Shining light on rhodopsin selectivity: How do proteins decide whether to transport \( H^+ \) or \( Cl^- \)?

DOI 10.1074/jbc.H120.016032
Keiichi Inoue*©
From the Institute for Solid State Physics, The University of Tokyo, Kashiwa, Chiba, Japan
Edited by Michael J. Shipston

The versatile microbial rhodopsin family performs a variety of biological tasks using a highly conserved architecture, making it difficult to understand the mechanistic basis for different functions. Besaw et al. now report structures of a recently discovered cyanobacterial \( Cl^- \)-pumping rhodopsin and its functionally divergent mutant that reveal how these transmembrane proteins create a gradient of activity with subtle changes. These insights are paralleled by a second recent report, which in combination answers long-standing questions about rhodopsin selectivity and will facilitate future engineering efforts.

Light serves as a source of energy and information across all domains of life. Miniscule photons can be used to initiate large changes in biomolecules, particularly in the microbial rhodopsins, where photon-induced isomerization of all-trans-retinal chromophore affects the biological functions of ion pumps, ions channels, phototactic sensors, enzymes, and so on. Microbial rhodopsins are part of a large family of photoreceptive membrane proteins with a conserved structure made up of seven-transmembrane \( \alpha \) helices (1). Because rhodopsins can perform such a wide variety of functions using this common compact structure, they have provided ideal model systems to study structure–function relationships in proteins using spectroscopy, biochemistry, and structural biology. Moreover, ion pumps and ion channels have been widely adopted as tools in optogenetics to manipulate the firing pattern of animal nerves with light, and further elucidation of the mechanism of ion-transporting rhodopsins is increasingly required to construct new ion-transporting optogenetic tools.

So far, four types of ion-pumping rhodopsins, including outward and inward \( H^+ \) pumps, an inward \( Cl^- \) pump, and an outward \( Na^+ \) pump, have been identified. Of these, the archaearal outward \( H^+ \) pump, called bacteriorhodopsin (BR), was the first to be discovered, in 1971. Then the archaearal inward \( Cl^- \) pump halorhodopsin (HR) was reported in 1977 and functionally characterized in 1982. BR has an Asp-Thr-Asp (DTD) motif in the third helix that plays a critical role during \( H^+ \) pumping; these three residues are highly conserved in closely related \( H^+ \) pumps. HR, in contrast, has a TSA motif, which is similarly critical for its chloride-specific activity. Interestingly, the mutation of the first D of the DTD motif to T in BR confers an inward \( Cl^- \) pumping function similar to HR (2), suggesting that this residue (Asp-85 in BR) might be responsible for determining whether an \( H^+ \) or \( Cl^- \) is transported. However, the reverse mutation of HR, changing the T of the TSA motif to D, did not result in the conversion to an \( H^+ \) pump (3). Hence, the determinants separating \( H^+ \) and \( Cl^- \) pump rhodopsins were not yet completely clear.

Recently, it was revealed that cyanobacteria have a new type of \( Cl^- \)-pumping microbial rhodopsins with a TSD motif. One of them, MastR (also known as MrHR) from *Mastigocladopsis repens*, functions as a \( Cl^- \) pump similar to the TSA-containing HR, but in this case, the Thr to Asp mutation (MastR T74D) was successful in creating an \( H^+ \) pump (4), thus achieving complete functional conversion between an \( H^+ \) pump (BR) and a \( Cl^- \) pump (MastR) by swapping only one residue. Moreover, another TSD-containing HR from *Synechocystis* sp. PCC 7509 called SyHR was shown to be able to pump a sulfate ion (\( SO_2^- \)) that other HRs are unable to transport (5). These findings suggest that TSD-containing HRs have molecular mechanisms for ion transport that differ from the archaearal TSA-containing HRs. To understand the mechanisms of these unique abilities, however, demands their three-dimensional structure at atomic level.

Besaw et al. now answer this call, revealing the X-ray crystallographic structure of MastR and its \( H^+ \) pumping mutant (6) in bicelles at 2.33 and 2.50 Å, respectively. In the structure of WT MastR, the substrate \( Cl^- \) is bound to Thr-74 and Ser-78, corresponding to the T and S in the motif, and to the Schiff base part of the retinal via a water molecule. In MastR T74D, the Asp occupies the position adopted by \( Cl^- \) in the WT protein, and strong hydrogen bonds similar to those found in BR are formed between the Asp, water molecule, and retinal (Fig. 1). This result supports the idea that a strong hydrogen-bonding network is essential for the \( H^+ \) pump, in agreement with previous spectroscopic and theoretical studies (1). Another structure of the same protein was also reported by Yun et al. (7). They further focused on the difference in an extracellular loop between MastR and SyHR. The replacement of residues in the extracellular loop in the former with the corresponding sequence from the latter resulted in MastR being able to transport \( SO_2^- \). A structure of the \( SO_2^- \)-transporting mutant revealed a more positively charged entrance to the putative extracellular ion pathway compared with the WT, suggesting that this entrance determines the anion selectivity of TSD-type HRs. Besaw et al. similarly point to differences in loops between MastR and BR that seem to explain their ion selectivity, suggesting an even broader role for the extracellular entrance.

These two structural studies illuminate the long-sought functional determinants between \( H^+ \) and \( Cl^- \) pumps and structural elements enabling divalent-anion pumping. Moreover, the new structures provide an exciting opportunity to revisit our understanding of rhodopsin evolution in general, in which the structural elements important for the function of the ancestral molecule were conserved.
in the descendants with diversified functions, as discussed by Besaw et al. There are, however, unsolved mysteries of ion transport by TSD-containing HRs remaining. For example, we do not know the structure of the SO$_2^-$-bound state during SO$_2^-$ transport. Also, although both studies suggested possible ion transport pathways in their respective proteins, these structures represent the dark state, so the precise structure of the transiently opened pathway has not been revealed. To solve these problems, further structural studies on the SO$_2^-$-bound protein and photoactivated intermediates are required. Freeze-trapping methods will help to capture intermediate structures under light illumination (8), but recently developed techniques, such as time-resolved serial millisecond crystallography (TR-SMX), available at synchrotron facilities (9), and the time-resolved serial femtosecond crystallography (TR-SFX) technique with X-ray free electron lasers (XFEL) (10), will provide not only structural insights into each photointermediate but also the dynamics of conformational change between them. It will be exciting to see how these and other explorations shed light on this fascinating protein family.

**Conflict of interest**—The author declares that he has no conflicts of interest with the contents of this article.

**Abbreviations**—The abbreviations used are: BR, bacteriorhodopsin; HR, halorhodopsin; PDB, Protein Data Bank.

**References**

1. Ernst, O. P., Łodowski, D. T., Elstner, M., Hegemann, P., Brown, L. S., and Kandori, H. (2014) Microbial and animal rhodopsins: Structures, functions, and molecular mechanisms. *Chem. Rev.* **114**, 126–163 CrossRef Medline

2. Sasaki, J., Brown, L. S., Chon, Y. S., Kandori, H., Maeda, A., Needleman, R., and Lanyi, J. K. (1995) Conversion of bacteriorhodopsin into a chloride ion pump. *Science* **269**, 73–75 CrossRef Medline

3. Muroda, K., Nakashima, K., Shibata, M., Demura, M., and Kandori, H. (2012) Protein-bound water as the determinant of asymmetric functional conversion between light-driven proton and chloride pumps. *Biochemistry* **51**, 4677–4684 CrossRef Medline

4. Hasemi, T., Kikukawa, T., Kamo, N., and Demura, M. (2016) Characterization of a cyanobacterial chloride-pumping rhodopsin and its conversion into a proton pump. *J. Biol. Chem.* **291**, 355–362 CrossRef Medline

5. Niho, A., Yoshizawa, S., Tsukamoto, T., Kurihara, M., Tahara, S., Nakajima, Y., Mizuno, M., Kuramochi, H., Tahara, T., Mizutani, Y., and Sudo, Y. (2017) Demonstration of a light-driven SO$_2^-$ transporter and its spectroscopic characteristics. *J. Am. Chem. Soc.* **139**, 4376–4389 CrossRef

6. Besaw, J. E., Ou, W. L., Morizumi, T., Eger, B. T., Sanchez Vasquez, J. D., Chu, J. H. Y., Harris, A., Brown, L. S., Miller, R. J. D., and Ernst, O. P. (2020) The crystal structures of a chloride-pumping microbial rhodopsin and its proton-pumping mutant illuminate proton transfer determinants. *J. Biol. Chem.* **295**, 14793–14804 CrossRef Medline

7. Yun, J. H., Park, J. H., Jin, Z., Ohki, M., Wang, Y., Lupala, C. S., Liu, H., Park, S. Y., and Lee, W. (2020) Structure-based functional modification study of a cyanobacterial chloride pump for transporting multiple anions. *J. Mol. Biol.* **432**, 5273–5286 CrossRef Medline

8. Matsui, Y., Sakai, K., Murakami, M., Shiroy, Y., Adachi, S., Okumura, H., and Koyama, T. (2002) Specific damage induced by x-ray radiation and structural changes in the primary photoreaction of bacteriorhodopsin. *J. Mol. Biol.* **324**, 469–481 CrossRef Medline

9. Weinert, T., Skopintsev, P., James, D., Dworkowski, F., Panepucci, E., Kekilli, D., Furrer, A., Brünle, S., Mous, S., Ozerov, D., Nagy, P., Wang, M., and Standfuss, J. (2019) Proton uptake mechanism in bacteriorhodopsin captured by serial synchrotron crystallography. *Science* **365**, 61–65 CrossRef Medline

10. Nango, E., Royant, A., Kubo, M., Nakane, T., Wickstrand, C., Kimura, T., Tanaka, T., Tono, K., Song, C., Tanaka, R., Arima, T., Yamashita, A., Kobayashi, J., Hosaka, T., Mizohata, E., et al. (2016) A three-dimensional movie of structural changes in bacteriorhodopsin. *Science* **354**, 1552–1557 CrossRef Medline