Bacteriorhodopsin — the movie

Werner Kühlbrandt

For 30 years and more, the mechanism of a microbial proton pump has been subject to increasingly sophisticated analysis. The full picture of how the pump operates is now emerging.

Bacteriorhodopsin, a small, robust protein from the cell membrane of a salt-loving microorganism, pumps protons out of cells and provides them with the energy to live. But how exactly does it work? The basic function of bacteriorhodopsin as a light-driven proton pump was recognized[1] not long after its discovery in the 1960s[2], yet the details of the molecular motions and mechanisms resulting in directed proton transport are being described only now.

Elsewhere in this issue (page 645 on), no fewer than three papers[3–5] address this question. Together with earlier work[6–10], they give us the first complete motion picture of bacteriorhodopsin in action. The path of the proton through the membrane can now be followed in exquisite detail.

Over the years, bacteriorhodopsin has become the object of scrutiny for an entire branch of science. It has fostered more than 30 years of ingenious experimentation, driving the development of some of the most advanced techniques in biochemistry, biophysics and structural biology. Thousands of papers have been published about it, and whole scientific conferences are devoted to it. This is not just because it is the molecule that enables an exotic organism to flourish at unhealthy salt concentrations, using sunlight as the sole source of energy. Rather, two further circumstances have driven the huge interest in bacteriorhodopsin.

First, it serves as a simple model for certain cell-membrane receptors, known as G-protein-coupled seven-helix receptors, which include most well-known drug targets in humans and which probably operate by a similar switch mechanism[3]. Second, it is the prototype of a membrane transporter. These are biological macromolecules performing the amazing feat of transporting ions against an electrochemical potential — up to 250 millivolts in the case of bacteriorhodopsin, which translates into a 10,000-fold difference in proton concentration on either side of the membrane. Such transport processes are fundamental to all forms of life.

Bacteriorhodopsin is a deceptively simple molecular machine. It consists of seven membrane-spanning helical structures (A to G in Fig. 1), linked by short loops on either side of the cell membrane. The membrane forms a barrier around every cell which is normally impermeable to ions and nutrients needed to sustain life. Each bacteriorhodopsin contains one molecule of a linear pigment called retinal, one end of which is attached to the nitrogen atom of a lysine residue in helix G. The other end is wedged deep in the protein. Retinal changes its structure in response to visible light. The polypeptide harnesses the light energy trapped by the retinal and uses it to push a single proton through the seven-helix bundle, from the cell interior to the outside. As it does this, the molecule passes though several intermediates, referred to as K, L, M, N and O, which have different colours and have been well characterized by spectroscopy. The ground state and the L, M and N intermediates are shown in Fig. 1.

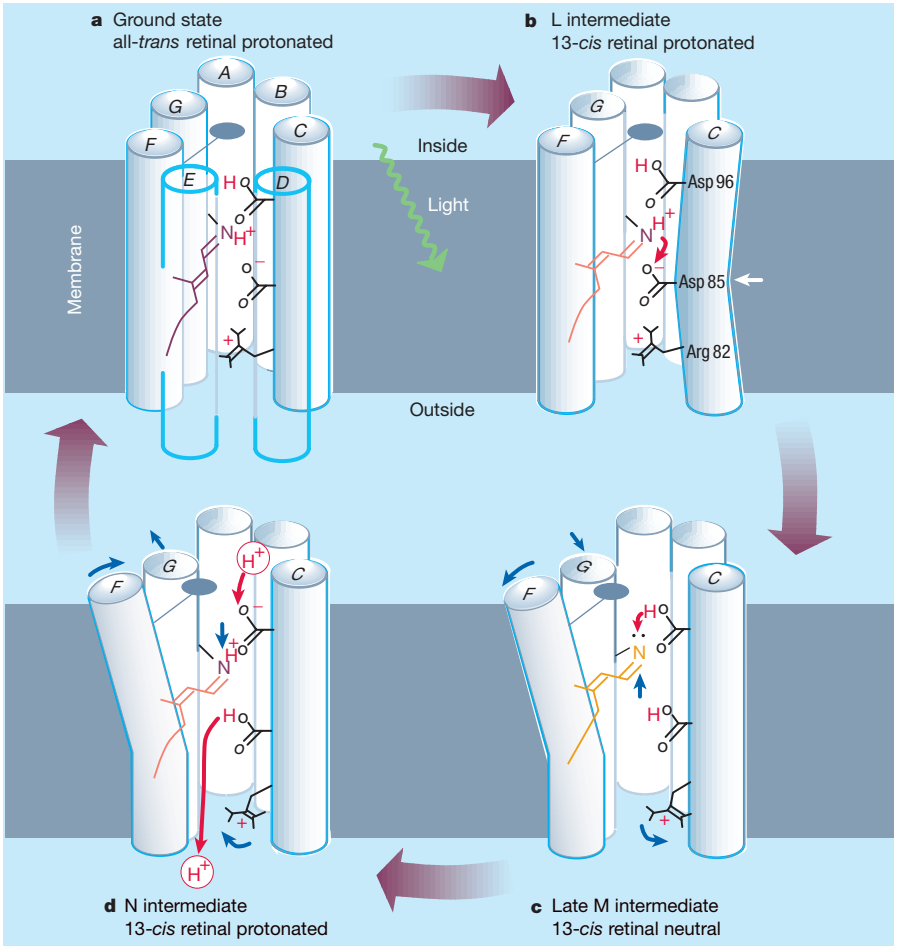

Figure 1 **Molecular mechanism of proton (H^+) pumping in bacteriorhodopsin. Retinal is bound in the space between the seven membrane-spanning helices (A to G) by a lysine in helix G.** a, **Light-induced isomerization of the protonated retinal from all-*trans* (purple) to 13-*cis* (pink) triggers the transfer of the proton to aspartate 85, aided by a slight movement of this residue in the L intermediate** (b) **towards the nitrogen atom. In the M state** (c), **the deprotonated retinal (yellow) straightens, pushing against helix F and causing it to tilt. This opens a channel on the inner, cytoplasmic side of the membrane through which aspartate 96 is reprotonated** (d), **having given up its proton to the nitrogen on the retinal. Aspartate 85 transfers its proton through a network of hydrogen bonds and water molecules to the outside medium, past arginine 82, which has moved slightly. Red arrows, proton movements; blue arrows, movements by groups of atoms. Helices D and E are omitted in** b–d **for clarity. The 'paddle' attached to helix F represents the bulky side chains, which move to open the cytoplasmic proton channel.**

The structure of the ground (default) state was determined ten years ago[6] by electron

cryo-microscopy of two-dimensional crystals that form in the cell membrane. In the past few months, structures of the K (ref. 8) and M (ref. 9) intermediates have been determined by X-ray crystallography of microcrystals[11]. This was followed by the structure of the N intermediate determined by electron microscopy of two-dimensional crystals[10]. The M and N intermediates were of bacteriorhodopsin mutants that have slow proton-pumping cycles and are therefore easier to handle. They are now joined by another electron-microscopic structure of a mutant representing the key M intermediate[3], and by X-ray structures of the wild-type (non-mutant) M (ref. 5) and L intermediates[4].

The structures of the various intermediates are all slightly different from each other and from the ground state, and it is fascinating to see how they combine to illustrate the proton-pumping cycle. The main events are shown in Fig. 1. First, the retinal is hit by a photon and changes its configuration from the all-*trans* to the 13-*cis* form, which takes about one picosecond (K state). In the 13-*cis* state, one end of the retinal is twisted around a double bond which causes this part of the pigment to move relative to the protein scaffold. As a result, the proton located on the nitrogen finds itself in an energetically unfavourable environment and within about 50 microseconds moves to aspartate 85, by way of tyrosine 89 (ref. 3) or aspartate 212 and a tightly bound water molecule[5], to form the L intermediate. The proton transfer is helped by a small movement of helix C, which brings the side chain of aspartate 85 closer to the nitrogen atom, as shown by the structure of the L state[4].

The retinal turns from pink to yellow and straightens as it becomes deprotonated. Because the retinal is tethered at both ends, the nitrogen atom moves upwards towards the cell interior by 0.7 to 1 Å (refs 3, 9), pushing against some bulky residues on helix F. By lever action, the upper part of helix F swings out by about 3.5 Å in late M, while the top of helix G moves partly into its place[3,10].

In the transition to the N state, the retinal is reprotonated from aspartate 96 and flexes again. The movement of helices F and G opens a narrow channel through which aspartate 96 is reprotonated[3,5,10]. In the N to O transition, the proton on aspartate 85 is transferred to an extended network of hydrogen bonds including several water molecules in the lower half of the channel[5,9]. The end of the positively charged arginine 82 moves downwards slightly during M (refs 5, 9), facilitating the release of the proton on the outside. Finally, the retinal relaxes to the all-*trans* form, helices F and G swing back to their original position, and another proton-pumping cycle can begin.

The primary motions of the proton-pumping action in bacteriorhodopsin are surprisingly small, involving movements of groups of atoms by 1 Å or less in response to the flexing and unflexing of the retinal. This affects the proton affinity of neighbouring side chains through a change of their local chemical environment. The key event is the straightening of retinal as it sheds its proton in M, as shown by a mutant[3] that has helices F and G stuck in the open state but still pumps protons, albeit inefficiently. The retinal thus acts as a valve in the middle of the membrane, imparting a unique direction to the pumping process.

One or two puzzles remain. Several experiments[7,12,13] have indicated that there is a single, large conformational change in the protein scaffold during the pumping cycle. This change occurs during the transition from early to late M (ref. 7) and is manifest as a movement of helices F and G in the structures of the N and the M intermediates determined by electron cryo-microscopy of two-dimensional crystals[3,10]. Yet the X-ray structures of the M state either describe a smaller, different movement[5] or completely fail to show it because the relevant parts of helices F and G are not visible[9]. This may be because the molecule has not yet gone all the way from early to late M, or is unable to complete the transition, because in three-dimensional crystals this helix movement is obstructed. Further investigation will clarify this point.

The molecular mechanism of proton pumping by bacteriorhodopsin will be an inspiration for the study of other equally fascinating but considerably more complex membrane transporters. Well-known examples include the Ca-ATPase[14], a calcium pump active in muscle contraction, and the ubiquitous transporters that control the levels of neurotransmitter substances in the brain. These transport proteins and the G-protein-coupled drug receptors all undergo similarly subtle conformational changes as part of their molecular mechanisms. To fully understand how they work, we now need to investigate their structures at the same level of detail as bacteriorhodopsin. ■

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Quasicrystals

Electrons in a strange sea

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Did you start your day using electricity — for instance, by switching on a light, turning on a computer or using a toaster? If so, you made use of the fact that electrons can move through a metal such as copper with reasonably high efficiency. This efficiency comes about because the electrons float throughout the metal in an 'electron sea' — another way of saying that the electronic quantum states, which describe the confinement of electrons in metals, are highly delocalized (sometimes called extended or band-like). But our understanding of the way in which electrons move in metals has just been presented with a new challenge, as described by Rotenberg *et al.*[1] on page 602 of this issue. The quandary arises from trying to understand electronic states in a special type of metallic alloy known as a quasicrystal.

Electrons cannot move with complete freedom in a metal — their motion is moderated by a number of factors, most important of which is the arrangement of atomic nuclei. Before quasicrystals were discovered, all known crystals displayed a periodic arrangement of their atoms. In other words, the structure was built up from single building blocks — the particular arrangement of atoms in a unit cell — stacked in three dimensions. Electrons moving through such an arrangement of nuclei can be described by Bloch functions, a mathematical treatment that shows how the spatial distributions of the electron energies (or momenta) reflect the symmetry of the atomic lattice.

Until now, quasicrystals have defied similar treatment because the rules by which nature arranges their atoms are different from the 'rules of periodicity', even though the positions of their atoms are predictable — that is, quasicrystals are aperiodic but well-ordered. A simple example of this type of order, in one dimension, is the Fibonacci sequence. Each number in the sequence is generated from the sum of the preceding two, and many quasicrystals follow an analogous construction rule in three dimensions to produce long-range atomic order without conventional periodicity. As a result, some quasicrystals have fivefold or tenfold