Mechanism of proton transport from crystallographic structures of the nine states of the bacteriorhodopsin photocycle. J. K. LANYI, Dept. Physiology & Biophysics, University of California, Irvine, CA 92697, U.S.A.

In the last few years we have been able to trap the K (*1*), L (*2*), M_1 (*3*), M_2 (*4*), M_2' (*5,6*) and N' (*7*) intermediates of the bacteriorhodopsin photocycle in crystals, and determine their structures by x-ray diffraction to 1.43-1.62 Angstrom resolutions. With models proposed earlier for N and O from crystallography of non-illuminated mutants (*8, 9*), structures are now available for the initial bacteriorhodopsin state (*1*, *10*) as well as the eight intermediate states. We applied stringent criteria for evaluating whether the often small changes in the electron density maps are in the data or come from the refinement, i.e., are they real and meaningful? The structures reveal the reasons for protonation of the retinal Schiff base by Asp85, proton release to the extracellular membrane surface, the switch event that allows reprotonation of the Schiff base from the cytoplasmic side, side-chain and main-chain motions initiated in the cytoplasmic region, formation of a single-file chain of hydrogen-bonded water molecules that conducts the proton of Asp96 to the Schiff base, and reprotonation of Asp96 from the cytoplasmic surface. The refined models describe in atomic detail how the transformations of the photoisomerized retinal change its interaction with wat402, Asp85, and Trp182, and how the displacements of main-chain and functional residues, and the water molecules sequestered in the extracellular and cytoplasmic regions facilitate the transfer of a proton from one membrane surface to the other. The observed changes can be summarized as a detailed atomic-level model for the transport in this pump, that describes it as the gradual relaxation of the distorted retinal that causes a cascade of displacements of water and protein atoms that spreads to the rest of the protein and results in vectorial proton transfers to and from the Schiff base. Such local-global coupling of conformational changes may be the general principle for how ion pumps and receptors function (*11*).

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