Point of VIEW

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First Glimpse of the Crystal Structure of YaeT's POTRA Domains

Rajeev Misra*

School of Life Sciences, Arizona State University, Tempe, Arizona 85287

Members of the Omp85 family of proteins are involved in the translocation of proteins into and across the outer membrane (OM) of Gram-negative bacteria (Omp85-YaeT), chloroplast (Toc75), and mitochondria (Sam50-Tob55) (*1*). Their structures are predicted to contain two distinct regions: a C-terminal transmembrane β-barrel and an N-terminal soluble region carrying 1–5 polypeptide-transport-associated (POTRA) domains (*2*). A recent paper by Kim et al. (*3*) provides the first crystal structure of YaeT's POTRA domains. The heteromeric complex of YaeT constitutes essential machinery involved in the assembly of β-barrel outer-membrane proteins (OMPs) (*4*–*7*). YaeT and the four lipoproteins, YfgL, NlpB, YfiO, and SmpA, which interact with YaeT (*8*), have been recently renamed BamA–BamE, respectively, to indicate that they are components of the β-barrel assembly machinery (Bam).

A truncated YaeT polypeptide of *Escherichia coli* containing the first four POTRA domains (P1–P4) and the first seven residues of the fifth POTRA domain (P5) was crystallized as a dimer. (A YaeT polypeptide carrying all five POTRA domains could not be crystallized.) The four POTRA domains have a similar basic 3D structure composed of a three-stranded β-sheet superimposed by two α-helices that are arranged in the order of N-β1-α1-α2-β2-β3-C (Figure 1). A parallel β-strand interfacing between the β2 edge residues of P3 and vestigial residues of the P5 domain provided contacts between the two subunits through main-chain hydrogen bonds. On the basis of the data provided in the paper (*3*), the YaeT dimers are likely to be an artifact of crystallization, but similar interactions between YaeT and incoming OMPs through β-strand augmentation could serve to nucleate β-barrel OMP assembly or mediate interactions with other components of the assembly machine.

The significance of individual POTRA domains of YaeT in OMP assembly was investigated by mutagenesis. YaeT constructs deleted for any one POTRA domain had a severe OMP assembly defect, which shows that all five POTRA domains are required for OMP assembly. Further biochemical analysis provided insights into the possible role of individual POTRA domains in OMP assembly. Deletion of P1 produced a severe OMP assembly defect but did not compromise YaeT's interaction with the four accessory lipoproteins, an indication that P1 may directly receive incoming OMPs presumably bound to the periplasmic chaperones SurA or Skp (Figure 1). Deletion of P2, P3, or P4 produced very similar biochemical defects, and they all abolished YaeT's interaction with YfgL but not with the other three lipoproteins. However, because YfgL is not an essential lipoprotein and strains lacking YfgL do not produce as severe OMP assembly defects (*9*) as seen in strains deleted for YaeT's P2, P3, or P4 domains (*3*), it seems that the OMP assembly defect in ΔP2, ΔP3, and ΔP4 mutants is probably the result of an additional impairment of interaction between YaeT and OMP.

ABSTRACT The Omp85/YaeT family of proteins, which are conserved from bacteria to human, catalyzes insertion and assembly of proteins in the outer membrane. The structure consists of a transmembrane β-barrel domain and a soluble polypeptide-transport-associated (POTRA) domain. The POTRA domain is critical for substrate recognition and perhaps substrate folding, while the β-barrel domain assists in membrane insertion. The resolution of the crystal structure of the POTRA domain of the *Escherichia coli* YaeT protein provides a possible molecular mechanism by which the diverse group of substrates is recognized. Knowledge gained from the crystal structure may also spur the development of a novel class of chemotherapeutic inhibitors.

***Corresponding author, rajeev.misra@asu.edu.**

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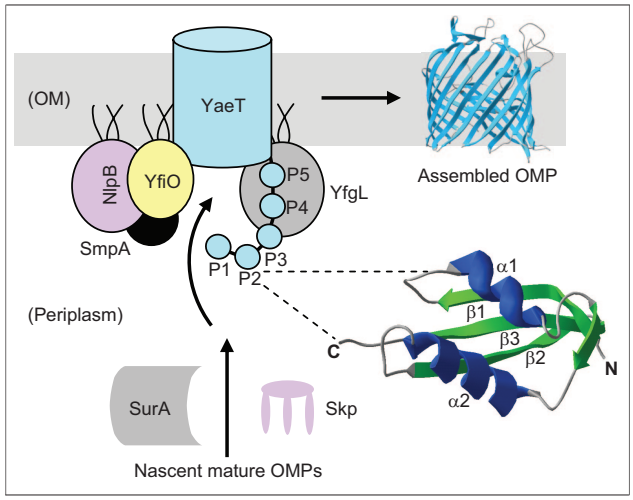

Figure 1. A cartoon depicting the steps of OMP assembly in *E. coli*. Nascent mature OMPs refer to OMPs that have had their N-terminal signal sequences removed during Sec-mediated translocation across the inner membrane. These OMPs probably attain secondary structures in the soluble environment of the periplasm and interact with periplasmic chaperones SurA and Skp. OMP–chaperone complexes then interact with the POTRA domains (P1–P5) of YaeT, where OMPs achieve further folding and possibly assembly into β-barrels before inserting into the OM. The four lipoproteins YfgL, YfiO, NlpB, and SmpA are components of the YaeT β-barrel assembly machinery. A 3D structure of the P2 POTRA domain of YaeT is shown.

The structure of P3 showed a small protrusion along the edge of the β2-strand. Because this edge binds to the vestigial residues of the P5 domain in the crystal structure, the authors speculated that the β2-strand of P3 may provide a site of interaction *via* β-strand augmentation. Attempts to obliterate such interactions by shifting the β2-strand edges outward by two or four residues impaired YaeT's interactions with YfgL. Because the mutant YaeT proteins still complemented the chromosomally deleted *yaeT* null allele, the β2-strand region of P3 does not seem to play a critical role in YaeT–OMP interactions. However, unlike small insertion mutations in P3, deletion of the entire P3 severely impairs OMP assembly, so it is likely that P3 plays a greater role in OMP assembly than simply facilitating YaeT's interaction with YfgL. If P3 provides a site of OMP interaction *via* β augmentation, as it does for YfgL, this would mean that two separate polypeptides could occupy the same assembling platform within the P3 POTRA domain. (Atomic structure of the POTRA domain–OMP peptide cocrystal will be needed to decipher the precise interacting surfaces between the two molecules.) Deletion of P2 or P4 also abolishes the interaction of YaeT with YfgL. It is unclear whether residues within these two POTRA domains also make specific contacts with YfgL or whether the loss of YfgL interaction is the result of gross spatial separation between the interacting surfaces of the two proteins. Deletion of the β-barrel-proximal P5 abolishes YaeT's binding to all four lipoproteins of the assembly machinery, an indication of its importance in the assembly of the YaeT complex itself.

The structure of the POTRA domains resembles that of the PDZ domain, which is well-known for its interactions with a diverse group of peptide sequences (*10*). PDZ-domain-mediated substrate interactions are thought to involve short C-terminal peptide motifs (*10*). Significance of the last β-strand of OMPs, particularly the terminal phenylalanine residue, in OMP assembly has been well documented (*11*). Moreover, binding of short peptides containing C-terminal OMP residues increased the *in vitro* channel activity of YaeT in planar lipid bilayer assays (*12*). Thus, it is likely that the C-terminal β-strand of OMPs interacts with YaeT's POTRA domains by β augmentation. This initial binding may trigger the assembly of the remaining β-strands into a barrel. Whether internal OMP β-strands, several of which contain aromatic residues at the termini, also bind to POTRA domains remains unclear. Internal sequence recognitions have been reported for PDZ domains (*13*).

It is interesting to note that the number of POTRA domains varies in the Omp85 superfamily of transporters (*2*). Members of the two-partner secretion (Tps) system have extremely narrow substrate specificity and tend to have one or two POTRA domains (*2, 14*). On the other hand, YaeT/Omp85 proteins, which are involved in the transport of numerous proteins with diverse sequences, are known to contain five POTRA domains. Thus, the number of POTRA domains in a transporter may reflect the number and diversity of substrates with which a given transporter interacts.

The crystal structure of four of the POTRA domains of YaeT have provided valuable insight into the possible mechanism of how incoming OMPs interact with YaeT for their assembly. However, additional work will be needed to better understand the molecular nature of YaeT–OMP interactions, β-barrel OMP assembly, and the subsequent insertion of partially or fully assembled OMP barrels into the OM. The role of YaeT's OM-embedded β-barrel domain in OMP assembly remains elusive. It is possible that by keeping the POTRA domains close to the OM, the β-barrel domain serves as a scaffold to facilitate the insertion of POTRA-bound OMPs into the OM. YaeT has been shown to form voltage-activated channels in planar lipid bilayers (*12*). If YaeT channels are formed *in vivo*, it is conceivable that POTRA-bound OMPs enter YaeT's channel and are released laterally in the OM, much like the mechanism proposed by the SecYEG-mediated translocation of inner-membrane proteins (*15*). However, such a lateral opening would require that a large number of hydrogen bonds, holding the barrel structure, be broken and reformed, a thermodynamically challenging scenario.

The resolution of the crystal structure of YaeT's POTRA domains is just the beginning of the knowledge needed to fully comprehend the highly dynamic process of β-barrel OMP assembly. Several issues stay unresolved. For example, it remains to be determined whether P1-bound OMPs are subsequently handed over to other POTRA domains in a sequential and coordinated manner, or whether each POTRA domain independently participates in OMP assembly and OMP binding to them is determined by their availability and the OMP's folding/assembly status. Another key pending issue that needs resolution is how soluble POTRA domains coordinate their activities with the membrane-bound β-barrel domain of YaeT to insert OMPs into the OM. These and other questions concerning the mechanistic aspect of YaeT are bound to be resolved in the near future.

YaeT/Omp85 is essential for the transport of OMPs in both prokaryotes and eukaryotes. Substrate specificity of the YaeT/Omp85 family of proteins is likely determined by their POTRA domains. The structure of YaeT's POTRA domains provides an excellent opportunity to develop chemotherapeutics in the form of peptides that compete with native substrates and thereby inhibit YaeT's activity.

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