

Nature's toolkit for microbial rhodopsin ion pumps

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Microbial rhodopsins function as light-driven [see Heberle et al.'s *Retinal Proteins—You can teach an old dog new tricks* (1)], are found in all three domains of life (2–4),

and were recently reported in viruses as well (5, 6). The first discovered microbial rhodopsin, the light-driven proton pump bacteriorhodopsin (BR), was identified in halophilic (high-salt loving) archaea more than 40 y ago (in 1971). Later on, the light-driven chloride pump halorhodopsin (HR) was discovered in the same archaeal microorganisms (for a historical perspective, see ref. 2). Both pumps were discovered using biophysical and physiological techniques. The widespread bacterial light-driven proton pump proteorhodopsin (PR), however, was discovered using metagenomics (7), and was initially detected based on a weak homology to BR (less than 30% identity on the protein level). Although diverse PRs are found in most marine microorganisms in the photic zone (8–11), BRs and HRs are restricted to halophilic archaea and are found only in hypersaline environments. The work of Yoshizawa et al. (12) in PNAS is now reporting on a new light-driven chloride pump, dubbed CIR (for Cl⁻ rhodopsin), which differs from HRs and is found in a marine bacterium (the flavobacterium *Nonlabens marinus*). Interestingly, this bacterium also possesses a PR protein and a light-driven sodium pump (NaR) [recently found in other flavobacteria (13–16)], which makes it, according to the authors, the first reported microbe to possess three different types of rhodopsin pumps.

The newly reported bacterial CIR has less than 25% identity to archaeal HR or BR proteins. In addition, none of the known amino acids implicated in chloride transport in HR are conserved in CIR. HR shares less than 35% identity to BR on the protein level, and in HR the corresponding amino acid to aspartate at position 85 of BR (the proton acceptor site) is threonine (yielding a protein with the motif TSA; see Fig. 1 for details). A single amino acid change in BR in which aspartate 85 is replaced by threonine

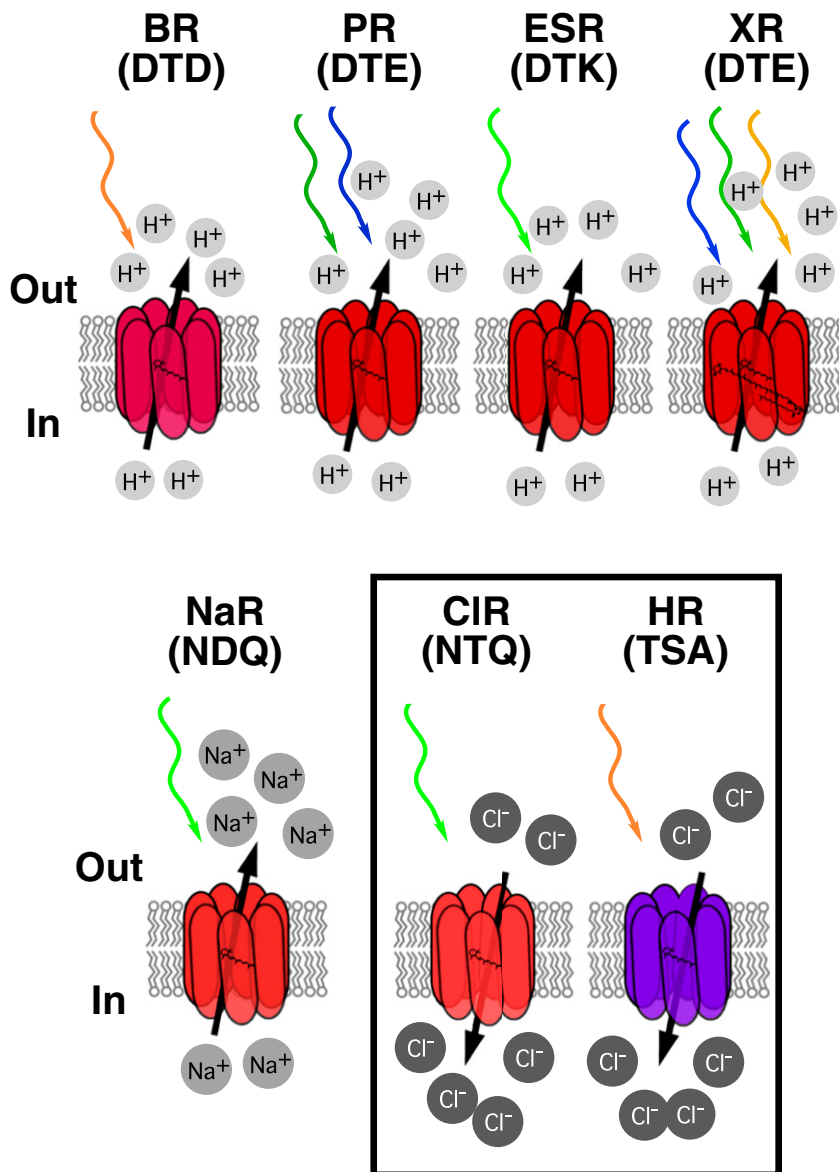


Fig. 1. Schematic representation of different microbial rhodopsin ion pumps. Amino acids shown in brackets are amino acids found at positions 85, 89, and 96 in each of these rhodopsins (BR numbering). Colored arrows represent different wavelengths at which each rhodopsin absorbs. In the case of PR, the arrows represent the absorption of blue and green rhodopsins (29); XR absorbs at different wavelengths due to its additional carotenoid molecule. ESR, *E. sibiricum* rhodopsin.

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(changing the DTD motif of BR to TTD), turns BR from a proton pump into a chloride pump (17). Interestingly, in the new CIR, position 85 is occupied by asparagine (protein motif NTQ). Mutation of BR in which aspartate 85 is changed to asparagine (yielding a BR mutant protein with the motif NTD) does not turn into a chloride pump (17, 18), and it would therefore be extremely interesting to understand what residues participate in chloride pumping in the new CIR.

Cultured members of the flavobacteria group have been instrumental in the study of microbial rhodopsins, from the first cultured bacteria showing PR phototrophic activity [*Dokdonia* sp. strain MED134 (19)] through the identification of an unusual PR from nonmarine permafrost bacteria [*Exiguobacterium sibiricum* (20)], to the identification of NaR [*Nonlabens dokdonensis* (basonym: *Donghaeana dokdonensis*) (13, 15); *Dokdonia eikasta* (basonym: *Krokinobacter eikastus*) (16); *N. marinus* (12)], and now the new CIR [*N. marinus* (12)]. Perhaps the next challenge is to find functional xanthorhodopsin (XR) homologs in these abundant marine bacteria.

XR is a PR-like proton pump that uses a light-harvesting carotenoid antenna in addition to the retinal chromophore (21). Since its discovery in *Salinibacter ruber*, a halophilic bacterium, XR was discovered and shown to be functional also in *Gloeobacter violaceus*, a thylakoid-less cyanobacterium (22). Genes for several XR-like proteins have been reported in the literature lately (14, 23–27), some even in flavobacteria; however, none

have yet been shown to behave as XR, namely to have a light-harvesting carotenoid molecule that can transfer energy to retinal.

Current explorations for new microbial rhodopsin ion pumps are restricted to

homology searches to known rhodopsins [with only three functional detections reported to date (21, 28, 29)]. Additional creative functional screens should therefore be developed to find novel rhodopsin features.

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