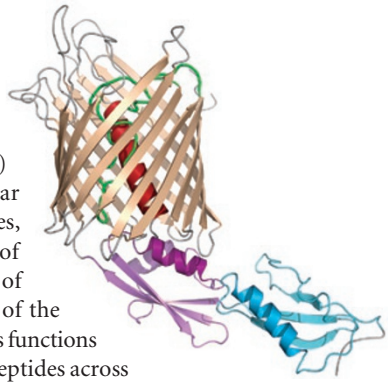
To the outer membrane and beyond



Gram-negative bacteria, mitochondria and chloroplasts use members of the Omp85 superfamily to assemble β-barrel outer membrane proteins (OMPs) with a variety of cellular functions, including enzymes, adhesins and transporters of small molecules in or out of cells or organelles. A subset of the Omp85 superfamily members functions in the translocation of polypeptides across the outer membrane. All Omp85 members have a β-barrel domain and a soluble periplasmic region with low sequence conservation and a variable number of polypeptide transport–associated (POTRA) domains, which are thought to be involved in protein-protein interactions. Two recent papers provide structural and functional information on different members of the Omp85 superfamily, affording insight into the mechanisms by which they assemble or translocate their substrates and the different roles for POTRA domains within the superfamily. Jacob-Dubuisson, Villeret and colleagues have solved the crystal structure of FhaC from *Bordetella pertussis*, which transports this bacterium's major adhesin, filamentous hemagglutinin (FHA). The structure of FhaC features its β-barrel domain plugged by an extracellular loop, L6, whose deletion abolishes FHA transport and alters FhaC's conductance properties. The authors use mutagenesis to map FhaC's two periplasmic POTRA domains. They propose a model where the N-terminal region of FHA is recognized by the POTRA domains, which serve as an anchor to orient the central part of FHA toward the β-barrel. This region of FHA would push the L6 loop from the interior of the barrel, effectively opening the channel for FHA translocation across the outer membrane. Kahne, Silhavy and collaborators have solved the structure of a periplasmic region from YaeT, the core component of the OMP assembly complex in *Escherichia coli*. YaeT has five POTRA domains, and the crystallization construct contained full-length domains 1–4, all with similar folds. The effects of deletions of individual POTRA domains support a role of YaeT as a scaffold for the other components of the OMP assembly complex. YaeT crystallized as a dimer, which is probably not physiologically relevant, stabilized by contacts between two β-strands from different subunits in a parallel orientation. Such a mode of contact, called β-strand augmentation, might be involved in YaeT's interaction with the other components of the OMP assembly complex, as well as its substrates, unfolded OMPs. YaeT may thus serve as a template for the folding of β-strands. (*Science* **317**, 957–961 and 961–964, 2007) *IC*

Dissecting membrane fusion

Vesicle fusion, which occurs in various processes including organelle trafficking and neurotransmission, requires Rab proteins, Rab effectors and SNAREs. SNAREs are able to bring the two membranes to be fused into physical proximity, but accessory proteins and special lipids are also needed to promote the bilayer rearrangements required for membrane fusion. Previous studies have shown that excessive amounts of SNAREs can promote *in vitro* liposome fusion independent of the accessory proteins. However, this is often done with a considerable amount of lysis, whereas little lysis occurs under normal fusion conditions *in vivo*. Wickner and colleagues have now examined this phenomenon further using the yeast lysosomal fusion pathway, which requires four SNARE proteins (located in *trans* on the vesicles to be fused), the Rab GTPase Ypt1p and the homotypic fusion and protein sorting (HOPS) complex. Using a *ypt1* knockout strain that also overexpresses all four SNAREs, the authors were able to produce lysis and fusion events independent of Ypt1p, requiring only the addition of Sec18p, a protein known to promote *trans*-SNARE pairing. Results of additional experiments using a modified liposome fusion assay suggest that formation of a SNARE–HOPS complex may be the rate-limiting step for fusion. Because overexpression of SNARE components can promote fusion rates similar to those observed for Rab-dependent events, the authors suggest that the role of Ypt1p may be to somehow facilitate SNARE–HOPS assembly through spatial concentration of these proteins. Although the data also reveal that normal levels of SNARE, Rab and Rab effector proteins promote efficient fusion with minimal amounts of vacuolar lysis, how this is achieved remains unknown and will undoubtedly be the focus of future studies. (*Proc. Natl. Acad. Sci. USA* **104**, 13551–13558, 2007) *MM*

Snapshot of Mg^{2+} transporter

Although Mg^{2+} is vital to many biological processes, the basis of Mg^{2+} transporter selectivity and control remains obscure. There are three major prokaryotic families of Mg^{2+} transporters, and the structure of a CorA family member has previously been solved. Nureki and colleagues now present the 3.5-Å structure of *Thermus thermophilus* MgtE, representative of a second widely conserved Mg^{2+} transporter family. MgtE is dimeric, with each subunit contributing five transmembrane helices and two cytosolic subdomains. The loops between the transmembrane domains contain small helices, an unusual characteristic; one helix even forms part of the periplasmic entrance of the 30-Å long pore. The conserved Asp432 from each dimer seems to interact with a region of strong electron density, one of five potential Mg^{2+} ions bound to the structure, and may be crucial for selectivity. In contrast to other transporters, the authors propose that the putative Mg^{2+} binding site might recognize the fully hydrated ion. Higher-resolution structures of the cytosolic domains in the presence and absence of Mg^{2+} indicate that when Mg^{2+} is not bound to this region, the more open cytosolic structure may allow ions to pass through the pore, suggesting a basis for channel regulation and Mg^{2+} homeostasis. A similar model had been suggested for the architecturally distinct CorA transporter, pointing to Mg^{2+}-triggered conformational change as a general regulatory mechanism for these transporters (though, importantly, mammalian MgtE transporters lack this proposed regulatory cytoplasmic domain). Though the structure seems to support a channel-like mechanism and shows no evidence for a counter-transported ion, MgtE resembles a secondary active transporter when viewed from its cytoplasmic side. This structure thus suggests a number of testable hypotheses, and further work can now assess the proposed mechanistic models. (*Nature*, advance online publication 15 August 2007, doi:10.1038/nature06093) *SL*

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