Crystal structure of heliorhodopsin

Rhodopsins consist of two distinct protein types, microbial rhodopsins (type-1) and animal rhodopsins (type-2). Type-1 microbial rhodopsins comprise seven transmembrane helices and an all-*trans* retinal chromophore attached to a lysine residue of the protein through a protonated Schiff base linkage and exhibit diverse functions such as pumps, channels, enzymes, and signaling. Recently, a distinct abundant group of microbial rhodopsins, heliorhodopsins (HeRs), were discovered by metagenomics [1] (Fig. 1). HeRs are widely present in bacteria, archaea, algae, and algal viruses. Although HeRs are phylogenetically distant from the type-1 rhodopsins, HeRs are predicted to have seven transmembrane helices and a retinal chromophore linked to lysine at the seventh transmembrane helix through a protonated Schiff base linkage, as in the type-1 rhodopsins. However, the distribution of charged residues and the functional analysis have suggested that HeRs are embedded in the membrane with their N termini facing the cell cytoplasm, which is the opposite orientation to those of the type-1 and type-2 rhodopsins.

HeRs exhibit a photocycle involving the K, M, and O intermediates upon light absorption, and have a long-lived photoactivated state ($\tau \sim 1$ s). HeR photocycles accompany retinal isomerization and proton transfer, as in the type-1 and type-2 rhodopsins, but protons are never released from the protein, even transiently. HeRs lack pump and channel activities, suggesting that HeRs function as light sensors through putative interaction partners. Moreover, the HeR-possessing species lack a retinal biosynthetic pathway in their genomes. Thus, HeRs are considered to work by efficiently binding an exogenous retinal, similar to actinorhodopsin [2]. HeRs and type-1 rhodopsins exhibit distant sequence similarities, and thus little is known about the overall fold and the photoactivation mechanism of HeRs.

We screened multiple HeRs and identified the Thermoplasmatales archaeon SG8-52-1 HeR (TaHeR) as a promising candidate. We purified and crystallized the full-length TaHeR by meso crystallization. We determined the TaHeR structure at 2.4 Å resolution [3]. The diffraction data was collected with an automated data-collection system, ZOO, at SPring-8 **BL32XU**.

The structure comprises seven transmembrane helices (TM), six loops and short N and C termini (Fig. 2(a)). Analysis of HeR 48C12 showed that the N terminus faces the cell cytoplasm [1], and we therefore refer to the loops on the N-terminal side as intracellular loops (ICL) 1–3, and those on the C-terminal side as extracellular loops (ECL) 1–3. ECL1 forms a long loop containing two antiparallel β-strands and ICL1 forms a three-turn α-helix. HeR forms a dimer with the symmetric protomers aligned along the crystallographic two-fold axis. High-speed atomic force microscopy (HS-AFM) images revealed that HeR also forms a dimer in a lipid bilayer, indicating that the dimer reflects the physiological condition (Fig. 2(b)).

To investigate the structural similarity between HeR and type-1 rhodopsin, we compare the structures of TaHeR and bacteriorhodopsin (BR), a representative type-I rhodopsin. The loop structures of HeR are completely different from those of BR (Fig. 2(c)). By contrast, the transmembrane region is more similar, although HeR and BR share only 8% sequence identity. An all-trans retinal is covalently bound to Lys238, forming the Schiff base, as in the type-1 microbial rhodopsins. Overall, HeR shares an essentially common fold with the type-1 rhodopsins. Despite these similarities, HeRs are embedded in the membrane in an inverted orientation relative to the other rhodopsins. The results of immunofluorescent staining analysis supported this inverted topology when HeR was expressed in mammalian cells. The intracellular and extracellular faces are positively and negatively charged, respectively (Fig. 2(d)), consistent with the 'positive-inside' rule.

From the Schiff base to the intracellular side, there are extensive water-mediated hydrogen-bonding interactions that play a key role in the photoactivation of HeR. By contrast, all of the residues directed toward the transmembrane region in the extracellular half are quite hydrophobic (Fig. 3(a)). No carboxylate residue is observed, which is critical for proton transfer in

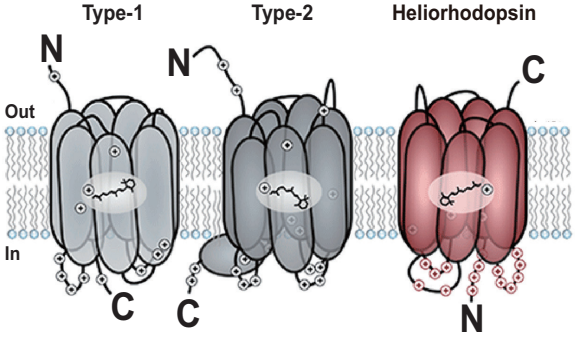

Fig. 1. Schematic of membrane topologies of type-1 rhodopsin, type-2 rhodopsin and heliorhodopsins.

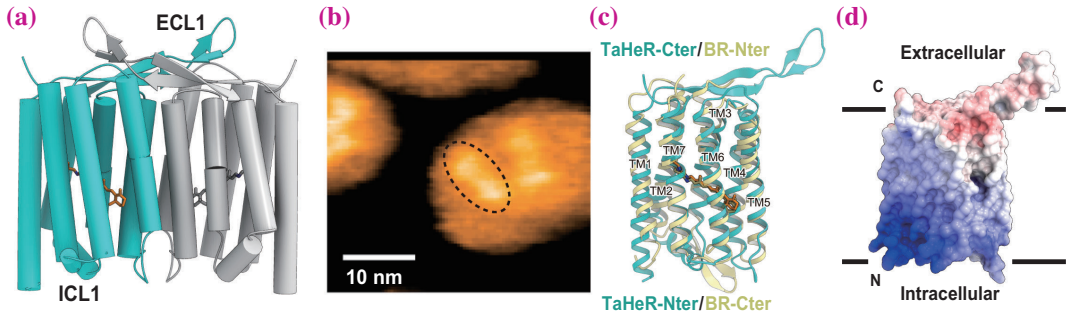

Fig. 2. Overall structure of TaHeR. **(a)** Schematic representation of TaHeR dimer. **(b)** Typical HS-AFM image of TaHeR dimers in the lipid membrane. **(c)** Superimposition of TaHeR and BR. **(d)** Electrostatic surface viewed from the membrane plane.

the proton-pumping rhodopsins. These hydrophobic residues fill the space in the extracellular half, preventing the permeation of protons and ions.

Unexpectedly, a lateral fenestration exists between TM5 and TM6, located above the β-ionone ring of the retinal chromophore, in the surface representation of the TaHeR structure (Fig. 3(b)). The β-ionone ring is exposed to the membrane environment through this fenestration, and the monoolein used in the crystallization fits within the fenestration and directly interacts with the β-ionone ring (Fig. 3(c)). The residue above the β-ionone ring is Gly171, which contributes to the formation of the fenestration. The G171W mutant showed no regeneration of the retinal chromophore in retinal bleaching and regeneration upon hydroxylamine (HA) analysis (Fig. 3(d)), and thus prevented retinal access through the lateral fenestration, suggesting that HeRs efficiently capture the exogenous retinal, which functions as light sensors, from the lipid environment through lateral fenestration.

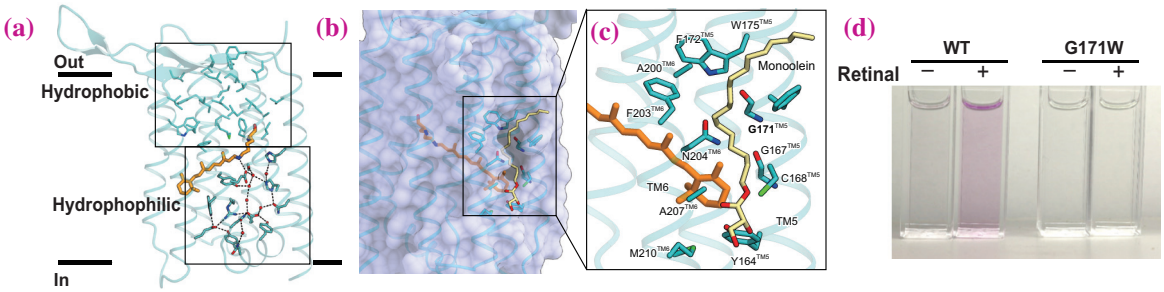

Fig. 3. **(a)** Intramolecular interactions viewed from the membrane plane. **(b)** Surface representation of the TaHeR structure. **(c)** Residues constituting the lateral fenestration and the monoolein are shown as sticks. **(d)** Pictures of TaHeR wild-type and G171W solutions without (–) and with (+) retinal.

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