Trends in Biochemical Sciences

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Review

From Chaperones to the Membrane with a BAM!

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Outer membrane proteins (OMPs) play a central role in the integrity of the outer membrane of Gram-negative bacteria. Unfolded OMPs (uOMPs) transit across the periplasm, and subsequent folding and assembly are crucial for biogenesis. Chaperones and the essential β-barrel assembly machinery (BAM) complex facilitate these processes. *In vitro* studies suggest that some chaperones sequester uOMPs in internal cavities during their periplasmic transit to prevent deleterious aggregation. Upon reaching the outer membrane, the BAM complex acts catalytically to accelerate uOMP folding. Complementary *in vivo* experiments have revealed the localization and activity of the BAM complex in living cells. Completing an understanding of OMP biogenesis will require a holistic view of the interplay among the individual components discussed here.

OMP Assembly Is a Prerequisite for Membrane Integrity

The outermost membrane of Gram-negative bacteria is the ultimate protective barrier of the cell, serving as the first line of defense that guards against extracellular threats. Composed of both lipids and thousands of **outer membrane proteins** (OMPs, see Glossary), biogenesis of **outer membrane** (OM) components and consequent OM integrity is essential for cell viability [1]. Targeting these processes is a promising route for directed drug design against bacterial pathogens. Understanding of the OMP assembly machinery in *Escherichia coli* has grown immensely owing to recent discoveries using several orthogonal techniques that include the publications of the crystal structures of key proteins in the past 12 months. In this Review we highlight recent discoveries involving the roles of chaperones and OM-localized folding factors in OMP assembly because understanding the underlying mechanisms for these processes will facilitate their efficient therapeutic targeting.

β-Barrel OMPs Take a Long and Winding Road to Their Native Environment

Typical OMPs share a β-barrel folded topology composed of a closed cylinder of antiparallel β-strands. The hydrophilic interiors of OMP β-barrels are often water-accessible, while the exteriors are hydrophobic and lipid-exposed. Adjacent β-strands interact through hydrogen bonding and are covalently connected by short periplasmic turns and longer extracellular loops. Accurate OMP biogenesis is a prerequisite for the formation of this native and functionally-active conformation.

The biological assembly pathway of OMPs is particularly challenging because these polypeptides must cross multiple compartments in an unfolded form to reach the OM (Figure 1, Key Figure). OMPs are synthesized by ribosomes in the cytoplasm of Gram-negative bacteria. The open reading frames of OMP sequences contain an N-terminal signal sequence that targets these newly translated chains to the **SecYEG** (light purple in Figure 1) translocon via interactions with **SecB** [2]. Subsequently, **unfolded outer membrane proteins** (uOMPs, red in Figure 1) are secreted across the bacterial inner membrane through SecYEG using the energy of the **SecA** motor [3]. After entry into the periplasm, uOMPs remain unfolded until they reach the

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Chaperones hold uOMPs in dynamic conformational ensembles to prevent potentially deleterious uOMP aggregation.

The BAM complex functions as an enzyme to accelerate uOMP folding *in vitro*.

Distortions in the β-barrel domain of BamA, as well as defects in the surrounding lipid bilayer, may contribute to the catalytic function of BamA.

Multiple conformations of the BamA soluble polypeptide-transport associated (POTRA) motifs may play a role in the BamA enzymatic mechanism.

The BAM complex facilitates insertion of uOMPs into the outer membrane in a spatially discrete nature *in vivo*.

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Key Figure

Depiction of Outer Membrane Protein (OMP) Biogenesis Components in *E. coli* Shown To Scale

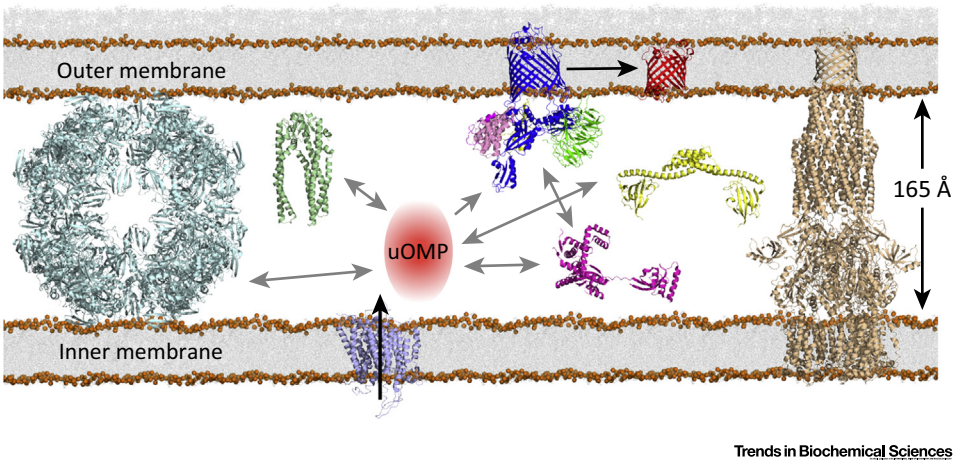

Figure 1. Unfolded OMPs enter the periplasm via the SecYEG translocase (light purple, PDB: 5ABB), which is located in the bacterial inner membrane. Once in the periplasm, unfolded OMPs (uOMPs) interact with several chaperones, including: DegP (cyan, PDB: 3CSO), FkpA (yellow, PDB: 1Q6U), SurA (magenta, PDB: 1M5Y), and Skp (pale green, PDB: 1U2M). The outer-membrane localized BAM complex (BamABCDE shown in blue/green/magenta/pink/yellow respectively, PDB: 5D0O) facilitates uOMP folding in the OM (e.g., OmpLA; red, PDB: 1QD5). The multiprotein Acr complex (tan, PDBs: 1EK9, 2F1M, 2DHH) was used to position the inner and outer membranes. The structures of these membranes were derived from molecular dynamics (MD) simulations of smaller patches that were concatenated to make this image [72]; phosphate atoms of lipid head-groups are shown in orange spheres. Arrows indicate known interactions, although the exact sequence and mechanisms of these interactions are unknown. Note the peptidoglycan is excluded from this figure. This image was created in PyMOL [73].

asymmetric outer membrane into which they fold (Figures 1,2). It is crucial to note that uOMPs requires a lipid bilayer to fold into their native conformation, and this means that they must traverse the ~165 Å aqueous periplasm in an unfolded but folding-competent state [4]. Herein lies a dilemma: uOMPs are prone to aggregate in aqueous environments [5,6], and the thermodynamically favorable but kinetically slow process of uOMP aggregation directly competes with the productive folding pathway of uOMPs. This potential dead-end fate for an unfolded OMP is avoided *in vivo* by the presence of periplasmic chaperones. This issue of uOMP aggregation is confounded by the fact that the periplasm lacks ATP [7]. Therefore, periplasmic chaperones must prevent uOMP aggregation and its associated cell stress in the absence of an external energy source. To accomplish this important cellular feat, the thermodynamics and kinetics of chaperone–uOMP interactions must be fine-tuned to maintain uOMP proteostasis in the absence of ATP [8–11].

How Do Periplasmic Chaperones Safeguard uOMPs Without an External Energy Source?

One mechanism periplasmic chaperones employ to prevent aggregation is to protect uOMPs by sequestering them within a defined internal uOMP cavity. **Seventeen-kilodalton protein** (Skp, light green in Figure 1) and the serine endoprotease **DegP** (cyan in Figure 1) are both oligomeric chaperones with defined internal binding cages that accommodate uOMPs (Figure 1) [12–14]. Most structural studies have focused on uOMP–Skp interactions [8,9]. In the apo (i.e., uOMP

Glossary

Atomic force microscopy-based single molecule force spectroscopy (AFM-SMFS): a branch of scanning probe microscopy in which the tip of a cantilever mediates mechanical unfolding of a single folded protein molecule.
β-Barrel assembly machinery (BAM): *E. coli* OM-localized multiprotein complex composed of the OMP BamA (formally YaeT, Omp85) and lipoproteins BamBCDE (formally YfgL, NlpB, YfiO, and SmpA, respectively).
DegP: *E. coli* serine endoprotease, a periplasm-localized oligomeric protein that is known to bind to uOMPs and degrade misfolded OMPs.
FkpB binding protein A (FkpA): *E. coli* periplasm-localized dimeric *cis/trans* prolyl isomerase that is known to bind to uOMPs.
Lipopolysaccharide (LPS): a polymer of sugars that is covalently linked to the outer leaflet Lipid A of the outer membrane of Gram-negative bacteria.
Molecular dynamics (MD): a computational technique that applies Newton's laws of motion to derive conformational trajectories of systems of atoms with time.
Nuclear magnetic resonance (NMR): a solution-based spectroscopic technique in which electromagnetic radiation is used to probe an atomic chemical environment and discern information about biomolecular structure.
Outer membrane (OM): a region of Gram-negative bacteria; this membrane is asymmetric in that the inner leaflet is composed of phospholipids and the outer leaflet contains lipid A/LPS.
Outer membrane proteins (OMPs): β-barrel proteins that reside in the outer membrane of Gram-negative bacteria.
Phosphoethanolamine (PE): a zwitterionic lipid head-group that contains a primary amine and phosphate linked by two carbons.
Phosphoglycerol (PG): a negatively charged lipid head-group that contains a glycerol moiety linked to phosphate.
Polypeptide-transport associated (POTRA) motif: ~70 residue soluble periplasm-localized motif with conserved architecture in *E. coli* BamA protein.