CHAPTER ONE

Structure and Function of POTRA Domains of Omp85/TPS Superfamily

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Contents

Abstract

The Omp85/TPS (outer-membrane protein of 85 kDa/two-partner secretion) superfamily is a ubiquitous and major class of β-barrel proteins. This superfamily is restricted to the outer membranes of gram-negative bacteria, mitochondria, and chloroplasts. The common architecture, with an N-terminus consisting of repeats of soluble

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polypeptide-transport-associated (POTRA) domains and a C-terminal β-barrel pore is highly conserved. The structures of multiple POTRA domains and one full-length TPS protein have been solved, yet discovering roles of individual POTRA domains has been difficult. This review focuses on similarities and differences between POTRA structures, emphasizing POTRA domains in autotrophic organisms including plants and cyanobacteria. Unique roles, specific for certain POTRA domains, are examined in the context of POTRA location with respect to their attachment to the β-barrel pore, and their degree of biological dispensability. Finally, because many POTRA domains may have the ability to interact with thousands of partner proteins, possible modes of these interactions are also explored.

1. INTRODUCTION

The OMs (outer membranes) of gram-negative bacteria, mitochondria, and chloroplasts are unique in their incorporation of β-barrel proteins, while all other biological membranes accommodate α-helical transmembrane proteins exclusively. β-Barrel OMPs (outer-membrane proteins) function as: porins, energy-dependent transporters, protein secretors, protein importers, autotransporters, adhesins, lipases, proteases, and lipid transporters (Wimley, 2003). Correct assembly of these OMPs is dependent upon a conserved family of proteins, Omp85 (outer-membrane protein of 85 kDa) (Voulhoux et al., 2003).

Omp85s share remarkable architectural similarity to a related set of proteins known as TpsB (two-partner secretion B), which are involved in the secretion of TpsA proteins through OMs (Clantin et al., 2007; Kim et al., 2007; Sanchez-Pulido et al., 2003). These two related protein families make up a superfamily of proteins that function as PTBs (polypeptide-transporting β-barrel proteins) (Jacob-Dubuisson et al., 2009). PTBs share a highly conserved domain organization, with an N-terminal region composed of soluble POTRA (polypeptide-transport-associated) domain repeats and a C-terminal transmembrane β-barrel (Sanchez-Pulido et al., 2003). TpsB proteins are defined as class I PTBs and most contain two POTRA domains, while Omp85 proteins are more variable having between one and seven POTRA repeats, and are defined as class II PTBs (Arnold et al., 2010; Kim et al., 2007; Koenig et al., 2010; Sanchez-Pulido et al., 2003).

While it is unclear which class of PTBs evolved first, duplication could have given rise to the two different classes and the different number of POTRA repeats found (Remmert et al., 2010). The fact that PTB members are present in gram-negative bacteria, mitochondria, and chloroplasts

suggests that Omp85 arose early in the evolution of prokaryotes, before the first endosymbiotic event (Habib et al., 2007; Reumann and Keegstra, 1999; Walther et al., 2009). Mitochondrial Sam50 most likely diverged from an Omp85 present in an α-proteobacteria, while the plastid Toc75 is thought to have evolved from a TpsB-like protein present in the cyanobacterial ancestor (Gentle et al., 2004).

While only one complete structure of a TpsB member of the Omp85/TPS superfamily has been experimentally determined (Clantin et al., 2007), the structures of multiple POTRA domains have been solved (Arnold et al., 2010; Gatzeva-Topalova et al., 2008; Kim et al., 2007; Knowles et al., 2008, 2009; Koenig et al., 2010; van den Ent et al., 2008; Zhang et al., 2011). Additionally, the mechanism(s) by which Omp85/TPS and POTRA domains interact allows for specific interactions with multiple partners, thousands of different partners in the case of Toc75 (Iniative, 2000), is fundamental to chloroplast evolution and function and merits further study. Members of the Omp85/TPS superfamily, and even specific POTRA domains, have been proven to be essential for cell growth in both bacteria (Bos et al., 2007; Tashiro et al., 2008) and organelle containing eukaryotes (Hust and Gutensohn, 2006; Patel et al., 2008) yet their exact role in survival is not known.

1.1. Omp85/TPS superfamily in gram-negative bacteria

An OM enveloping a peptidoglycan layer is the defining characteristic of gram-negative bacteria (Costerton et al., 1974). Integrity of the OM depends on the presence of OMPs and a variety of LPS (lipopolysaccharides), which are unique to the outer leaflet of the OM (Gentle et al., 2005; Voulhoux et al., 2003; Wiese and Seydel, 1999). These components are cytosolically synthesized, transported across the inner membrane, through the periplasm, and finally assembled into the OMs (Tamm et al., 2004; Tokuda and Matsuyama, 2004). The protein responsible for OMP insertion was discovered when depletion of Omp85 led to defects in OMP biogenesis (Voulhoux et al., 2003). The family of proteins that functions to insert OMPs into OMs is known as Omp85 proteins, and they make up class II PTBs.

Class I PTBs consists of TpsB proteins that secrete specific TpsA proteins in the TPS (two-partner secretion) systems (Jacob-Dubuisson et al., 2009; Kajava and Steven, 2006). Members of the TpsB family include ShlB of *Serratia marcescens* and FhaC of *Bordetella pertussis* (Surana et al., 2004).

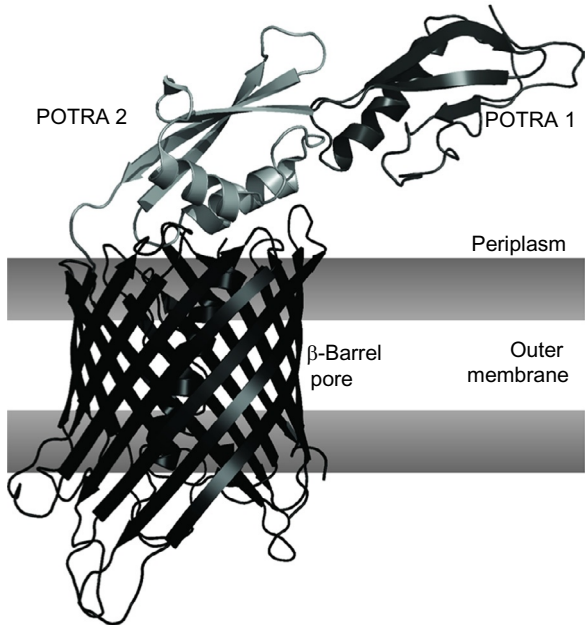

Figure 1.1 Structure of FhaC. FhaC, the TpsB transporter of filamentous hemagglutinin (FHA) from *Bordetella pertussis* has been crystallized and the structure solved at a resolution of 3.15 Å (pdb id: 2QDZ). The pore of the structure is shown in the outer membrane, while the POTRA domains are in the periplasm.

The TpsB protein, FhaC has been crystallized and is represented in Fig. 1.1, and demonstrates the conserved structure present in all members of the Omp85/TPS superfamily (Ertel et al., 2005; Sanchez-Pulido et al., 2003). This protein is the only member of the Omp85/TPS superfamily to be structurally solved in its entirety, only POTRA domains of other members have been structurally solved.

1.2. Omp85/TPS superfamily in organelles

The presence of β-barrel proteins in the OMs of mitochondria and chloroplasts is consistent with their evolutionary origins from α-proteobacteria and cyanobacteria, respectively (Gentle et al., 2005; Patel et al., 2008). Furthermore, mitochondrial Sam50 most likely diverged from an Omp85 present in the α-proteobacteria, while plastid Toc75 is thought to have evolved from a TpsB-like protein present in the cyanobacterial ancestor (Gentle et al., 2004).

1.2.1 Sam50/Tob55, a mitochondrial homolog of bacterial Omp85

Nuclear-encoded and cytosolically synthesized proteins targeted to the mitochondria are translocated through the mitochondrial membranes via the TOM and TIM (translocons at the outer/inner envelope of the mitochondria) (Paschen et al., 2005). β-Barrel proteins destined for the mitochondrial OM follow this path, and are inserted into the OM via the TOB (topogenesis of mitochondrial OM β-barrel proteins), also known as the SAM (sorting and assembly of mitochondria) complex (Paschen et al., 2005). Sam50/Tob55 appears to be the only Omp85 homologue in mitochondria, and was first discovered in *Saccharomyces cerevisiae* (Gentle et al., 2004; Kozjak et al., 2003; Paschen et al., 2005).

1.2.2 Toc75, a plastid homolog of bacterial Omp85

The genome of modern day plastids encodes between 60 and 200 proteins, while the total chloroplast proteome of *Arabidopsis thaliana* is estimated to comprise approximately 2100–4500 proteins (Leister and Kleine, 2008). Therefore, plastids depend on their host nuclei to encode over 95% of the proteins needed to correctly function (Abdullah et al., 2000; Inoue and Potter, 2004). The import of cytosolically translated precursor proteins into the plastid is accomplished by the TOC/TIC (translocon at the outer/inner envelope of the chloroplast) complexes along with soluble chaperones (Schnell et al., 1994), an excellent review of which can be found (Strittmatter et al., 2010). Toc75, the most abundant protein in the OM of the plastid envelope, is a member of the Omp85/TPS superfamily and is believed to form the β-barrel channel in the TOC complex (Gentle et al., 2005). This review will highlight similarities among the Omp85/TPS superfamily and their N-terminal POTRA domains, with an intensive look at phototrophic members of the superfamily. Structural and organizational similarities and differences of POTRA domains will be noted, and possible mechanisms of action will be explored.

2. Omp85/TPS SUPERFAMILY ARCHITECTURE

β-Barrel proteins have been implicated in protein translocation and in the assembly of OMPs in both organelles and bacteria (Moslavac et al., 2005). Bacterial and organellar members of the Omp85/TPS superfamily share a common domain organization (Gentle et al., 2005). The N-terminal regions of the OMP85/TPS superfamily were discovered to share a common three-dimensional structure consisting of repeatable units

made up of two α-helices and three β-strands termed POTRA domains (Sanchez-Pulido et al., 2003). The C-terminus is a highly conserved β-barrel domain comprising 10–16 β-strands (Sanchez-Pulido et al., 2003).

2.1. Highly conserved C-terminal β-barrel domain

The C-terminal β-barrel pore of OMP85/TPSs includes the presence of even numbers of amphipathic, antiparallel β-strands connected by loops of different sizes, forming a cylindrical barrel-like structure across the membrane bilayer. Each strand usually contains 8–11 residues, which is long enough to span biological membranes (Moslavac et al., 2005; Paschen et al., 2005). FhaC, an exporter of filamentous hemagglutinin (FHA) in *B. pertusis* is the only member of the OMP85/TPS superfamily with a solved structure that includes its C-terminus (Clantin et al., 2007), the rest of the structures are of only the N-terminal POTRA domains. It has a 16-stranded β-barrel pore with a width of around 0.8 nm at its restriction point, and has been shown to have both open and closed states (Clantin et al., 2007).

2.2. Soluble N-terminus, containing POTRA domains

Preceding the β-barrel domain is a variable sized domain composed of repeats of POTRA domains. The POTRA domains are always found at the N-termini of OMP85/TPSs and are involved in the assembly of proteins into or translocation across the OMs of mitochondria, chloroplasts, and gram-negative bacteria (Delattre et al., 2011; Gentle et al., 2004, 2005; Knowles et al., 2009; Moslavac et al., 2005; Paschen et al., 2003; Reumann et al., 1999; Sanchez-Pulido et al., 2003; Voulhoux et al., 2003). These domains are also present in the FtsQ/DivIB bacterial division protein family, which is the only known case where POTRA domains are not followed by a transmembrane β-barrel (Sanchez-Pulido et al., 2003).

A typical individual POTRA domain consists of 70–95 amino acids containing three β-strands and two α-helices (Sanchez-Pulido et al., 2003). The repeating POTRA domains are assigned consecutive numbers starting from the N-terminus (Sanchez-Pulido et al., 2003). All members of the Omp85/TPS superfamily have between one and seven repeats of POTRA domains (Arnold et al., 2010; Sanchez-Pulido et al., 2003). The number of POTRA domains varies from one protein and/or organism to another, for example, one POTRA repeat is present in Sam50, FtsQ/DivIB, ShlB, and CGI51; three POTRA repeats are present in YtfM and Toc75; five POTRA repeats are found in D15 of *Haemophilus influenza*, Omp85 of

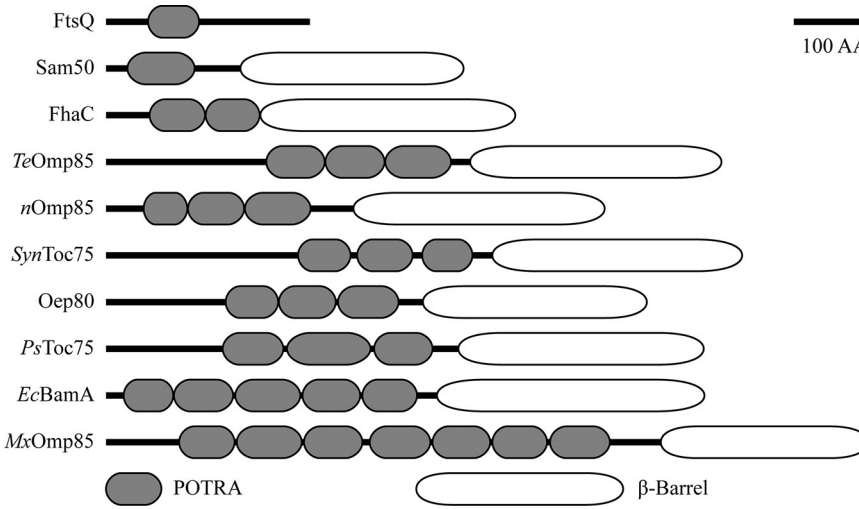

Figure 1.2 Conserved structure of the Omp85/TPS superfamily. POTRA domain-containing proteins are represented in this cartoon. POTRA domains are depicted as gray, while β-barrels are represented as white. FtsQ from *E. coli* is a protein that functions in cell division. Sam50 from *Saccharomyces cervisae* is part of the sorting and assembly machinery that functions in OMP assembly and is found in the mitochondria. FhaC from *B. pertussis* exports FHA. *Te*Omp85, *Mx*Omp85, BamA, and *n*Omp85 from *T.e.*, *M. xanthus*, *E. coli*, and *Nostoc*, respectively, and function in OMP assembly. *Syn*Toc75 from *Synechocystis* sp. PCC 6803 once exported virulence factors. Oep80 from *A. thaliana* functions in the chloroplast in OMP assembly. Toc75 from *Pisum sativum* functions in chloroplast protein import. Proteins and domains are represented to scale.

Neisseria meningitidis, and other bacterial Omp85s; while as many as seven repeats are seen in Mxan5763 of *Myxococcus xanthus* (Arnold et al., 2010; Sanchez-Pulido et al., 2003). This is represented in Fig. 1.2. A recent study of 567 POTRA-domain-containing proteins demonstrated that regardless of total number of POTRA domains present, the most C-terminal POTRA domain was the most conserved followed by the most N-terminal POTRA domain (Arnold et al., 2010). This also suggests that OMP85/TPSs containing multiple POTRA domain repeats may have evolved from a relatively simple ancestor, such as Sam50 containing only one POTRA repeat (Arnold et al., 2010). Notably, it was also demonstrated that POTRA domains from the cyanobacteria *Thermosynechococcus elongatus* (*Te*Omp85) are more closely related to POTRA domains from *A. thaliana* Toc 75 (*At*Toc75) than to proteobacterial Omp85s (Arnold et al., 2010).

These domains appear to be quite antigenic and are often recognized by antibodies raised against the entire protein (e.g., antibodies raised against the POTRA-containing N-terminal portion of the OMP D15, an Omp85

family protein, provide immunity against meningitis caused by *H. influenza*; Loosmore et al., 1997), this high antigenicity has led to the suggestion that they may be a good target to use in vaccines development against: cholera (Ruffolo and Adler, 1996), meningitis (Manning et al., 1998; Yang et al., 1998), gonorrhea (Manning et al., 1998), and syphilis (Cameron et al., 2000; Poolman et al., 2000; Robb et al., 2001).

POTRA domains show very little primary sequence homology, in fact, only glycine and hydrophobic residues forming the core of the domain are conserved between POTRAs (Sanchez-Pulido et al., 2003). However, the secondary sequence was both predicted (Sanchez-Pulido et al., 2003) and shown by crystallization and/or NMR of POTRA domains of BamA (Kim et al., 2007), FhaC (Clantin et al., 2007), FtsQ (van den Ent et al., 2008), *Te*Omp85 (Arnold et al., 2010), and *n*Omp85 (Koenig et al., 2010) to have a conserved structure, which was experimentally determined to have the consensus structure: β–α–α–β–β (Fig. 1.3). A table showing the relationship between primary structure identity and similarity of three-dimensional structure has been constructed for all of the structurally solved POTRA domains (Table 1.1). This table shows that the structural similarities of POTRA domains are not fully explained by sequence, as the most structurally similar POTRA domains are POTRA3 of *Te*Omp85 and *Nostoc*

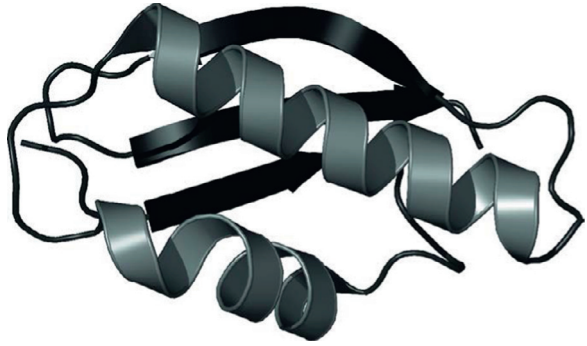

Figure 1.3 POTRA2 of *Te*Omp85. The primary, secondary, and tertiary structure of POTRA2 of *Te*Omp85 (pdb id: 2X8X) are shown. Under the primary sequence, shown in capital letter, is the secondary structure, represented in b for β-strand, and h for α-helix, the - represents unstructured residues.

Table 1.1 Similarity of POTRA domains

Percent identity																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1		13.9	4.5	5.9	13.2	20.0	11.5	16.4	20.3	12.8	13.6	16.2	10.4	19.2	15.3	1	Bp-FhaC-1
2	6.13		11.8	11.6	6.8	15.3	9.3	12.3	14.7	13.3	15.6	14.9	12.0	7.0	15.9	2	Bp-FhaC-2
3	3.77	4.52		86.8	10.3	7.4	15.9	11.8	10.9	10.1	13.3	13.4	8.7	6.0	6.2	3	Ec-FtsQ-1
4	4.04	4.91	2.06		8.7	10.3	14.3	11.6	12.5	8.6	16.7	14.7	8.6	7.5	6.2	4	Ye-FtsQ-1
5	6.08	4.51	3.06	3.61		16.0	23.5	63.6	26.1	18.8	19.4	11.0	8.0	13.7	16.9	5	No-Omp85-1
6	2.80	5.59	4.37	3.83	2.97		14.3	17.6	54.1	15.6	13.4	21.3	19.2	13.2	13.9	6	No-Omp85-2
7	8.99	3.69	12.05	10.56	1.70	8.46		23.4	16.9	52.4	26.5	12.0	16.9	18.7	31.5	7	No-Omp85-3
8	4.89	2.89	3.07	3.64	1.28	3.69	1.52		21.7	20.8	22.4	9.6	12.0	15.1	16.9	8	Te-Omp85-1
9	3.07	3.46	2.85	3.15	3.32	1.70	11.73	2.69		21.1	19.4	19.7	21.6	14.1	16.4	9	Te-Omp85-2
10	6.77	3.56	7.46	6.21	1.84	3.51	1.11	1.68	6.83		29.4	16.0	10.4	17.3	34.2	10	Te-Omp85-3
11	3.45	2.98	2.17	3.21	1.52	4.00	7.90	1.32	2.42	5.35		12.1	14.9	14.7	26.5	11	Ec-YaeT-1
12	3.74	4.02	2.29	2.52	3.00	3.19	3.72	2.41	2.19	3.53	1.70		14.8	14.5	11.1	12	Ec-YaeT-2
13	3.84	3.91	3.43	3.26	3.97	3.13	4.83	3.98	2.64	4.89	3.53	2.33		15.2	6.8	13	Ec-YaeT-3
14	3.93	4.33	2.66	3.44	2.41	2.52	2.47	2.79	2.49	2.70	2.41	2.04	3.75		10.8	14	Ec-YaeT-4
15	3.57	3.05	2.72	3.39	1.64	3.77	1.34	1.65	3.31	1.53	1.18	2.75	3.12	2.27		15	Ec-YaeT-5
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
RMSD of superimposition (Å)																	

Color scheme								
% Identity	<10	>10	>15	>20	>25	>30	>50	>60
RMSD	>9.0	<9.0	<7.0	<5.0	<3.5	<2.5	<2.0	<1.5

sp. PCC7120 (*n*Omp85), which have a 52.4% identity, but superimpose with an RMSD of 1.11 Å.

POTRA domains were originally identified by searching global hidden Markov models, which identified the POTRA domains by their shared secondary structure (Sanchez-Pulido et al., 2003). The interaction between the adjacent POTRA domains is poorly understood yet is believed to be highly dynamic, based on NMR data of the protein in solution (Knowles et al., 2008). Comparisons of the linkages between tandem POTRA domains in different published crystal structures support this dynamic coupling (Ward et al., 2009). Interestingly, it appears that the POTRA domain closest to the pore tends to have a fixed structure and orientation with respect to the pore (Koenig et al., 2010).

Although the structure and function of the Omp85/TPS superfamily is well conserved, how individual POTRA domains mediate the assembly of OMPs or translocation of polypeptides is still unclear. Solved crystal structures of two POTRA domains of FhaC (Clantin et al., 2007), five POTRA domains of BamA (Kim et al., 2007), three POTRA domains of *n*Omp85 (Koenig et al., 2010), and *Te*Omp85 (Arnold et al., 2010) provide insights into the organization and molecular mechanisms by which these domains may function (Bos et al., 2007).

3. FUNCTION OF Omp85 FAMILY OF PROTEINS

Despite their organizational similarity TpsBs and Omp85s differ fundamentally in their roles: Class I or TpsB members are involved in secretion of other proteins/substrates through the outer bacterial membrane, while Class II or Omp85 members have been shown to function in the biogenesis (Gatzeva-Topalova et al., 2010; Paschen et al., 2005) or insertion of β-barrel proteins into OMs presumably from the periplasmic or equivalent surface (Koenig et al., 2010). Members of the Omp85 family have been shown to be essential for assembly of OM β-barrel proteins into gram-negative OMs (Genevrois et al., 2003; Voulhoux et al., 2003; Wu et al., 2005). Homologues of this family have been found in the genome of all sequenced gram-negative bacteria (Gentle et al., 2004; Voulhoux and Tommassen, 2004), and in the membranes of mitochondria and plastids (Gentle et al., 2005; Voulhoux et al., 2003).

3.1. Role of Omp85 pore

β-Barrels from eukaryotic organelles are thought to facilitate the translocation of precursor proteins through the membranes as well as to aid in assembly of other OMPs (Schleiff and Soll, 2005). However, it should be noted that these organelle-localized members may have an inverted topology relative to their bacterial ancestral members (Reumann et al., 1999). Bacterial proteins destined for the OM are cytosolically translated as precursors with an N-terminal sequence that targets them for secretion via the SecYEG complex (Cross et al., 2009). A model for OMP assembly suggests that nascent OMPs, partially folded via chaperones in the periplasm, are translocated through the SecYEG complex in the cytoplasmic membrane and transferred to an Omp85 protein in the OM (Cross et al., 2009). Because the Omp85 pore is not large enough to accommodate the formation of a new β-barrel (Gentle et al., 2004; Voulhoux et al., 2003), Omp85 may serve as a scaffold that would assist in the insertion of incoming transmembrane spanning β-strands individually or in pairs thereby facilitating the insertion of the OMPs into the membrane at the protein–lipid interface (Gentle et al., 2005). Deletions of POTRA domains (Clantin et al., 2007; Jacob-Dubuisson et al., 2004) support a role of YaeT as a scaffold for other components of the OMP assembly complex.

3.2. Role of POTRA domains

As suggested by their name, POTRA domains play a key role in protein transport (Clantin et al., 2007; Sanchez-Pulido et al., 2003); however, the

mechanism(s) by which they aid the transport of proteins into or across membranes is unclear. A possible mode of interaction for POTRA domains is β-augmentation, a nonsequence-specific interaction between a β-strand of one protein and a β-strand of a second protein (Harrison, 1996), which has been observed in POTRA crystal structures (Gatzeva-Topalova et al., 2008; Kim et al., 2007; Koenig et al., 2010). Unfortunately, there is no information on which of the β-strands would be involved in an *in vivo* interaction since there are no structures of any POTRA domain with a substrate. While the mechanism(s) by which POTRA domains interact is unresolved, what is apparent is that individual POTRA domains of Omp85 proteins interact with OMP precursors (Bennion et al., 2010; Bredemeier et al., 2007; Habib et al., 2007; Kim et al., 2007; Robert et al., 2006; Sklar et al., 2007a; Vuong et al., 2008) and have unique structural characteristics (Gatzeva-Topalova et al., 2008; Koenig et al., 2010; Ward et al., 2009). Furthermore, POTRA domains are necessary for proper functioning of Omp85 proteins (Bos et al., 2007; Bredemeier et al., 2007; Clantin et al., 2007; Ertel et al., 2005; Habib et al., 2007; Kim et al., 2007), with specific POTRA domains vital to the survival of the organism (Bos et al., 2007).

While most of the structurally solved POTRA domains superimpose well, with an average RMSD of 3.7 Å (Table 1.1 and Fig. 1.4), there are differences in their structures that may help in the discovery of the niche that each individual POTRA repeat fills. There is a β-cap on POTRA1 and a

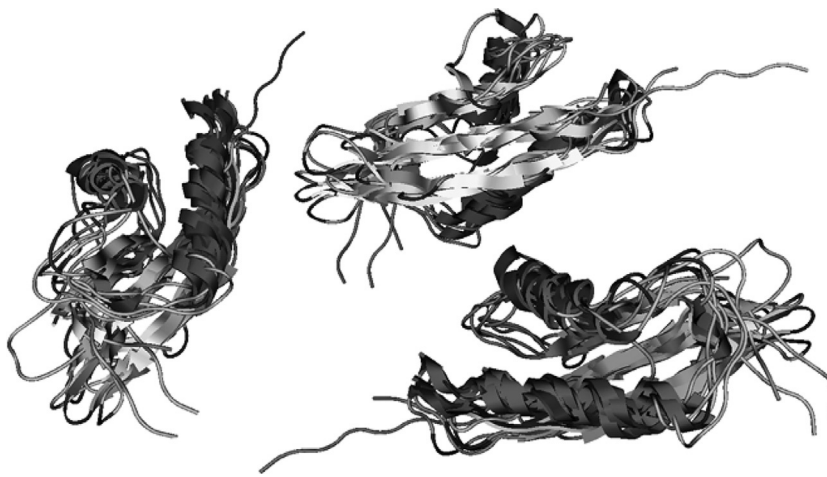

Figure 1.4 Overlay of structurally solved POTRA domains. Solved crystal structures of all solved POTRA domains have been superimposed onto each other. Three different views are present that show the similarity of the tertiary structure of these domains.

β-bulge on POTRA2 of cyanobacterial Omp85s and POTRA3 of BamA (Arnold et al., 2010; Koenig et al., 2010). These may be used as interaction sites for the POTRA domains, and could even function in β-augmentation. POTRA3 of cyanobacterial Omp85s has a loop L1, which may function to help gate the pore (Arnold et al., 2010; Koenig et al., 2010). Phylogenetic analysis shows that POTRAs cluster by their positional number, supporting the hypothesis that their order remains important (Bos et al., 2007). It is interesting that the average RMSD of the most C-terminal POTRA domains of Omp85s is 1.50 Å and the percent identity is 39.4% (Table 1.1). This suggests the most C-terminal POTRA domain is evolutionarily conserved in structure as well as by sequence to a lesser degree. The fact that POTRA5 of Omp85 in *N. meningitidis* is essential for cell viability (Bos et al., 2007) lends support to this hypothesis.

A number of specific interaction partners have been found for specific POTRA domains. The single POTRA domain of Sam50 has been shown to specifically bind β-barrel proteins destined for the mitochondrial OM (Habib et al., 2007). BamA is reported to bind C-terminal peptides of OMPs (Robert et al., 2006). In fact, POTRA5 is necessary for interactions with YfiO, NlpB, and SmpA (all proteins involved in the OM β-barrel protein biogenesis), while POTRA1 is required for an interaction with YfgL (Kim et al., 2007). POTRA1 also interacts with SurA (Sklar et al., 2007b; Vuong et al., 2008), a periplasmic chaperone, using an arginine residue (Bennion et al., 2010). The POTRA domains of *n*Omp85 have an affinity for the C-terminal pore of the protein, and act as a docking site for incoming precursor proteins (Bredemeier et al., 2007). Interestingly, these interactions have been shown to be species-specific (Robert et al., 2006). The identification of interacting partners of POTRA domains of the Omp85 family will help with to uncover roles and mechanism(s) for them.

Deletion studies, which uncovered the necessity of POTRA5 for *N. meningitides*, have been instrumental in the discovery of roles for POTRA domains. In fact, deletion of any POTRA repeat leads to a decrease in either the functionality of the protein machinery they are part of or a loss in cell viability (Bos et al., 2007; Bredemeier et al., 2007; Clantin et al., 2007; Ertel et al., 2005; Habib et al., 2007; Kim et al., 2007). *Escherichia coli* harboring deletions of POTRA1 or POTRA2 of BamA exhibit poor cell growth, while cells with deletions of POTRA3 or POTRA4 do not survive wild-type BamA depletion, and cells without POTRA5 do not survive even when they possess copies of wild-type BamA (Kim et al., 2007). This suggests not only an essential role for POTRA5 but also a dominant negative

interaction *in vivo*. Incorrect β-barrel assembly is correlated with POTRA domain deletions (Bredemeier et al., 2007; Habib et al., 2007). It has been shown that Sam50ΔPOTRA is able to bind, but not release β-barrel precursors, this indicates a role for the POTRA domain in the release of precursor proteins from the SAM complex (Stroud et al., 2011). This was also evidenced by analyzing membrane fractions where *n*Omp85ΔN-term yielded only one stabilized assembly of monomeric Omp85, while membranes containing wild-type *n*OMP85 show formation of a homo-trimeric Omp85 complex (Bredemeier et al., 2007). The deletion studies, structural information, and binding assays help to shed light on the functional roles of POTRA domains of Omp85s.

4. FUNCTIONS OF TWO-PARTNER SECRETION B PROTEINS

TpsBs are involved in polypeptide translocation across membranes (Koenig et al., 2010). FhaC mediates the translocation of FHA, the major adhesin of the whooping cough agent *B. pertussis* to the bacterial surface (Guedin et al., 1998). Clantin et al. (2004) reported the structure of the OM transporter FhaC, a member of the TpsB family of proteins, at a resolution of 3.1 Å. The C-terminal domain of FhaC forms a transmembrane pore comprised of 16 antiparallel β-strands (Clantin et al., 2004), while the N-terminal periplasmic module consists of two globular POTRA domains composed of 75 residues each (Clantin et al., 2007).

4.1. Role of TpsB pore

The β-barrel of FhaC is occluded by a 20-residue long α-helix (H1), residing between the pore and the POTRA domains, and a C-terminal extracellular loop (L6) (Clantin et al., 2007). Although the role of in-plugs are not well understood, it has been observed that one possible function is to stabilize individual β-strands that would be unstable in a lipid bilayer due to their distribution of hydrophobic residues (Naveed et al., 2009). *In silico* analyses of Omp85/TPS superfamily members uncovered conserved motifs within the β-barrel, referred to as motifs 3 and 4, which were seen in FhaC (Fig. 1.5; Clantin et al., 2007; Moslavac et al., 2005). These motifs are defined by two conserved transmembrane β-sheets and may function in the gating of the β-barrel pore (Moslavac et al., 2005). The L6 loop of motif 3, which could insert itself into the pore, is well conserved between FhaC and *Te*Omp85, and has been shown to play an important role in regulation

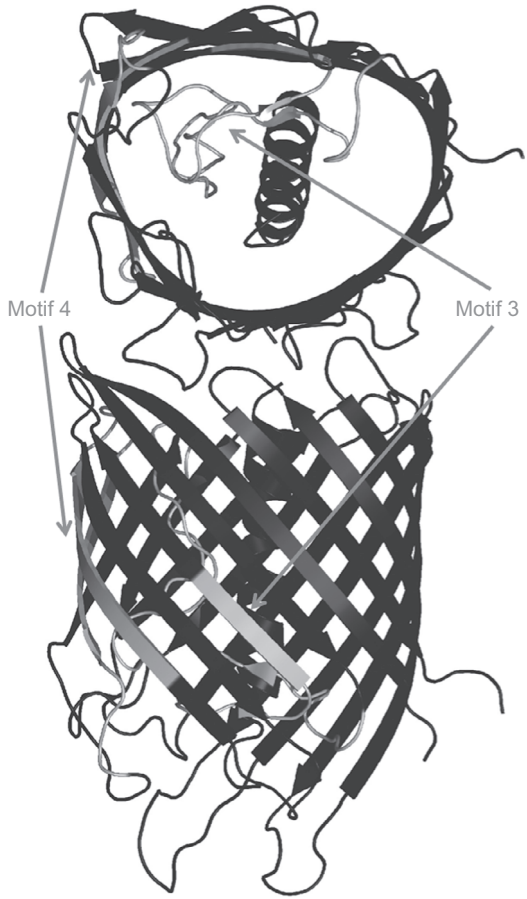

Figure 1.5 Conserved motifs of β-barrel from FhaC. The structure of the only β-barrel pore of the Omp85/TPS superfamily to be solved is shown (pdb id. 2QDZ). The pore here is occluded by an α-helix. Motifs 3 and 4, which are conserved in members of the Omp85/Tps superfamily, are shown in gray.

of polypeptide translocation (Arnold et al., 2010; Clantin et al., 2007). This suggests that L6 may be responsible for regulation of the assembly of OMPs in other *cy*Omp85 (cyanobacterial Omp85) as well.

4.2. Role of POTRA domains of TpsB

TpsA proteins all harbor a conserved, 250-residue long N-terminal TPS domain essential for secretion (Clantin et al., 2004; Hodak et al., 2006) and are involved in adhesion (Borlee et al., 2010), cytolysis, contact-dependent growth inhibition (Aoki et al., 2005), biofilm formation,

proteolysis, and host-cell invasion (Jacob-Dubuisson et al., 2004). The POTRA domains present in the N-terminus of the TpsB proteins interact with the TpsA proteins (Clantin et al., 2007; Hodak et al., 2006).

4.2.1 FhaC

One of the most well-characterized TpsB proteins is FhaC (Clantin et al., 2004, 2007). The structure of FhaC is shown in Fig. 1.1. Using this structure as a template for mutations it was shown that while FhaC still forms a channel with deletion of either POTRA domain, the POTRA domains are very important for the function of FhaC. Deletion of either POTRA domain will abolish secretion of its substrate, FHA, while insertion of residues into the POTRA domains strongly affects secretion (Clantin et al., 2007). POTRA2 is presumed to use β-augmentation during the transport of FHA, and substitutions in this domain have the most severe effect on secretion (Delattre et al., 2011). It has been shown using surface plasmon resonance that POTRA domains (specifically helix 2 of POTRA1) are involved in FHA recognition (Clantin et al., 2007). This interaction was shown to be quite tight with a K_d of ~4.0 μM. The isoelectric points of POTRA1 and FHA are 4.6 and 10.0, respectively, which has led to the suggestion that their interaction may contain a significant electrostatic component (Delattre et al., 2011).

5. PHOTOTROPHIC MEMBERS OF Omp85/TPS

Both oxygenic photosynthetic prokaryotes (i.e., cyanobacteria) and eukaryotes (i.e., plants and algae) contain members of the Omp85/TPS superfamily. These proteins are localized to the OM of the cyanobacteria (Bolter et al., 1998) or the plastid envelope (Bauer et al., 2000; Reumann et al., 1999) in algae or plants, respectively. It has been shown that these proteins are evolutionarily related, having been retained during the process of endosymbiosis (Keegstra et al., 1984). For example, in the model plant *A. thaliana*, the first plant with a sequenced genome (Iniative, 2000), there are three functional Omp85/TPS homologs: sorting assembly machinery of 50 kDa (Sam50) in the mitochondria OM (Gentle et al., 2004; Kozjak et al., 2003; Paschen et al., 2003), outer envelope protein 80 (OEP80), which mediates assembly of proteins into the OM of the chloroplast (Schleiff et al., 2003a), and Toc75-III (Hinnah et al., 1997; Schleiff et al., 2003c; Schnell et al., 1994), which is the central component of the protein translocon of the outer chloroplast envelope.

5.1. Cyanobacterial members of Omp85/TPS superfamily

The first Omp85/TPS member in cyanobacteria was identified in *Synechocystis* sp. PCC 6803 via a limited sequence homology against Toc75 of higher plants (Bolter et al., 1998). Currently there are over 100 (Altschul et al., 1997, 2005) different members of cyanobacterial Omp85/TPS genes that have been identified from a large number of organisms suggesting that this is a universal gene in cyanobacteria. Cyanobacterial *Syn*Toc75 is proposed to export virulence factors across the OM and is essential for the viability of the organism (Bolter et al., 1998; Reumann et al., 1999). *Syn*Toc75 and *Ps*Toc75 share 24% identity (41% similarity) and are similar in size, with *Ps*Toc75 consisting of 809 residues and *Syn*Toc75 containing 861 (Reumann et al., 1999). Furthermore, electrophysiological studies showed that *Syn*Toc75 and Toc75 both form cation-selective channels, which supports roles for the proteins in transport (Bolter et al., 1998; Hinnah et al., 1997). Recent studies show that cyanobacterial Omp85s differ in structure and composition from proteobacterial Omp85s, and are more closely related to chloroplastic Toc75s, with the phototrophic members containing only three POTRA domains instead of five (Arnold et al., 2010).

5.1.1 *Omp85 of* Nostoc *sp. PCC 7120*

Omp85 of *Nostoc* sp. PCC 7120 (*n*Omp85) was identified as a homolog of *Ps*Toc75, the proteins share 19.4% identity and 25.4% similarity (Ertel et al., 2005). The three POTRA domains of *n*Omp85 were recently crystallized with a resolution of 3.8 Å (Koenig et al., 2010). Based on this structure it is clear that the POTRA domains of *n*Omp85 share the common tertiary structure of a three stranded β-sheet packed against two helices as observed in other structures. However, there are several differences including: the N-terminus of POTRA1 is capped by two small β-sheets, random coil is integrated in helix α1 and strand β2 in POTRA2, and POTRA3 has an extended loop (L1) that forms two β-turns between β2 and α2 (Fig. 1.6). POTRA1 and POTRA2 may play roles in substrate recognition and hetero-oligomerization as evidenced by β-augmentation of POTRA1 seen in crystal structures (Arnold et al., 2010; Gatzeva-Topalova et al., 2010) and the two potential protein interaction surfaces present on POTRA2 (Koenig et al., 2010). The structure also revealed the presence of a flexible hinge region containing Pro and/or Gly residues between POTRA1 and POTRA2 corresponding to a flexible region between POTRA2 and POTRA3 of

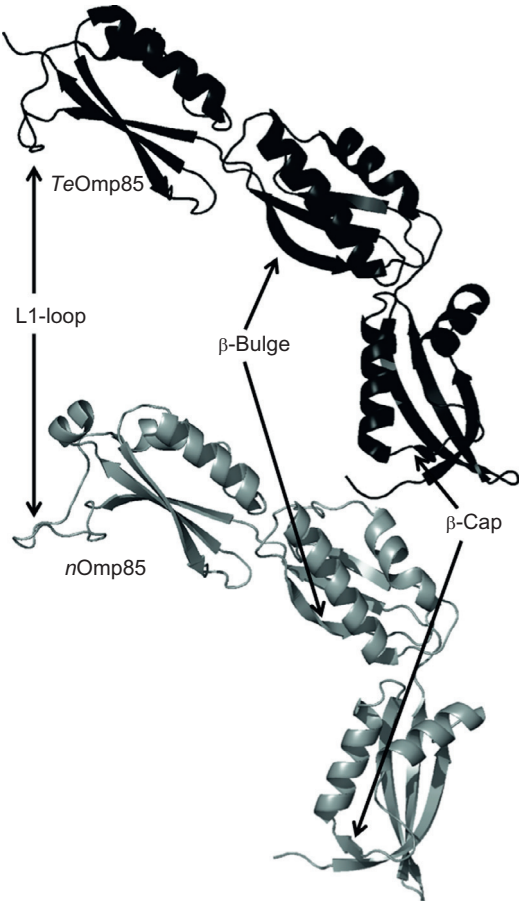

Figure 1.6 POTRA domains from *n*Omp85 and *Te*Omp85. The tertiary structures of the POTRA domains of *n*Omp85 and *Te*Omp85 are shown (pdb ids. 3MC8, 2X8X, respectively). The two POTRA domains can be superimposed with an r.m.s.d. of 0.37 nm, although their primary sequence is only around 50% identical. The features unique to cyanobacterial POTRA domains (L1-loop, β-bulge, and β-cap) are featured.

E. coli BamA, suggesting an equivalent hinge region (Kim et al., 2007; Koenig et al., 2010). Thus, the presence of flexible linker regions might be of importance for substrate-recognizing role of POTRA domains.

5.1.2 Omp85 of T. elongatus

Recently, Arnold et al. (2010) reported the first structure of all three POTRA domains of Omp85 from the cyanobacterium *T. elongatus* (*Te*Omp85) at 1.97 Å resolution. Mature *Te*Omp85 contains an

unstructured proline-rich region located N-terminal to the POTRA domains, followed by three POTRA domains and then a C-terminal pore containing 16 β-strands (Arnold et al., 2010). Residues 67–141 were identified as POTRA1, 142–217 as POTRA2, and 218–301 as POTRA3. POTRA domains of *n*Omp85 and *Te*Omp85 superimpose with an RMSD of 3.7 Å and the similarity between them can be seen in Fig. 1.6.

While POTRA domains are very similar in structure, mutants affecting the POTRA have similar effects on the proteins containing them. The deletion of POTRA domains leads to a destabilization of the pore at higher voltage, which suggests that the POTRA domains play a role in the stabilization of the channel (Arnold et al., 2010). This stabilization of *Te*Omp85 is due to interactions between POTRA3 and β-strands that are part of the pore (Arnold et al., 2010). The electrophysiological measurements of conductance of *Te*Omp85 were the highest reported. They were outside the upper range of other cyOmp85s, *Ps*Toc75, and TpsB proteins; and much higher than those of proteobacterial Omp85s and Sam50s (Arnold et al., 2010). These differences may indicate a similar evolutionary origin between the chloroplastic Omp85 and plant Toc75 homologues.

5.2. Chloroplast members of Omp85/TPS superfamily

Modern chloroplast has a genome (plastome) that codes for a very small amount of genes between 33 (in *Rhizanthella gardneri*) (Delannoy et al., 2011) and 243 (*Cyanidioschyzon merolae*) proteins (Ohta et al., 2003). This limited coding of the plastome is compensated by the large number of proteins that plastids use that are nuclear encoded. For example, *A. thaliana* has a proteome estimated at 3574 (Iniative, 2000). Proteins destined for the chloroplast obtained targeting sequences and the chloroplast had to develop machinery to translocate the cytosolically translated proteins through the outer and inner membranes (Inoue, 2007; Keegstra and Cline, 1999; Keegstra et al., 1984; Ueda et al., 2006). The majority of chloroplast-destined proteins are recognized via cleavable N-terminal targeting sequences termed transit peptides (TPs) discovered over 30 years ago (Chua and Schmidt, 1978; Highfield and Ellis, 1978). There is very little homology in primary sequence or secondary structure in solution among the thousands of known and predicted TPs (Bruce, 2000, 2001; Chotewutmontri et al., 2012).

Different precursor proteins are specifically targeted to six locations in the chloroplast: the OM, intermembrane space, inner membrane, stroma,

thylakoid membrane, or the lumen (Dyall et al., 2004). Regardless of their final destination, most proteins must cross the inner and outer envelope membrane. This translocation is facilitated by the coordinated activity of two protein-translocation complexes known as TOC and TIC (Cline, 2000; Jarvis and Soll, 2001). However, it is the TOC complex that binds precursor first and therefore ensures specificity and directionality of chloroplast protein import (Chen and Li, 2007). Toc34, Toc75, and Toc159 are the components that make up the core TOC complex (Bolter et al., 1998; Schnell et al., 1994; Seedorf et al., 1995). The core TOC complex is sufficient for *in vitro* translocation into lipid vesicles and is estimated to be between 500 kDa and 1 MDa (Chen and Li, 2007; Kikuchi et al., 2006, 2009; Schleiff et al., 2003b,c). Toc75 is a β-barrel translocon at the outer envelope of the chloroplast that was first identified in pea (Perry and Keegstra, 1994; Schnell et al., 1994). In *Arabidopsis* the presence of two paralogs of Toc34, *At*Toc33 and *At*Toc34, along with the four paralogs of Toc159, *At*Toc159, *At*Toc132, *At*Toc120, and *At*Toc90 suggest that distinct classes of TOC complexes could exist (Iniative, 2000; Ivanova et al., 2004; Jackson-Constan and Keegstra, 2001).

5.2.1 Toc75, a plastidic outer-membrane protein of Omp85/TPS superfamily

Toc75 is the only TOC component of cyanobacterial origin (Bolter et al., 1998; Kalanon and McFadden, 2008; Reumann and Keegstra, 1999; Reumann et al., 2005), and is the most abundant protein in the OM of the chloroplast (Nielsen et al., 1997; Seedorf et al., 1995). Toc75 is resistant to thermolysin treatment, salt and high pH extraction and therefore was identified as an integral OMP (Schnell et al., 1994). Toc75 is the only known chloroplast OMP with a cleavable bipartite N-terminal targeting sequence, where the most N-terminal part is a classical TP, cleaved by the SPP, and the C-terminal portion is cleaved in the intermembrane space by an envelope-bound type I signal peptidase (Inoue and Keegstra, 2003). The mature portion of Toc75 possesses two distinct domains typical of OMP85/TPSs: an N-terminal portion consisting of three POTRA domains, and a C-terminal pore made up of 16 β-strands (Sanchez-Pulido et al., 2003).

Toc75 has been shown to oligomerize with itself and other members of the TOC core complex (Nielsen et al., 1997; Reddick et al., 2007; Seedorf et al., 1995), as well as interacting with precursor protein (Hinnah et al., 1997). More specifically, the N-terminus of *Ps*Toc75 was shown to interact

with precursor to the small subunit of RuBisCO (prSSU), but not the mature form of the protein (mSSU) (Ertel et al., 2005). The same study shows that the most N-terminal region is involved in hetero-oligomerization with Toc34 and the C-terminus is involved in the pore formation. This study preceded the structural model of Toc75 containing POTRA domains. Evidence for Toc75 acting as the pore for protein translocation includes: *in vitro* experiments showing that Toc75 interacts with precursor proteins during import, cross-linking data showing an association with envelope-bound import intermediates, and arrest of import into purified chloroplasts by antibodies against Toc75 (Ma et al., 1996; Perry and Keegstra, 1994; Schnell et al., 1994; Tranel et al., 1995). Additionally, patch-clamp analysis provided compelling evidence for the role of Toc75 as a channel-forming protein (Hinnah et al., 1997). Toc75 formed narrow, voltage-gated, cation-selective transmembrane channels with a predicted hydrophilic pore with a constriction zone estimated to be 15.4 Å in diameter (Hinnah et al., 2002). Current through Toc75 was abrogated when a chloroplastic, but not mitochondrial or synthetic, precursor was added, suggesting a specific interaction with the TP (Hinnah et al., 1997). Thus, much evidence supports the role Toc75 and its POTRA domains play in protein import into the chloroplast.

5.2.2 Oep80, a chloroplastic paralog of Toc75

Pisum sativum has two Toc75 paralogs; Toc75, the translocation pore, and Toc75-V, a direct ortholog of an OMP present in the cyanobacterial ancestor (Eckart et al., 2002). Arabidopsis has three Toc75 paralogs that are represented by expressed sequence tags; *At*Toc75-I (shares 60% identity with *At*Toc75-III), *At*Toc75-III (the main pore with 74% identity to *Ps*Toc75), and *At*Toc75-IV (consisting of only 407 amino acids that align with the C-terminus of the other Toc75s) (Jackson-Constan and Keegstra, 2001). *At*Toc75-I is a pseudo-gene with a transposon inserted inside the sequence, and it is not expressed (Baldwin et al., 2005). *At*Toc75-IV does not have a cleavable N-terminal TP, and most likely lacks POTRA domains, as it was not sensitive to protease treatment (Baldwin et al., 2005). The *At*Toc75-III homozygous knockout mutant is embryo lethal (Baldwin et al., 2005).

The fourth *Arabidopsis* homologue of *Ps*Toc75 named *At*OEP80, which shares only 22% identity to *Ps*Toc75 (Eckart et al., 2002; Hinnah et al., 1997), exists as a 70-kD protein (Hsu et al., 2012). A pea ortholog of *At*OEP80, *Ps*Toc75-V, appears to be a 66-kDa protein and is not purified with the TOC complex (Eckart et al., 2002). Inoue et al. demonstrated that

*At*OEP80 is targeted to the OM of the chloroplast by information in its mature sequence and does not utilize the general import pathway for membrane insertion (Inoue and Potter, 2004). *At*OEP80 has been predicted to form a 16 β-stranded porin-like channel with three POTRA domains, and is hypothesized to be involved in the membrane biogenesis (Eckart et al., 2002; Inoue and Potter, 2004; Sveshnikova et al., 2000). T-DNA insertions into the *oep80* gene result in embryo lethality, although at a stage later than the *toc75* knockout. This indicates that while both Toc75 homologs are essential for the viability of plants, they may have distinct functions in chloroplast development (Patel et al., 2008).

Phylogenic analysis of Toc75 from various organisms led to the conclusion that Toc75 and OEP80 represent two independent gene families, both derived from cyanobacteria that had already diverged prior to the endosymbiotic event (Eckart et al., 2002). It is postulated that Toc75 acquired a new role in order to enslave the endosymbiont, while OEP80 retained the function of the ancestral Omp85, which is essential for viability in both bacteria and chloroplasts (Patel et al., 2008). The OEP80 (originally named *At*Toc75-V) is responsible for insertion of OMPs into the chloroplast envelope, and is a member of the Omp85 family that is thought to have diverged from Toc75 before the endosymbiotic event (Inoue and Potter, 2004). The presence of two distinct OMP85/TPS groups makes the chloroplast outer envelope unique among the evolutionary conserved biological membranes of mitochondria and gram-negative bacteria, which appear to have only one homologue each (Inoue and Potter, 2004).

5.3. Unique features of phototrophic Omp85 POTRA domains

The Omp85s of cyanobacteria all possess three POTRA domains, equivalent to the number of POTRA domains present in Toc75 of chloroplasts (Koenig et al., 2010; Sanchez-Pulido et al., 2003). POTRA domains tend to cluster according to their position, and in a cluster analysis of POTRA domains the N- and C-terminal POTRA domains of Toc75, Omp85, and cyanobacterial Omp85 form two distinct groups (Arnold et al., 2010). The similarity of the most N- and C-terminal POTRA domains is also shown in Table 1.1. The C-terminal POTRA domains have the highest homology, suggesting that their function is probably the most conserved and the most important. A knockout of the most C-terminal POTRA domain of Omp85 in *N. meningitidis* is lethal (Bos et al., 2007). This knockout and clustering data suggest that the most C-terminal POTRA domain (especially of

Toc75, Omp85, and cyanobacterial Omp85) may be necessary for organism or plastid survival. The other POTRA domains may have diverged for their specific functions, for example, POTRA3 of *n*Omp85 has a loop between its first β-strand and first α-helix that is conserved in the plastid and cyanobacteria Omp85 members, but is not found within the mitochondria or proteobacteria members (Koenig et al., 2010).

Interestingly, the increased length of POTRA2 in Toc75 of higher plants (e.g., 116 amino acids for POTRA2 of *Ps*Toc75) is not conserved across POTRA2 domains in cyanobacteria and there has yet to be an algal or plant POTRA domain structurally solved. POTRA2 in *n*Omp85 was determined to be 80 residues and POTRA2 in *Te*Omp85 is 70 residues (Arnold et al., 2010; Koenig et al., 2010). The helix α1 and the β-strand β1 of POTRA2 in *n*Omp85 are both interrupted, resulting in a β-bulge that is also present in the third POTRA domain of BamA (Kim et al., 2007; Koenig et al., 2010). Also conserved is a stable orientation for the POTRA2 and POTRA3 domains, and more flexibility between the POTRA2 and POTRA1, which mirrors the flexibility between POTRA2 and POTRA 3 of BamA (Gatzeva-Topalova et al., 2008; Kim et al., 2007; Koenig et al., 2010). POTRA1 of *n*Omp85 has a small two-stranded β-sheet near its N-terminus, which forms a β-cap (Koenig et al., 2010). The first β-strand of the cap is N-terminal to the β1 of POTRA1 and the second β-strand of the cap between α1 and α2 of POTRA1 (Fig. 1.6). The differences unique to POTRA domains that are part of cyanobacterial Omp85s seem conserved and perhaps allow for novel or specialized functioning of these domains.

The hinge region between POTRA2 and POTRA1 could allow POTRA1 the large degree of freedom needed to make adjacent interactions. POTRA1 could change position until it encounters another POTRA1 at which point the ability of POTRA1 to self-dimerize would allow time for the β-barrel pores of the two Toc75s to come into contact with each other. Alternatively, this interaction could compete for an in-plug formation that could occlude the protein-conducting channel. The outer edge of the β-barrels could interact with each other, perhaps via β-augmentation, and this would help to form the 4:4:1 stoichiometry of Toc75:Toc34:Toc159 that is present in the TOC core complex (Agne et al., 2009).

6. POTRA DOMAINS MODE OF ACTION

Although the domain organization of OMP85/TPSs is well conserved, the mechanism by which these domains mediate the assembly of OMPs and polypeptide translocation is not clear. However, crystal

structures (Arnold et al., 2010; Clantin et al., 2004; Kim et al., 2007; Koenig et al., 2010) of several POTRA domains allow for structure-based predictions on how these domains may operate.

6.1. Role and model of action for individual POTRA domains

The unique features of the POTRA domains mentioned above could be biophysical evidence for the unique roles that POTRA domains seem to play in various OMP85/TPSs. The ability of POTRA1 to participate in β-augmentation has been noted in BamA and *n*Omp85 (Gatzeva-Topalova et al., 2008; Kim et al., 2007; Koenig et al., 2010). This β-augmentation has been shown to be able to occur in both parallel and anti-parallel orientations (Heuck et al., 2011; Koenig et al., 2010). TPs have a region toward their C-terminus that has been predicted to form an amphiphilic β-strand (von Heijne et al., 1989), which means that POTRA domains and TPs could interact via β-augmentation.

POTRA1 has been shown to interact with substrates that the Omp85 or TPS translocates through or inserts into biological membranes. For example, POTRA1 of BamA in *E. coli* has been shown to interact with nascent LptD, LamB, and OmpF (all OMPs destined for the OM in *E. coli*), and has been implicated in assembly of BamA into the OM (Bennion et al., 2010; Knowles et al., 2008). POTRA domains belonging to TpsB transporters interact with their TpsA partner (Clantin et al., 2007; Hodak et al., 2006). In particular POTRA1 of FhaC was shown to interact with FHA, although not as well as the entire periplasmic domain of FhaC does (Delattre et al., 2011). This means that POTRA2 of FhaC must interact with the FHA substrate as well. POTRA2 of Toc75 is predicted to be very large and may contain a β-bulge-like POTRA2 of *n*Omp85 or POTRA3 of BamA. If this is the case, POTRA2 of Toc75 will have more residues that could possibly interact with TPs or other members of the TOC machinery.

It has been shown that POTRA domains gate the pore, as constructs without the POTRA-containing N-terminus of *n*Omp85 allow sucrose to diffuse freely into the liposomes they are reconstituted into (Ertel et al., 2005; Koenig et al., 2010). More specifically the most C-terminal POTRA domain, POTRA3, of *n*Omp85 contains a loop deemed L1 loop between the first β-strand and first α-helix. This loop is conserved in chloroplast and cyanobacteria but not mitochondria or proteobacteria, and has been shown to have a gating effect on the pore of *n*Omp85 (Koenig et al., 2010). Another possibility for the observed gating effect on the pore of the translocon is that there are β-strands in the pore that are unstable, and

interaction with other residues could help to stabilize them. In this case residues from the POTRA domains could act as the stabilizing proteins and would be called "in-plug" domains (Naveed et al., 2009).

6.2. Models of POTRA interaction with peptide substrates

While some of the roles for individual POTRA domains have been defined, we will take a look at proteins with structures similar to POTRA domains so that we can analyze possible modes of interaction for the POTRAs.

6.2.1 MHC class I

Class I major histocompatibility complex (MHC) molecules carry short peptides from proteins degraded in the cytoplasm of nucleated vertebrate cells to the cell surface via secretory vesicles (Falk et al., 1991). The tertiary structure of this MHC is strikingly similar to a POTRA domain (Fig. 1.7), although there is a difference in the way that the two different domains behave with respect to interacting with their respective substrates. Class I MHC molecules bind peptides very strongly *in vitro* with fast association rates (Springer et al., 1998) and slow dissociation rates (Buus et al., 1986). In fact, class I MHC molecules are capable of forming complexes with half-lives lasting tens of hours with many different peptide substrates (Khan et al., 2000). This differs from POTRA domains because POTRA domains do not form stable complexes with their substrates. If the binding affinity of POTRA

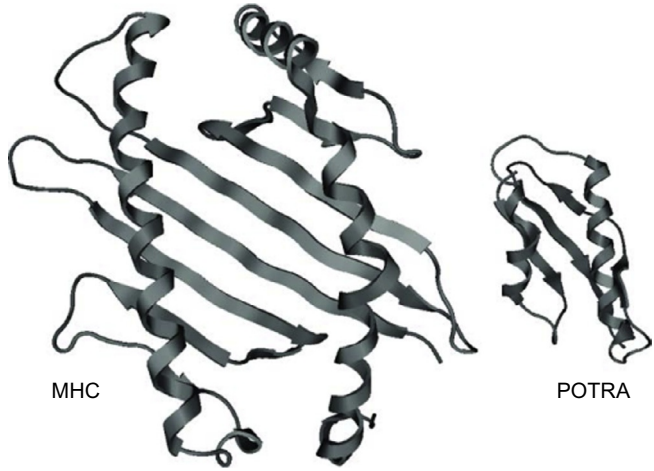

Figure 1.7 Tertiary structure of MHC and POTRA domain. Solved crystal structures of Class I major histocompatibility complex (MHC) from *Homo sapiens* and POTRA2 from *Te*Omp85 (pdb ids. 1DUZ, 2X8X, respectively) are shown.

domains were too high, it would severely hamper the ability of their proteins to export/import substrates or to insert proteins into the OMs. It is possible that the shorter length of α-helices in POTRA domains contributes to a lower affinity for their substrates when compared to MHC molecules. Another way the mode of interaction of POTRA domains and MHC molecules differ is that MHC interactions are specific. Side chains of peptides in the binding groove of MHC molecules reside in specific pockets, showing a sequence dependence on peptide binding (Garrett et al., 1989; Guo et al., 1993; Saper et al., 1991). However, POTRA domains cannot interact specifically with TPs, because there are thousands of different TPs (Bruce, 2000), and only three POTRA domains in Toc75 (Sanchez-Pulido et al., 2003).

6.2.2 Hsp70/DnaK

Another set of molecules that use β-strands to bind their substrates are the molecular chaperones in the 70-kDa heat shock protein (Hsp70) family, present in the cells of all organisms (Zhu et al., 1996). These proteins have constitutive and stress-induced functions (Saito and Uchida, 1978), and are involved in events such as translocation, nascent polypeptide folding, and antiaggregation functions (Rothman, 1989). There are ATP and substrate-binding domains present on Hsp70s (Welch and Feramisco, 1985). The crystallized structure of the substrate-binding domain of DnaK, the Hsp70 that functions in DNA replication in *E. coli* (Saito and Uchida, 1978), has a β-sandwich, composed of eight β-strands inside of five α-helices (Zhu et al., 1996). Peptides interact with the substrate-binding domain by forming hydrogen bonds with the β-sandwich domain of DnaK (Mayer and Bukau, 2005). Hsp70-peptide complexes, like class I MHC interactions, have long half-lives (Schmid et al., 1994). However, DnaK can bind peptides in extended conformations, and the hydrogen bonds formed between DnaK and peptide backbones are generic with few key specificity determining pockets (Zhu et al., 1996).

6.2.3 PDZ domains

PDZ domains are 80–110 residue-containing domains present in the C-terminal portions of signaling proteins of bacteria, yeast, plants, and animals that use β-augmentation to interact with peptides (Boxus et al., 2008; Cho et al., 1992; Ponting, 1997). PDZ domains derived their name from the first three different proteins that they were noticed in: postsynaptic density protein of 95 kDa (PSD95), *Drosophila* disc large tumor suppressor (DlgA),

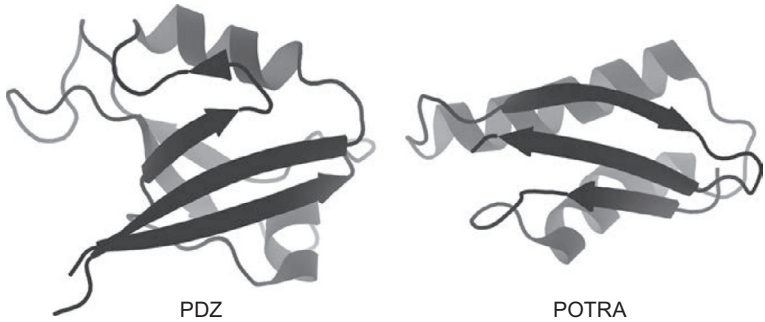

Figure 1.8 Tertiary structure of PDZ and POTRA domain. Solved crystal structures of PDZ domain of PSD-95 from *Rattus norvegicus* and POTRA2 from *Te*Omp85 (pdb ids. 3GSL, 2X8X, respectively).

and zonula occludens-1 protein (Zo-1) (Kennedy, 1995). PDZ domains have six β-sheets and two α-helices. Here, β-augmentation occurs between an exposed β-strand of the PDZ domain and a ligand, including a synthetic peptide used in a crystallization experiment (Lee et al., 2011). Interestingly, the structure of PDZ domains and POTRA domains is highly similar, with both domains ranging in size from 70 to 120 residues (Fig. 1.8), unlike the larger MHC domains. Both β-augmentation interactions in this crystal structure are antiparallel, but parallel interactions can occur (Kim et al., 2007; Koenig et al., 2010). Regions of 100–150 residues present in insulin receptor substrates 1 and 2 also form hydrogen bonds with β-stranded peptides near the exposed edge of a β-sheet (Harrison, 1996).

7. CONCLUDING REMARKS

This review covers the recent progress in the structure and function of the Omp85/TSP superfamily of OMPs. We show that this family is composed of two discreet groups with divergent roles. Moreover, the distinct roles of these two subgroups are ancient and most likely precede the earliest stages of endosymbiosis that gave rise first to mitochondria and then to plastids. Interestingly, both groups are conserved in their general structure and contain a variable number of POTRA domains at their N-terminus followed by a 16-stranded β-barrel structure. Further work is needed to elucidate the role of the individual POTRA domains, however both structural alignments and functional studies suggest that it is the POTRA domain most proximal to the β-barrel that is most conserved and essential. Future work will be needed to elucidate the individual roles of the POTRA domains.

A very interesting area to emerge recently is the apparent duplication and multiple roles that the OMP85 members play in photosynthetic organisms including cyanobacteria and in the OM of the plastid envelope. This is an area that has had considerable advances from genomic analysis but also from recent structural work. Of great interest is the process of how cyanobacterial OMP85 members were redeployed to function in the process of protein import into the plastid. This likely required a topological inversion and meant that the ancestral function essential for cyanobacterial survival was dispensed and the duplicated protein found a new niche and now functions in the biogenesis of the plastid. As new structural data becomes available this will be an exciting area of membrane and organelle biogenesis.

Finally, the role of these Omp85/TPS proteins in the translocation, insertion, and assembly of proteins will be important for a wide range of membrane proteins including other TOC components, OMP assembly machinery, and virulence factor exporters. However, the details of this chaperone role of the POTRA domains in protein folding and assembly will require new structural and genetic approaches in the future.

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