Review

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Light-driven ion-translocating rhodopsins in marine bacteria

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Microbial rhodopsins are the photoreceptive membrane proteins found in diverse microorganisms from within Archaea, Eubacteria, and eukaryotes. They have a heptahelical transmembrane structure that binds to an all-*trans* retinal chromophore. Since 2000, thousands of proteorhodopsins, genes of light-driven proton pump rhodopsins, have been identified from various species of marine bacteria. This suggests that they are used for the conversion of light into chemical energy, contributing to carbon circulation related to ATP synthesis in the ocean. Furthermore, novel types of rhodopsin (sodium and chloride pumps) have recently been discovered. Here, we review recent progress in our understanding of ion-transporting rhodopsins of marine bacteria, based mainly on biophysical and biochemical research.

Microbial rhodopsins

The word 'rhodopsin' originates from the Greek words 'rhodo' and 'opsis', which indicate rose and sight, respectively. Thus, the classical meaning of rhodopsin is the red-colored pigment in the retina of eyes. The chromophore molecule that absorbs light is retinal, which is the origin of the red color. Similar colored retinal-binding proteins were then found in microbes, largely expanding the definition of 'rhodopsin'. The modern meaning of rhodopsin encompasses photoactive proteins containing a retinal chromophore in animals and microbes [1]. Rhodopsins are now found in all domains of life and are classified into two groups, animal and microbial rhodopsins. Animal rhodopsins contain an 11-*cis* retinylidene chromophore via protonated Schiff-base linkage to a conserved lysine residue in the seventh helix [1,2]. Animal rhodopsins carry out light-signal transduction as G protein-coupled receptors (GPCR) [3–7], where photo-activated animal rhodopsins induce a GDP/GTP-exchange reaction of transducin (G_t).

By contrast, microbial rhodopsins mainly bind to an all-*trans* type chromophore, and are found in various unicellular microorganisms in the Archaea, Eubacteria, and eukaryotes. Unlike animal rhodopsins, the functions of microbial rhodopsins are diverse [1,8,9]. The first microbial rhodopsin, bacteriorhodopsin (BR), was discovered from the cytoplasmic membrane of the haloarchaeon *Halobacterium salinarum* in 1971 [10,11]. BR pumps protons from the cytoplasmic to the extracellular side of the cell by using light energy [12,13]. After the discovery of BR, the rhodopsins functioning as light-driven inward Cl^- pumps (halorhodopsin, HR), with both positive and negative phototactic sensors (sensory rhodopsin I and II) were found in the same species [8,14–19]. All of these microbial rhodopsins bind a common chromophore, all-*trans* retinal (Figure 1A), which isomerizes to the 13-*cis* form if retinal absorbs light. The isomerization triggers subsequent conformational changes of the protein, leading to various functions. Although several similar microbial rhodopsins were found, they were mainly restricted to the Haloarchaea before 2000. However, metagenomic research identified a new species of microbial rhodopsin from the gene of an uncultured γ-Proteobacteria from Monterey Bay [20]. A cell suspension of *Escherichia coli* expressing this protein showed acidification upon illumination, suggesting that this rhodopsin functions as a light-driven outward H^+ pump similar to BR (Figure 1B). The new rhodopsin was named 'proteorhodopsin' (PR) from the name of the hosting bacterium [20]. Since the first report of PR, thousands of PR genes have been identified from various oceans to date [21–26]. It is estimated that 50% of microbes in the photic zone have PR genes [27]. Interestingly, new PR-like genes have also been found in some viruses [28,29].

While many PR genes were identified as acting as H^+ pumps from various oceans [22,30–33], other types of function were not discovered. This is in contrast to non-oceanic microbial rhodopsins, whose functions are diverse and include: H^+ pumps, Cl^- pumps, cation channels, positive phototaxis sensors, negative phototaxis sensors, and gene regulation [1,2,19]. However, a second type of function was reported for a rhodopsin of marine bacteria in 2013, namely an outward sodium (Na^+) pump rhodopsin (NaR) [34]. The first NaR was identified from the gene of the flavobacterium *Krokinobacter eikastus*. This bacterium has two rhodopsin genes: *Krokinobacter* rhodopsin 1 (KR1) and 2 (KR2). KR1 is homologous in sequence to PR and acidification occurred upon illumination of *E. coli* cells expressing KR1 (Figure 1B). However, a pH increase was observed when light was illuminated onto *E. coli* cells expressing KR2. The pH change was not due to the active transport of H^+ inward, because it was enhanced by the addition of a protonophore, carbonylcyanide m-chlorophenylhydrazone (CCCP). This means that KR2 transports ions other than H^+ that generate the membrane potential

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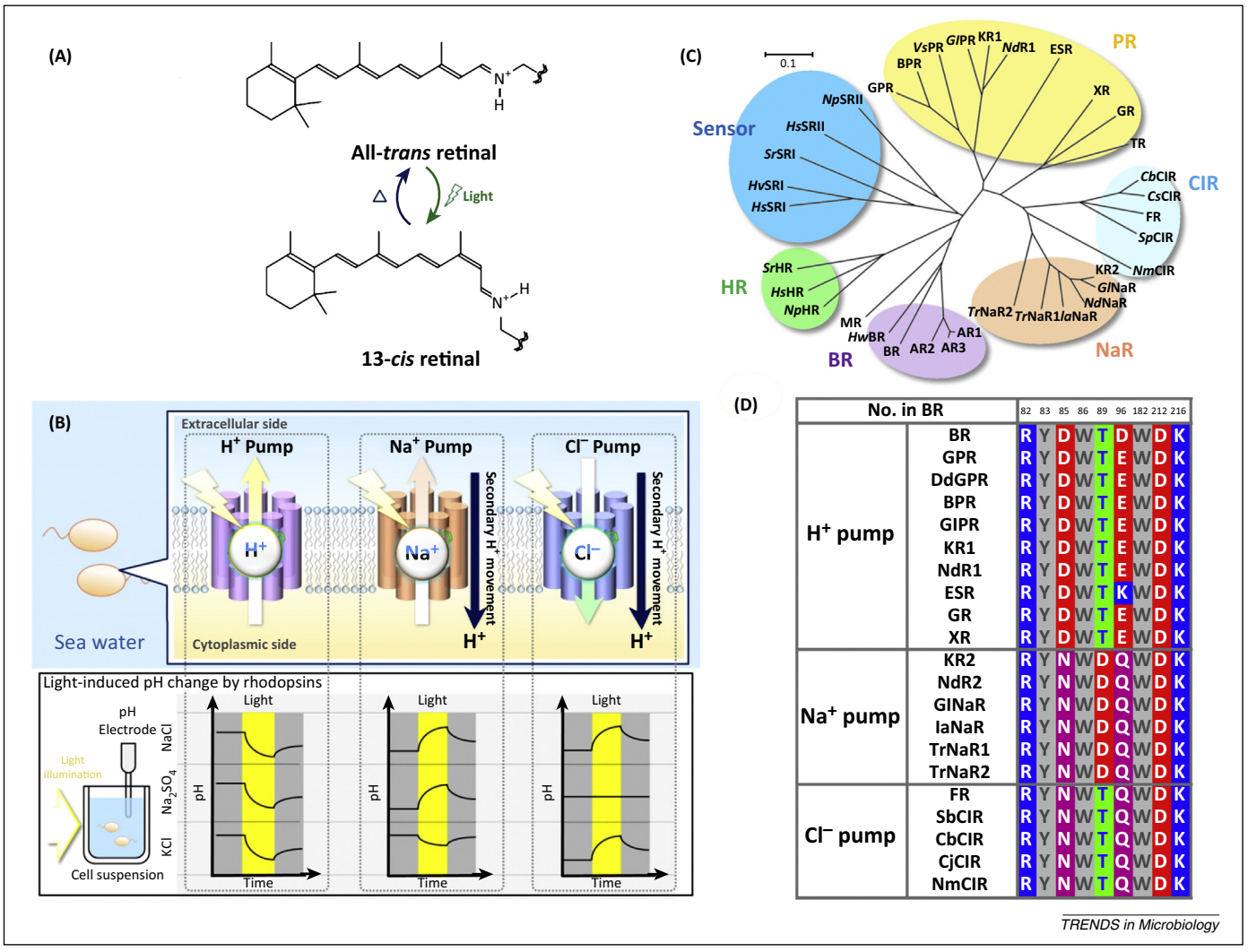

Figure 1. The diversity of microbial rhodopsins from marine bacteria. **(A)** The structure of all-*trans* retinal and 13-*cis* retinal bound to rhodopsin via the protonated Schiff base. All-*trans* retinal isomerizes to the 13-*cis* form upon the absorption of light. Re-isomerization from 13-*cis* is the thermal process that occurs at the end of the photocycle. **(B)** The ion-transport rhodopsins (outward H^+ pump, outward Na^+ pump, and inward Cl^- pump) found in marine bacteria. Their pumping ability upon illumination of light is assayed by the pH monitoring system under various solvent conditions (see main text) [34,37]. **(C)** Phylogenic tree of microbial rhodopsins. Unrooted CLUSTALW guide tree of selected microbial rhodopsins from public genome databases (http://www.ncbi.nlm.nih.gov/protein). The scale bar represents the number of substitutions per site (0.1 indicates ten nucleotides substitutions per 100 nucleotides). H^+ (proteorhodopsin; 'PR'), Na^+ (sodium pump rhodopsin; 'NaR'), and Cl^- (chloride pump rhodopsin; 'CIR') pumps of marine bacteria are indicated by yellow, orange, and cyan circles, respectively. 'BR', 'HR', and 'sensor' indicate H^+ (bacteriorhodopsin; BR), Cl^- (halorhodopsin, HR) pumps, and sensory rhodopsin, respectively from halophilic Archaea and Eubacteria. **(D)** The amino acid sequence alignment of microbial rhodopsins for the residues highly conserved in each type of rhodopsin (red, acidic; blue, basic; gray, aromatic; green, -OH bearing; magenta, asparagine and glutamine residues). PR, NaR, and CIR have DTE, NDQ, and NTQ motifs at the position corresponding to D85, T89, and D96 of BR (DTD), respectively. Abbreviations: AR1, archaerhodopsin-1; AR2, archaerhodopsin-2; AR3, archaerhodopsin-3; *Cb*CIR, *Cs*CIR, *Sp*CIR, and *NmCIR*, CIR from *Citromicrobium bathyomarinum*, *Citromicrobium* sp. JLT1363, *Sphingopyxis baekryungensis* DSM 16222, and *Nonlabens marinus*; *Gl*NaR, *Nd*NaR, *Ia*NaR, *Tr*NaR1, and *Tr*NaR2, NaR from *Gillisia limnaea*, *Nonlabens dokdonensis*, *Indibacter alkaliphilus*, and two NaR from *Truepera radiovictrix*, respectively; *Hs*SRI, *Hv*SRI, *Sr*SRI, sensory rhodopsin I from *Halobacterium salinarum*, *Haloarcula vallismortis*, and *Salinibacter ruber*, respectively; *Hs*SRII, *Np*SRII, sensory rhodopsin II from *H. salinarum* and *Natronomonas pharaonis*, respectively; *Hw*BR, BR from *Haloquadratum walsbyi*; MR, middle rhodopsin; *Np*HR, *Hs*HR, *Sr*HR, HR from *N. pharaonis*, *H. salinarum*, and *S. ruber*, respectively; TR, proteorhodopsin from *Thermus thermophilus*; *Vs*PR, *Gl*PR, *Nd*R1, proteorhodopsins from *Vibrio* sp. AND4, *G. limnaea* DSM 15749, and *N. dokdonensis* DSW-6, respectively; XR, xanthorhodopsin.

leading to inward H^+ movement. Similarly, a light-induced pH increase was reported for HR [15]. Given that HR transports anions, the pump efficiency depends on the size of anions present in the environment (Figure 1B, right) [15,35,36]. By contrast, even if anions were exchanged for larger ones, such as Br^- or even SO_4^{2-}, KR2 showed identical alkalization. However, the direction of the light-induced pH change was inverted in the solvent containing the salt of larger cations, such as KCl, RbCl, and CsCl [34]. The cation dependence of the pump function indicates that KR2 functions as an outward Na^+ pump in physiological sea water containing approximately 400 mM NaCl, while it pumps H^+ only in the presence of cation(s) larger than Na^+ (Figure 1B, middle).

Following NaR, the inward chloride pump rhodopsin (ClR) was discovered from the flavobacterium *Nonlabens marinus* S1-08^T [9,37]. This rhodopsin also shows an increase in pH upon illumination, similar to NaR. However, the dependence on ions is opposite to that of KR2. While pH increases even in solvents containing larger cations, such as K^+, it is abolished in the presence of larger anions, such as SO_4^{2-}. This indicates that ClR inwardly pumps Cl^- as well as HR does; in addition, the molecular mechanism of another light-driven chloride pump from the marine