omp85 family of proteins. Mol Microbiol. 2005; 58:1216-1225.
77. Sanchez-Pulido L, Devos D, Genevrois S, Vicente M, Valencia A. POTRA: a conserved domain in the FtsQ family and a class of beta-barrel outer membrane proteins. Trends Biochem Sci. 2003; 28:523-526.
78. Clantin B, Delattre AS, Rucktooa P, Saint N, Meli AC, Locht C, Jacob-Dubuisson F, Villeret V. Structure of the membrane protein FhaC: a member of the

Omp85-TpsB transporter superfamily. Science. 2007; 317:957-961.
79. Gatzeva-Topalova PZ, Walton TA, Sousa MC. Crystal structure of YaeT: conformational flexibility and substrate recognition. Structure. 2008; 16:1873-1881.
80. Kim S, Malinverni JC, Sliz P, Silhavy TJ, Harrison SC, Kahne D. Structure and function of an essential component of the outer membrane protein assembly machine. Science. 2007; 317:961-964.
81. Knowles TJ, Jeeves M, Bobat S, Dancea F, McClelland D, Palmer T, Overduin M, Henderson IR. Fold and function of polypeptide transport-associated domains responsible for delivering unfolded proteins to membranes. Mol Microbiol. 2008; 68:1216-1227.
82. Gatsos X, Perry AJ, Anwari K, Dolezal P, Wolynec PP, Likic VA, Purcell AW, Buchanan SK, Lithgow T. Protein secretion and outer membrane assembly in *Alphaproteobacteria*. FEMS Microbiol Rev. 2008; 32:995-1009.
83. Voulhoux R, Bos MP, Geurtsen J, Mols M, Tommassen J. Role of a highly conserved bacterial protein in outer membrane protein assembly. Science. 2003; 299:262-265.
84. Habib SJ, Waizenegger T, Niewienda A, Paschen SA, Neupert W, Rapaport D. The *N*-terminal domain of Tob55 has a receptor-like function in the biogenesis of mitochondrial β-barrel proteins. J Cell Biol. 2007; 176:77-88.
85. Stegmeier JF, Andersen C. Characterization of pores formed by YaeT (Omp85) from *Escherichia coli*. J Biochem. 2006; 140:275-283.
86. Sveshnikova N, Grimm R, Soll J, Schleiff E. Topology studies of the chloroplast protein import channel Toc75. Biol Chem. 2000; 381:687-693.
87. Bredemeier R, Schlegel T, Ertel F, Vojta A, Borissenko L, Bohnsack MT, Groll M, von Haeseler A, Schleiff E. Functional and phylogenetic properties of the pore-forming beta-barrel transporters of the Omp85 family. J Biol Chem. 2007; 282:1882-1890.
88. Ertel F, Mirus O, Bredemeier R, Moslavac S, Becker T, Schleiff E. The evolutionarily related beta-barrel polypeptide transporters from Pisum sativum and Nostoc PCC7120 contain two distinct functional domains. J Biol Chem. 2005; 280:28281-28289.
89. Kleinschmidt JH, Tamm LK. Secondary and tertiary structure formation of the β-barrel membrane protein OmpA is synchronized and depends on membrane thickness. J Mol Biol. 2002; 324:319-330.
90. Sklar JG, Wu T, Gronenberg LS, Malinverni JC, Kahne D, Silhavy TJ. Lipoprotein SmpA is a component of the YaeT complex that assembles outer membrane proteins in *Escherichia coli*. Proc Natl Acad Sci U S A. 2007; 104:6400-6405.
91. Wu T, Malinverni J, Ruiz N, Kim S, Silhavy TJ, Kahne D. Identification of a multicomponent complex required for outer membrane biogenesis in *Escherichia coli*. Cell. 2005; 121:235-245.
92. Misra R. First glimpse of the crystal structure of YaeT's POTRA domains. ACS Chem Biol. 2007; 2:649-651.
93. Wiedemann N, Truscott KN, Pfannschmidt S, Guiard B, Meisinger C, Pfanner N. Biogenesis of the protein import channel Tom40 of the mitochondrial outer membrane: intermembrane space components are involved in an early stage of the assembly pathway. J Biol Chem. 2004; 279:18188-18194.
94. Gentle I, Gabriel K, Beech P, Waller R, Lithgow T. The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria. J Cell Biol. 2004; 164:19-24.
95. Kozjak V, Wiedemann N, Milenkovic D, Lohaus C, Meyer HE, Guiard B, Meisinger C, Pfanner N. An essential role of Sam50 in the protein sorting and assembly machinery of the mitochondrial outer membrane. J Biol Chem. 2003; 278:48520-48523.
96. Walther DM, Rapaport D, Tommassen J. Biogenesis of beta-barrel membrane proteins in bacteria and eukaryotes: evolutionary conservation and divergence. Cell Mol Life Sci. 2009; 66:2789-2804.
97. Robert V, Volokhina EB, Senf F, Bos MP, Van Gelder P, Tommassen J. Assembly factor Omp85 recognizes its outer membrane protein substrates by a species-specific *C*-terminal motif. PLoS Biol. 2006; 4:e377.
98. Stojanovski D, Guiard B, Kozjak-Pavlovic V, Pfanner N, Meisinger C. Alternative function for the mitochondrial SAM complex in biogenesis of alpha-helical TOM proteins. J Cell Biol. 2007; 179:881-893.
99. Bos MP, Robert V, Tommassen J. Functioning of outer membrane protein assembly factor Omp85 requires a single POTRA domain. EMBO Rep. 2007; 8:1149-1154.
100. Jacob-Dubuisson F, Fernandez R, Coutte L. Protein secretion through autotransporter and two-partner pathways. Biochim Biophys Acta. 2004; 1694:235-257.
101. Guedin S, Willery E, Locht C, Jacob-Dubuisson F. Evidence that a globular conformation is not compatible with FhaC-mediated secretion of the *Bordetella pertussis* filamentous haemagglutinin. Mol Microbiol. 1998; 29:763-774.
102. Vollmer W, Höltje JV. The architecture of the murein (peptidoglycan) in gram-negative bacteria: vertical scaffold or horizontal layer(s)? J Bacteriol. 2004; 186:5978-5987.
103. Jürgens UJ, Drews G, Weckesser J. Primary structure of the peptidoglycan from the unicellular cyanobacterium *Synechocystis sp*. strain PCC 6714. J Bacteriol. 1983; 154:471-478.
104. Nakae T. Identification of the outer membrane protein of *E. coli* that produces transmembrane channels in reconstituted vesicle membranes. Biochem Biophys Res Commun. 1976; 71:877-884.
105. Hancock RE, Decad GM, Nikaido H. Identification of the protein producing transmembrane diffusion pores in the outer membrane of *Pseudomonas aeruginosa* PA01. Biochim Biophys Acta. 1979; 554:323-331.
106. Benz R, Böhme H. Pore formation by an outer membrane protein of the cyanobacterium *Anabaena variabilis*. Biochim Biophys Acta. 1985; 812:286-292.
107. Colombini M. Pore-size and properties of channels from mitochondria isolated from *Neurospora crassa*. J Membr Biol. 1980; 53:79-84.
108. Flügge UI, Benz R. Pore-forming activity in the outer membrane of the chloroplast envelope. FEBS Lett. 1984; 169:85-89.
109. Osborn MJ, Gander JE, Parisi E, Carson J. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. Isolation and characterization of cytoplasmic and outer membrane. J Biol Chem. 1972; 247:3962-3972.
110. Jürgens UJ, Weckesser J. Carotenoid-containing outer membrane of *Synechocystis sp*. strain PCC6714. J

Bacteriol. 1985; 164:384-389.
111. Schneiter R, Brugger B, Sandhoff R, Zellnig G, Leber A, Lampl M, Athenstaedt K, Hrastnik C, Eder S, Daum G, Paltauf F, Wieland FT, Kohlwein SD. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. J Cell Biol. 1999; 146:741-754.
112. Douce R, Joyard J. Biochemistry and function of the plastid envelope. Annu Rev Cell Biol. 1990; 6:173-216.
113. Molloy MP, Herbert BR, Slade MB, Rabilloud T, Nouwens AS, Williams KL, Gooley AA. Proteomic analysis of the *Escherichia coli* outer membrane. Eur J Biochem. 2000; 267:2871-2881.
114. Rey S, Acab M, Gardy JL, Laird MR, deFays K, Lambert C, Brinkman FS. PSORTdb: a protein subcellular localization database for bacteria. Nucleic Acids Res. 2005; 33:D164-D168.

(*Received August 20, 2009; Revised October 22, 2009; Accepted October 24, 2009*)