Forest of Brazil, the average size of restricted use, protected areas is a mere 9210 hectares, and a significant proportion of the total area and number of remnants are in the small size classes. Even in regions such as the Amazon forest, the deforestation frontier leaves a highly fragmented landscape in its wake (*5, 12*). Further thwarting conservation efforts are the raging fires that have become epidemic during the dry season, and many parks have experienced repeated incursions by wildfires (*18*).

Given current knowledge about edge effects, coupled with increasing matrix harshness, we believe that small fragments (<5000 hectares) in most tropical regions are in serious and immediate danger of suffering the receding edge phenomenon. If so, a large proportion of forest remnants in fragmented landscapes that are already protected or available for conservation are doomed to disappear without proper management. Clearly, the future of these reserves demands active and costly management commensurate with the preservation of the last vestiges of tropical ecosystems.

Preemptive planning is necessary to implement conservation on regional and global scales, where such opportunities still exist. For frontier tropical wilderness areas, the recognition of the dynamic nature of edges demands that conservation planning rapidly migrate to the landscape scale (*27*). This approach is exemplified by the proposed large-scale biodiversity corridors of the Brazilian rainforests (*28*) and the Mesoamerican corridor (*29*). Only through such measures will there be any hope of conserving tropical landscapes and saving forest fragments from a vanishing future.

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PERSPECTIVES: STRUCTURAL BIOLOGY

A Chloride Pump at Atomic Resolution

John L. Spudich

Membrane proteins are involved in selective transport across the cell membrane and the sensing and transmission of information from the extracellular environment to the cell interior. In *Halobacterium salinarum*, both transport and sensory signaling are accomplished by a family of four archaeal rhodopsins, homologous proteins that are modifications of a common design. The proteins each contain seven membrane-embedded helices with a conserved interior binding pocket surrounding a retinal prosthetic group (*1–3*). Photochemical reaction cycles similar in early steps are induced by retinal photoisomerization in each of the four proteins but have distinctly different outcomes. On page 1390 of this issue, Kolbe *et al.* (*4*) report a new structure that sheds light on how proton and chloride transport mechanisms are related in different members of this family.

Bacteriorhodopsin uses light to power transport of a proton from the cytoplasm to the extracellular medium (see the figure). Halorhodopsin is also a light-driven ion pump but has different specificity and acts in the opposite direction, moving a chloride ion from the extracellular medium into the cell cytoplasm. The resulting electrochemical ion gradients provide a source of energy to the cell and enable pH control. The sensory rhodopsins (SRI and SRII) are phototaxis receptors. Each of them is a subunit of a signaling complex that includes a transducer protein (HtrI and HtrII) that controls a phosphorylation cascade modulating the flagellar motors. Numerous homologs of these proteins have been found in other haloarchaea. A eukaryotic photoactive homolog, exhibiting a very similar photochemical reaction cycle to that of SRII, has also been identified in the fungus *Neurospora crassa* (*5*).

Experiments that convert one function to another suggest a common mechanism for the different functions of the archaeal rhodopsins. At acid pH, the proton pump bacteriorhodopsin transports chloride (*6*), and in the presence of azide the halide pump halorhodopsin transports protons (*7*). Moreover, a single mutation, $Asp^{85} \rightarrow Thr$, converts bacteriorhodopsin into a chloride pump with halorhodopsin-like photochemical reactions (*8*). Separation from its tightly bound transducer HtrI subunit converts SRI from a sensory to a transport rhodopsin, which pumps protons across the membrane in the same direction as does bacteriorhodopsin. HtrI interacts with SRI by transmembrane helix-helix contacts (*9*) and inhibits the pump by closing SRI's cytoplasmic proton channel (*10*).

To elucidate the underlying molecular mechanism that confers this multiplicity of basic membrane functions on variants of a single evolutionary progenitor protein, we need atomic resolution structures of the proteins in the dark and in their light-activated forms. Bacteriorhodopsin was first visualized by electron crystallographic analysis of two-dimensional (2D) crystals

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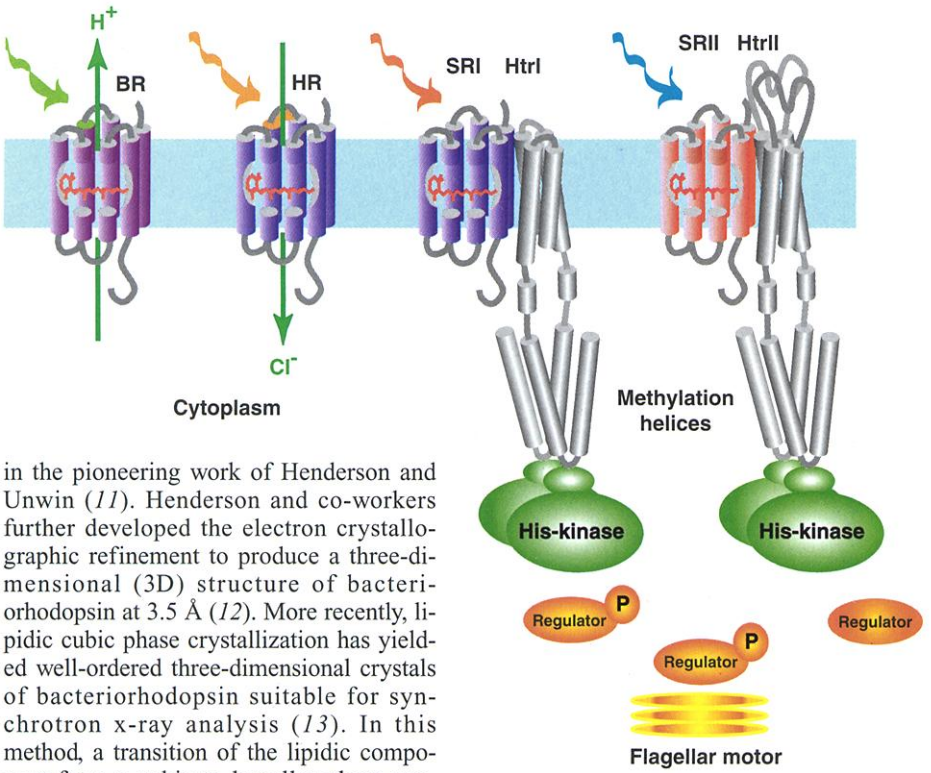

The four archaeal rhodopsins in *H. salinarum*. Schematic representations of bacteriorhodopsin (BR), halorhodopsin (HR), and the sensory rhodopsins I and II (SRI and SRII) with components in their signal transduction chains. The colors of the four rhodopsins are the approximate colors of the pigments. The sensory rhodopsins are complexed to their cognate transducer proteins, HtrI and HtrII, which have adaptative methylation and histidine kinase-binding domains that modulate flagellar motor switching through a cytoplasmic phosphoregulator. The transducers are drawn as dimers based on disulfide cross-linking studies. It is not certain whether the stoichiometry is one or two SR molecules per Htr dimer. The relative positions of the Htr and SR helices are arbitrary and chosen for illustration only.

in the pioneering work of Henderson and Unwin (*11*). Henderson and co-workers further developed the electron crystallographic refinement to produce a three-dimensional (3D) structure of bacteriorhodopsin at 3.5 Å (*12*). More recently, lipidic cubic phase crystallization has yielded well-ordered three-dimensional crystals of bacteriorhodopsin suitable for synchrotron x-ray analysis (*13*). In this method, a transition of the lipidic component from a cubic to lamellar phase produces bacteriorhodopsin 3D crystals that are essentially highly ordered stacks of 2D crystals, like those used for electron crystallography. The electron crystallography-derived structures provide the phase information, enabling atomic-resolution x-ray structure determination by molecular replacement. Six additional bacteriorhodopsin structures, with resolutions ranging from 2.3 to 3.5 Å, were determined by either electron or x-ray crystallography between 1997 and 1999 (*14*). Since then, Luecke *et al.* have produced an even higher resolution structure (1.55 Å) of bacteriorhodopsin (*15*).

A new landmark is now reported by Kolbe *et al.* (*4*). The authors grew halorhodopsin crystals in a lipidic cubic phase and accomplished the first x-ray structure determination of this anion pump at an incisive 1.8 Å resolution. The best structure available before this study, determined by electron crystallography at a resolution of 5 Å (*16*), was very similar to bacteriorhodopsin. A striking feature of the new x-ray structure is that the positions of the helix backbones are nearly identical to those in bacteriorhodopsin even at this higher resolution. The root mean square deviation from bacteriorhodopsin positions is only 0.74 Å for the 122 α-carbon positions in the most conserved helices (C to G). The precise positioning of the transmembrane helix backbones is evidently important to function.

A single chloride ion occupies a site within the photoactive center of the molecule, 18 Å beneath the membrane surface. Its solvation sphere is highly irregular, containing predominantly hydrogen atoms derived from aliphatic groups contacting the halide. A patch of five arginine residues occurs on the cytoplasmic side; however, no chloride is bound to this positive surface patch nor to the extracellular surface despite the high chloride concentration in the crystal. The position occupied by the chloride ion is nearly identical to that of the carboxylate of Asp^{85} in bacteriorhodopsin, the proton acceptor and principal counterion to the protonated Schiff base. This finding lends support to the idea that chloride and proton transport occur by an equivalent mechanism. Kolbe *et al.* propose that after retinal photoisomerization flips the NH dipole [see fig. 6C in (*4*)], the mobile chloride is electrostatically dragged across the protonated Schiff base before it is released on the cytoplasmic side of the protein. In bacteriorhodopsin [see fig. 6C in (*4*)], the anchored negative charge of Asp^{85} attracts the proton from the protonated Schiff base because of the same forces that cause the chloride to move in halorhodopsin.

The structure reveals that in the dark state of the protein, there is no chloride-conducting pathway on the cytoplasmic side. Therefore, a light-induced conformational change in halorhodopsin must open such a channel for chloride release, as is believed to occur for proton uptake during the bacteriorhodopsin photocycle (*17*). This conformational change is likely to be the mechanistic link between the two transport rhodopsins and the sensory rhodopsins (*2*). The unifying principle may be that the very similar electrostatic rearrangement in the photoactive site in each of the archaeal rhodopsins opens the protein structure on the cytoplasmic side and that this conformational change is used for the differing molecular functions.

The high-resolution structure of halorhodopsin reported by Kolbe *et al.* (*4*) paves the way for crystallographic characterization of photointermediate states during the chloride pumping cycle. There may be advantages to monitoring the passage of chloride through halorhodopsin over studying the transport of the proton through bacteriorhodopsin, because there is only one chloride. Such studies hold great promise for understanding anion transport in general and the unifying principles in the archaeal rhodopsin family in particular.

CREDIT: ADAPTED FROM (*18*)

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