BIOCHEMISTRY

Getting Into and Through the Outer Membrane

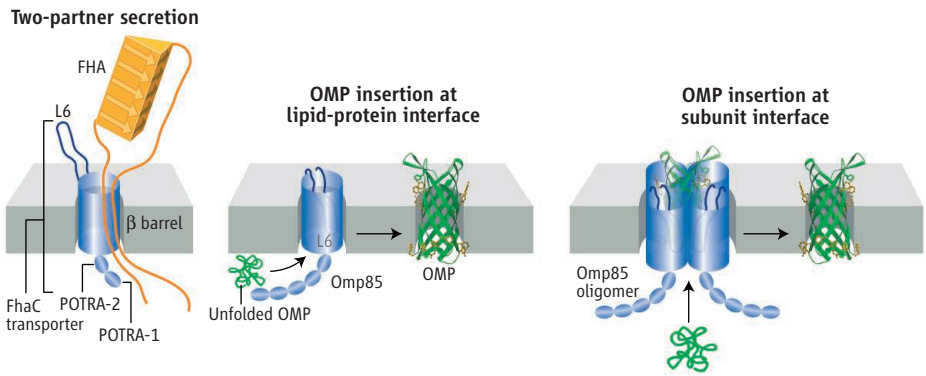
The structures of two related bacterial membrane proteins help to understand protein transport processes in the outer membranes of bacteria, mitochondria, and chloroplasts.

Jan Tommassen

Two membranes surround Gram-negative bacteria, as well as mitochondria and chloroplasts in eukaryotes. Transport of proteins into or through the outer of these membranes usually requires complex molecular machines. Omp85, an evolutionary conserved protein, is the central component of the machine required for folding and inserting outer membrane proteins (OMPs) (*1*). Moreover, some OMPs with sequence similarity to Omp85 are involved in transport processes such as protein secretion in bacteria and protein import into chloroplasts (*2*). In this issue, Kim *et al*. (p. 961) and Clantin *et al*. (p. 957) provide insights into the structures of members of this protein superfamily (*3*, *4*).

The bacterial Omp85 consists of a membrane-embedded β barrel and an amino (N)–terminal periplasmic extension encompassing five polypeptide transport–associated (POTRA) domains. It interacts directly with its substrate proteins (*5*) and is part of a complex that also contains four lipoproteins, YfiO, YfgL, NlpB, and SmpA, of which only one, YfiO, is essential (*6, 7*). The mitochondrial Omp85 homolog contains only one POTRA domain, which directly interacts with substrate proteins (*8*). The accessory lipoproteins are not found in the mitochondrial system.

Kim *et al.* (*3*) report the structure of a fragment of the Omp85-family member YaeT from *Escherichia coli*. The fragment encompasses four complete POTRA domains and, at the carboxyl (C) terminus, a short segment of the fifth one. Each POTRA domain consists of a three-stranded β sheet and two α helices. In the crystal, the fragment forms a dimer as a result of the augmentation of the β-sheet in POTRA-3 with a β strand formed at the C-terminal end of the other subunit. Although this dimer is a crystallization artifact, the dimer interface may reflect the way in which YaeT interacts with its substrates; this interaction involves a signature motif that forms a β strand at the C termini of these proteins (*5*). Thus, like the POTRA-5 segment in the crystallized YaeT fragment, this signature motif forms a β strand at the C termini of these proteins.



Protein traffic in the outer membrane. (**Left**) Clantin *et al.* suggest that in two-partner secretion, substrate binding to POTRA-1 opens a channel in the FhaC transporter by displacement of pore-blocking segments (L6). Extracellular folding of the secreted FHA protein into a β helix probably provides energy for transport. (**Middle**) Binding of an OMP to the POTRA domains of an Omp85 protein, such as YaeT studied by Kim *et al.*, results in outer membrane insertion, possibly at the protein/lipid interface. For simplicity, accessory proteins of the Omp85 complex are not shown. (**Right**) A central channel formed by oligomers of Omp85 superfamily members might provide an alternative route for insertion and/or translocation.

Kim *et al*. investigated the possibility that the subunit interface is involved in substrate recognition. Mutations in POTRA-3 that should prevent β augmentation did not interfere with YaeT function but with binding of the YfgL subunit of the machine. Thus, POTRA-3 may bind YfgL by β augmentation. In a series of mutants in which all POTRA domains were deleted one at a time, all deletions severely infringed function. Furthermore, deletion of POTRA-2, -3, -4, and -5 each resulted in loss of YfgL from the machine. Deletion of POTRA-5, which shows the highest sequence conservation of all POTRA domains (*1*), resulted in loss of all accessory lipoproteins.

Clantin *et al*. (*4*) solved the structure of FhaC, a member of the Omp85 superfamily involved in the secretion of filamentous hemagglutinin (FHA) in *Bordetella pertussis* via a pathway known as two-partner secretion. The structure shows a β barrel and an N-terminal extension consisting of an α helix and two periplasmic POTRA domains structurally resembling those of YaeT. The β barrel consists of 16 antiparallel β strands connected by short turns at the periplasmic side and long loops at the cell surface. The channel within the barrel is occluded by loop L6, which folds into the barrel, and by the N-terminal α helix, which spans the channel interior. The residual opening of 3 Å is too narrow to allow for transport of a protein, even in an extended conformation.

However, upon reconstitution of FhaC into planar lipid bilayers and application of a transmembrane potential, much wider channels were revealed with a conductivity of 1200 pS (*9*), corresponding to channel widths of 8 to 10 Å. Thus, the channel appears to be dynamic: Upon binding of FHA to POTRA-1 (*4*), the channel may open by extrusion of the α helix and/or loop L6, thus creating a protein translocation pathway (see the figure, left panel). Previous work indeed showed a topological rearrangement in L6 upon coexpression of FHA (*10*).

What can we learn from the FhaC structure about the C-terminal domain of Omp85 proteins? Omp85 showed much narrower channels in planar lipid bilayers than FhaC. Their conductivity of 120 pS (*5*) could correspond to the closed channels observed in the FhaC crystal structure. Omp85 sequences show a conserved motif that corresponds to the L6 loop of FhaC, but no segment corresponding to the N-terminal helix. Thus, assuming that Omp85 has a similar 16-stranded β barrel as FhaC, another loop besides L6 should contribute to closing the channel.

In the planar lipid bilayer experiments, substrate binding increased the channel activity of Omp85 (*5*), but this increased activity reflected a higher probability for the open state, rather than a widening of the low-conductance chan-

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nels. Hence, there is no indication that Omp85 channels can open by displacement of pore-blocking segments. Indeed, it is unlikely that OMPs would insert into the β barrel of Omp85, because it is difficult to envisage how such a barrel could subsequently open laterally to allow for OMP insertion into the membrane. Rather, OMPs will insert at the lipid/protein interface (see the figure, middle panel) or at the subunit interface of an oligomeric complex (see the figure, right panel).

Omp85 forms defined homo-oligomeric complexes in vitro (*5*). Similarly, HMW1B, an FhaC homolog involved in two-partner secretion in *Haemophilus influenzae*, has been purified from the outer membrane as a tetramer (*11*). In liposome-swelling assays, both proteins showed pore sizes of 2.5 to 2.7 nm—much wider than the channel within the FhaC β barrel. Furthermore, electron microscopy has shown that the HMW1B oligomer formed ringlike structures with a central cavity of 2.5 nm (*11*). The possible involvement of these wide channels in protein traffic (see the figure, right panel) needs to be investigated.

The mechanism and the pathway of protein traffic via members of the Omp85 superfamily is still far from understood. Future experiments should focus on the characterization of the oligomeric complexes and on the development of in vitro systems with purified components to study these processes.

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CELL BIOLOGY

Aneuploidy in the Balance

An extra chromosome slows yeast cell proliferation, suggesting that aneuploid human cells must overcome this effect during carcinogenesis.

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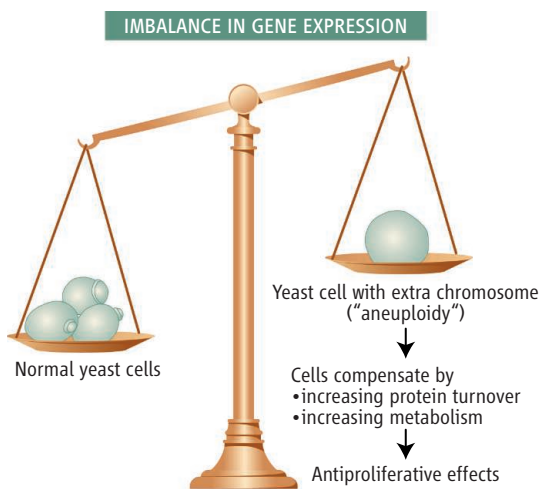
A central principle of genetics is that cells within an organism contain the same complement of chromosomes. The presence of too many or too few chromosomes, called aneuploidy, is associated with disease, and accounts for the majority of spontaneous miscarriages in humans, as well as hereditary birth defects such as Down syndrome (*1*). Precisely how aneuploidy affects cells is not well understood. Extra chromosomes cause a proportionate increase in gene expression (*2*), potentially altering a cell's dosage of proteins in damaging ways. On the other hand, most cancer cells are aneuploid, suggesting that some patterns of chromosome gain and loss enable cells to escape normal growth restraints and develop into malignant tumors—for example, by acquiring extra copies of an oncogene, or losing a tumor suppressor gene (*3*, *4*). But are the effects of aneuploidy strictly specific to a given over- or underrepresented chromosome, or does aneuploidy evoke a generalized physiological response regardless of what chromosome is affected? A new study by Torres *et al.* (*5*) on page 916 of this issue uncovers characteristics shared by all aneuploid cells, identifying a broad cellular response to aneuploidy that has ramifications for better understanding aneuploidy-linked diseases in humans.

Torres *et al.* analyzed the budding yeast *Saccharomyces cerevisiae*, a well-established and tractable system for studying chromosome segregation errors (*6*). In general, aneuploid yeast cells are at a substantial competitive disadvantage relative to cells with a normal complement of chromosomes (euploids) because they are eventually overtaken by spontaneously arising euploid revertants (*7*, *8*). However, aneuploidy can be beneficial in the presence of strong selective pressure (*9*, *10*). For example, where yeast has two similar genes on different chromosomes, cells in which one of these paralogs is deleted may compensate by the chance gain of an extra copy of the chromosome bearing the other paralog (*10*). Torres *et al.* engineered yeast strains to contain two copies of specific chromosomes (disomes) on an otherwise haploid genetic background. By varying the identity of the extra chromosome, the authors generated disomic strains encompassing 13 of the 16 yeast chromosomes. As expected, genes present on disomic chromosomes were transcribed at about twice their normal levels. However, after correcting for this effect, two groups of genes were coordinately up-regulated in many different aneuploid strains. One cluster, previously characterized as part of the environmental stress response, is also induced in many slow-growing but euploid strains. However, the other cluster, whose expression increased in aneuploid strains independently of growth rate, includes genes involved in ribosome biogenesis. Ribosome biogenesis consumes roughly half of the metabolic energy of a proliferating yeast cell, and it is tightly coupled to signaling pathways that regulate progress through the G_1 phase of the cell division cycle (*11*). Indeed, a substantial fraction of the aneuploid strains examined by Torres *et al.* exhibited a delay in cell cycle entry and an increase in cell size, demonstrating a



More genes but less fit. Yeast cells that gain an extra chromosome are at a proliferative disadvantage relative to normal cells, regardless of the specific chromosome gained. Aneuploid cells try to compensate for the gene imbalance by increasing protein turnover, which requires more energy and slows down proliferation. Cancer cells somehow overcome the antiproliferative effect of aneuploidy.

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