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Membrane proteins — pumping along

Editorial overview

Stephen H White and J Eric Gouaux

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The pace of discovery of membrane protein structures and their function has picked up remarkably during the past few years. If all goes well, we can expect to know the structures of a hundred membrane proteins before the year ends [1]. Most of these proteins concern themselves with getting ‘stuff’ across membranes, such as ‘signals’, metabolites of all kinds, and even proteins. They often use cellular energy to pump the stuff, either directly or indirectly (the big exception is ion channels). In some cases, the structures reveal exactly how pumping occurs; in others, the structures just tantalize us, which is part of the fun of studying membrane proteins. A problem with crystallographic structures is that we capture them in a static state, leaving open the question of exactly how they do their thing in the membrane bilayer. The big questions about all membrane proteins are how they are integrated into the membrane in the first place and the role of the membrane in shaping their structure [2]. The reviews presented in this year’s Membranes section cover these general topics, and more.

Progress on the question of how proteins are integrated into membranes, which involves pumping proteins across or into membranes, is the subject of the review by [White and von Heijne](#). They report from the perspective of a transmembrane helix’s journey toward the haven of three-dimensional structure. Like all journeys, there is a beginning, a mid-passage, and an arrival. The mid-passage — translocon-guided insertion into the membrane — is filled with mystery and cunning. A major mystery is the exact organization of the translocon assembly upon which the ribosome docks to transmit its elongating polypeptide into or across the membrane. The veil of mystery is beginning to be lifted through steadily improving image reconstructions of ribosomes mated with the translocon. The resolution of the images is still not quite good enough to solve the mystery completely, but the major components of the translocon assembly seem to have been identified. One mystery does seem to have been solved, however. It is now clear that the elongating chain must pass through a pore in the center of one of the four SecY/Sec61 heterotrimeric proteins that are the principal components of the translocon assembly, rather than through a pore formed by the juncture of the four proteins. The details about how a transmembrane helix finds its way from SecY/Sec61 into the membrane bilayer remain unsettled. Nevertheless, the basic rules that the translocon follows for identifying amino acid segments as transmembrane helices have been determined.

A potential helix’s journey to the translocon begins in the exit tunnel of the ribosome. [White and von Heijne](#) discuss the most recent evidence supporting the emerging idea that elements of secondary structure can form in the tunnel. The end of the journey of a transmembrane helix is really not an end at all, because the helices must somehow assemble by physicochemical processes to create the final three-dimensional structure. These processes

are easier to study in model systems. White and von Heijne examine the interesting progress being made toward understanding the behavior of helices in pure lipid bilayers and, in some cases, detergent micelles. A long-held view has been that the lipid bilayer adapts itself to helices, becoming thicker or thinner in the vicinity of the helix as necessary to match the hydrophobic length of the helix. Although there is evidence of this in some cases, it appears that it may be more common for the reverse to be true. That is, the helix adjusts its angle of tilt to optimize hydrophobic matching.

The active transport of ions across cell membranes is essential for cell homeostasis. The most familiar example is perhaps $\text{Na}^+\text{-K}^+\text{-ATPase}$, which pumps potassium into nerve and muscle cells and sodium out in order to maintain the electrochemical gradients necessary for the generation of action potentials by passive flow of ions down the gradients via voltage-sensitive ion channels. Because the pump works against the electrochemical gradient, energy must be expended by ATP hydrolysis. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ is an example of a P-type ATPase, a family so-named because members form a covalent phosphorylated-aspartyl intermediate in the course of pumping. It has been known for many years that there are four states during the transport cycle that exchanges potassium for sodium ions [3]. In order to understand the transport cycle at the molecular level, one needs a structure of the protein in each of the four states.

Elegant structural studies by the Toyoshima laboratory in Japan and the Møller laboratory in Denmark have now determined the structures necessary to see all four states of a $\text{Ca}^{++}\text{-ATPase}$, a P-type ATPase closely related to the $\text{Na}^+\text{-K}^+\text{-ATPase}$. This ATPase pumps Ca^{++} ions into the sarcoplasmic reticulum (SR) of muscle cells in exchange for H^+ to create a large reservoir of Ca^{++} . An action potential traveling along a muscle cell stimulates release of Ca^{++} into the myoplasm, which in turn activates the contractile machinery. The structural basis of active Ca^{++} transport by the rabbit SR is described by Møller *et al.* in their review of the $\text{Ca}^{++}\text{-ATPase}$.

Gram-negative bacteria such as *Escherichia coli* also use $\text{Na}^+\text{-K}^+\text{-ATPase}$ to create sodium and potassium ion gradients across their cytoplasmic membranes. But unlike muscle and nerve cells, which are bathed in a rather constant extracellular solution maintained by the kidney and other organs, bacteria face the challenge of living in wildly varying environments. Because the external concentrations of essential chemicals are often very low, active transport is essential. But the process is complicated, because bacteria also face the challenge of getting nutrients and essential metabolites across their outer membranes as well as their cytoplasmic membranes. Populating the outer membrane of Gram-negative bacteria are β -barrel membrane proteins and, even though

these proteins facilitate transport across the outer membrane, they do not directly hydrolyze ATP or other energy-rich substrates. The management of active transport across the outer membrane is thus a complex business — Baroque in the words of Wiener, who reviews TonB-dependent transport across the outer membrane of Gram-negative bacteria. As he explains, active transport by proteins such as BtuB [4] is achieved by coupling outer membrane transporters to a complex of inner membrane proteins that can hydrolyze energy-rich substrates. The central player is the so-called TonB protein. Just exactly how TonB and the TonB-dependent transporters (TBDTs) work together to transport essential compounds is a major mystery.

The trimeric porins of the outer membrane provide passageways through their β barrels for the passive movement of small solutes, a luxury not found in the TBDTs. Instead, in all of the structures solved to date, the β barrels of TBDTs are blocked by a hatch domain. A major challenge, as Wiener discusses, is to understand the role of the hatch domain and how the restriction of the domain is managed during passage of actively transported substances.

The TBDTs are generally involved in the transport of hydrophilic molecules. Gram-negative bacteria must also transport hydrophobic molecules, especially long-chain fatty acids (LCFAs). The only outer membrane proteins known to transport hydrophobic substances all belong to the so-called FadL family. van den Berg, who recently solved the structure of *E. coli* FadL [5], discusses LCFA transport in his review of the mechanism of transport of the FadL family. He notes that FadL differs significantly from other hatch-containing β barrels, such as BtuB, because they do not require exogenous energy input from the TonB or TolA systems. As he explains, his structure suggests that FadL orthologs probably use spontaneous conformational changes to transport substrates by diffusion. He solved the structure of FadL from two crystal forms. By a stroke of good fortune, the two structures each had a bound LDAO detergent molecule, but in different conformations due to conformational differences in the hatch domains. These differences provide important clues to the mechanism of transport.

Transmembrane signaling can be considered a transport process, but the stuff being transported is information rather than a solute of some kind. The classic examples of this class of membrane protein are the G-protein-coupled receptors (GPCRs), which are crucial in neurobiology and pharmacology. Indeed, they constitute one of the largest gene families in the human genome. Pharmaceutical scientists dream of having lots of structures in order to design new drugs. But, alas, GPCRs have proven exceptionally difficult to crystallize. The bright exception is rhodopsin, whose crystallization benefits from its very

high concentration in vertebrate rod outer segments. Since the first structure was reported in 2000 [6], remarkable progress has been made toward obtaining better structures by both X-ray and electron diffraction. Scherter, in his review of the structure of rhodopsin, notes that the three-dimensional structures obtained from two-dimensional crystals by electron diffraction are particularly important, because the position of rhodopsin and lipid molecules can be studied in an authentic membrane bilayer environment.

The lipid environment of rhodopsin is especially important for its function, as discussed by Feller and Gawrisch in their review of how the physical and chemical properties of highly polyunsaturated fatty acids (PUFAs), such as the unsaturated fatty acid docosahexaenoic acid (DHA), affect the properties of the membrane bilayer and, consequently, the function of rhodopsin and other GPCRs. The authors emphasize that long-standing concepts of the role(s) that PUFAs play in modulating the properties of membranes need to be revised. In particular, they provide evidence that PUFAs are dynamic and flexible lipids that confer unique properties on the membrane bilayer, such as a decrease in order of saturated fatty acid groups, and an increase in 'softness' to bending and surface area perturbations. Because rhodopsin and presumably other GPCRs undergo conformational changes upon activation, the ease with which the membrane bilayer deforms can, in turn, affect the stability of activated signaling states. Beyond these non-specific effects, rhodopsin also contains chemically specific binding sites for lipid molecules and occupancy of these sites, in turn, provides an additional and direct mechanism by which PUFAs and other lipids can modulate the activity of metarhodopsin-II, the key activated state of rhodopsin.

As noted at the beginning of this overview, a shortcoming of most membrane protein structural studies is that the proteins are not visualized in their native bilayer environments. The vast improvements in algorithms and hardware during the past several years, together with the growing number of high-resolution membrane protein structures, have made it possible for molecular dynamics (MD) simulations to be used rather routinely for examining membrane proteins in lipid bilayers. In their review, Schulten and colleagues summarize the excellent progress being made in this area, especially for proteins involved in passive solute transport, such as ion channels and water transporters. Noteworthy advances include probing conformational changes in membrane proteins via the application of different MD simulation strategies and the calculation of accurate electrical properties of membrane proteins. Studies of the mechanosensitive ion channel MscS are just one example of computational studies providing significant insight. In this case, the crystal structure of MscS revealed an open channel, but MD simulations show that the crystallographic con-

formation spontaneously collapses to a closed state, thus suggesting that the conformation studied by crystallography may correspond to a meta-stable, partially open state. α -Hemolysin, a bacterial 230 kDa toxin that creates large and non-specific pores in target cells membranes, has been studied by extensive MD simulations in which calculated current/voltage relationships compared well with those obtained from experiment. Even though the application of computational methods to membrane proteins requires careful thought, there is little doubt that these methods can contribute substantially to our understanding of the relationships between structure, dynamics and function for the proteins and lipids of the membrane bilayer.

It seems fair to say that studies of the molecules of the biological membrane are telling us that there is a great deal that we do not understand about the fundamental structural elements of membrane proteins, the conformational changes that these proteins undergo, and the extent to which the dynamic properties of the proteins are coupled to the physical and chemical nature of the lipid bilayer. From the experimental structural perspective, an important challenge for the future is the development of methods that will allow membrane proteins to be trapped in specific conformational states. As revealed from studies of the Ca^{++} ATPase, the availability of reagents to trap conformational states was crucial to the elaboration of the corresponding crystal structures. In many cases, however, such equivalent reagents are simply not available. The judicious application of computational studies, together with other experimental, non-crystallographic approaches (including NMR and EPR [7]), can be used to explore the conformational and electrostatic properties of membrane proteins, allowing us to venture along conformational reaction pathways that are not yet amenable to direct structure determination.

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Update

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Erratum

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Editorial overview

[*Current Opinion in Structural Biology* 2005, **15**:375–377][☆]

Stephen H White and J Eric Gouaux

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In our Editorial overview of the 2005 Membranes section of *Current Opinion in Structural Biology*, we stated, incorrectly, that “Gram-negative bacteria such as *Escherichia coli* also use Na⁺-K⁺-ATPase to create sodium and potassium ion gradients across their cytoplasmic membranes”. Professors Peter Jorgensen and Ronald Kaback have reminded us that this is not correct. *E. coli* do not have a Na⁺-K⁺-ATPase in their cytoplasmic membrane [1]. We apologize for this egregious cognitive lapse. But how do

E. coli maintain transmembrane gradients of sodium and potassium ions? Potassium ions are pumped into *E. coli* by a number of transport systems, the best studied being the ATP-driven Kdp transport system [2]. Sodium ions are actively exported from the cell by at least two Na⁺/H⁺ antiporters, NhaA and NhaB, which utilize the H⁺ electrochemical gradient generated by the respiratory chain or by the F₁-F₀ ATPase [3].

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