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Review

Molecular and evolutionary aspects of microbial sensory rhodopsins ☆

Keiichi Inoue [a,b], Takashi Tsukamoto [c], Yuki Sudo [b,c,d,*]

[a] Department of Frontier Materials, Nagoya Institute of Technology, Showa-ku, Nagoya 466–8555, Japan
[b] Japan Science and Technology Agency (JST), PRESTO, 4-1-8 Honcho Kawaguchi, Saitama 332–0012, Japan
[c] Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya, 464–8602, Japan
[d] Department of Life and Coordination-Complex Molecular Science, Institute for Molecular Science, 38 Nishigo-Naka, Myodaiji, Okazaki, Japan

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ABSTRACT

Retinal proteins (~rhodopsins) are photochemically reactive membrane-embedded proteins, with seven transmembrane α-helices which bind the chromophore retinal (vitamin A aldehyde). They are widely distributed through all three biological kingdoms, eukarya, bacteria and archaea, indicating the biological significance of the retinal proteins. Light absorption by the retinal proteins triggers a photoisomerization of the chromophore, leading to the biological function, light-energy conversion or light-signal transduction. This article reviews molecular and evolutionary aspects of the light-signal transduction by microbial sensory receptors and their related proteins. This article is part of a Special Issue entitled: Retinal Proteins - You can teach an old dog new tricks.

1. Introduction

1.1. Conversion of light energy into an electrochemical potential with microbial rhodopsins

Biological molecules containing vitamine-A aldehyde retinal (Fig. 1A) as a chromophore are generally called "Retinal (or Retinylidene) proteins" [1]. These retinal proteins have basically been classified into two groups, microbial (type-1) and animal (type-2) proteins [1,2]. In both types retinal works as a chromophore within the opsin. This article focuses on structure-function relationship among type-1 retinal proteins.

In 1971, a retinal protein has been found in the halophilic archaeon *Halobacterium salinarum* (formerly *halobium*) by Drs. Oesterhelt and Stoeckenius [3] (Fig. 1A). Similar to the visual rhodopsins, this molecule named bacteriorhodopsin (BR) is an integral membrane protein having both seven-transmembrane α-helices and a retinal chromophore linked to a specific lysine residue (Lys216) via a protonated Schiff base (PSB) linkage (Fig. 1A) [4]. Several sites in this protein have been of major attention in the research discussed in this review and those are shown in Fig. 1B with the alignment of putative amino acid sequences of other microbial type-1 retinal proteins. Most of them have an arginine and two aspartate residues at the position of BR Arg82, Asp85 and Asp 212. These residues and PSB form a quadrupole structure called "pentagonal cluster" [5], and it would be important for the structural stability of type-1 rhodopsins. BR acts as a light-driven outward proton pump across the membrane, and such a proton gradient is utilized by the ATP (adenosine triphosphate) synthase, indicating that organisms having light-driven pumps can produce ATP under light illumination [6,7]. It is well-known that ATP is a multifunctional nucleotide, used in cells and organisms as a coenzyme, and is often called the molecular unit of currency of intracellular energy transfer. In the past four decades since its discovery, BR has become a model for the simplest and most essential features necessary in an active proton transporter that is activated by a light stimulus. It should be noted that until recently, BR and related proteins capable of producing a chemiosmotic membrane potential in response to light had only been described in halophilic archaea [6].

In 2000, however, a type of retinal protein derived from γ-proteobacteria was discovered through genomic analysis in marine bacterioplankton, and was named "Proteorhodopsin (PR)" (Fig. 1A) [8]. PR exhibited a photocycle with intermediates and kinetics characteristic of BR, and showed active proton transport upon photoillumination. Since then, thousands of PR-like proteins have been discovered mainly from marine environment. This implies that archaeal-like retinal proteins are broadly distributed among different taxa, including members of the domain bacteria. Moreover, in 2005, a fungal retinal protein (*Leptosphaeria* Rhodopsin: LR) was found and identified from the eukaryota *Leptosphaeria maculans* (Fig. 1A) [9]. LR turned out to be very similar to BR in its photochemical behavior, and exhibits light-driven active proton transport. At present, a vast number of microbial type-1 retinal proteins, such as *Acetabularia* Rhodopsin (AR) [10], xanthorhodopsin (XR) [11], *Gloeobacter* Rhodopsin (GR) [12],

Abbreviations: BR, bacteriorhodopsin; HR, halorhdopsin; SRI, sensory rhodopsin I; SRII, sensory rhodopsin II; MR, middle rhodopsin; ASR, *anabaena* sensory rhodopsin; AR3, archaearhodopsin-3

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* Corresponding author at: Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya 464–8602, Japan. Tel.: +81 52 789 2993; fax: +81 52 789 3054.
E-mail address: z47867a@cc.nagoya-u.ac.jp (Y. Sudo).

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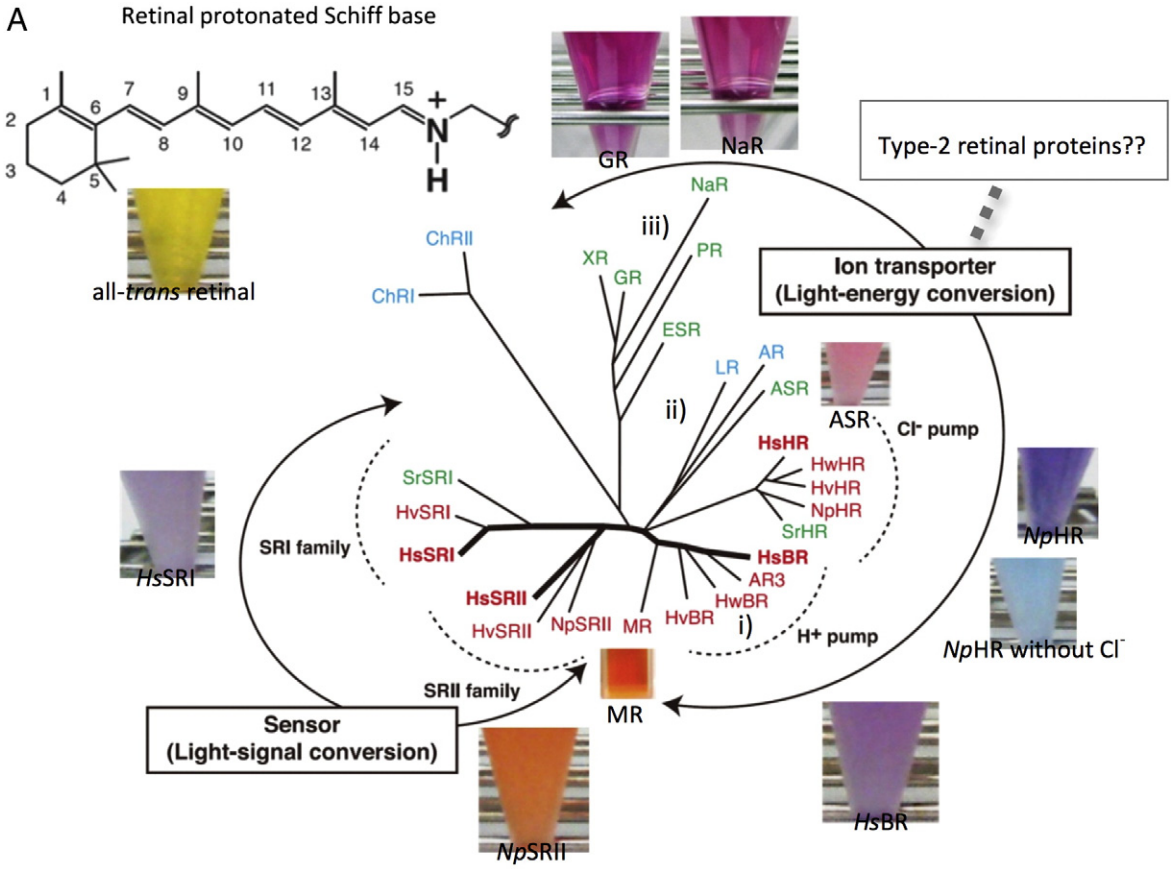

B

HsBR	82	85	96	125	128	141	144	145	176	177	185	193	194	200	204	205	210	211	212	215	216	223	226
(NpSRII)	(72)	(75)	(86)	(105)	(108)	(130)	(133)	(134)	(165)	(166)	(174)	(182)	(183)	(189)	(193)	(194)	(199)	(200)	(201)	(204)	(205)	(211)	(214)
(SrSRI)	(69)	(72)	(83)	(102)	(105)	(127)	(130)	(131)	(162)	(163)	(171)	(179)	-	(185)	(189)	(190)	(195)	(196)	(197)	(200)	(201)	(208)	(211)
HsSRI	R	D	Y	D	M	S	F	H	N	H	Y	P	A	T	V	A	F	L	D	A	K	F	R
HvSRI	R	D	F	D	M	S	F	H	N	H	Y	P	E	T	V	S	F	L	D	A	K	F	R
SrSRI	R	D	Y	D	M	A	C	H	N	H	Y	P	-	S	V	S	Y	M	D	A	K	V	S
HsSRII	R	D	Y	D	V	G	G	Y	N	I	Y	P	A	Q	Y	T	Y	L	D	S	K	A	G
NpSRII	R	D	F	N	V	G	A	F	N	L	Y	P	P	T	D	V	Y	L	D	T	K	I	D
ASR	R	D	S	Q	V	G	A	F	T	Y	Y	P	S	N	D	T	L	L	P	S	K	D	G
HsBR	R	D	D	D	M	S	A	M	N	V	Y	S	E	P	E	T	V	L	D	A	K	L	S
HsHR	R	T	A	D	M	S	F	F	V	L	Y	V	E	Q	V	T	V	L	D	A	K	L	W
NpHR	R	T	A	D	M	S	C	F	L	L	Y	V	E	P	Y	T	A	L	D	A	K	L	Y

Fig. 1. A) Chemical structure of the all-*trans* retinal protonated Schiff base and diversity of microbial type-1 retinal proteins. Retinal proteins show various colors, and exhibit two basic functions, the light-energy conversion and light-signal conversion. They widespread in archaea (red), eubacteria (green) and eukaryotes (blue). B) Alignments of some amino acid residues in type-1 retinal proteins. BR, SRII and SRI on the top of the panel indicate the molecules from *H. salinarum*, *N. phraonis* and *S. ruber*, respectively, with the numbers of their amino acid residues.

Exiguobacterium sibiricum Rhodopsin (ESR) [13,14] and sodium pump rhodopsin (NaR) [15] have been found in the genomes of many organisms except for higher plants and animals (Fig. 1A). Thus, it is now obvious that BR and related proteins are very common for large taxonomic groups within the three biological kingdoms, eukarya, bacteria and archaea (Fig. 1A), indicating that in many organisms the proton gradient produced by proton pumps could be utilized by the ATP synthesis, generating biochemical energy from light. The phylogenic analysis implies that these proton pumps are categorized to a few distinct clades including i) BR (*Hs*BR), AR3 (archaerhodopsin-3) and their related proteins, ii) fungal rhodopsins such as LR and iii) PR, XR, ESR and their related proteins, and they locate far from each other (Fig. 1A). All of proton-pumping rhodopsins have conserved carboxylic residues at the positions of BR Asp85 and Asp96 (Fig. 1B). Both of them are important for the proton transport and they function as a proton acceptor and a donor for the retinal Schiff base in the photoreaction of proton-pumping rhodopsins.

In 1977, a second retinal protein named halorhodopsin (HR) has been discovered in the archaeon *H. salinarum* by Drs. Matsuno-Yagi and Mukohata (Fig. 1) [16]. Dr. Lanyi et al. has demonstrated that HR acts as a light-driven inward chloride pump [17]. Functionally, HR is the mirror image of BR: anions such as Cl^-, Br^- and I^- instead of cations are transported, and the translocation is in the extracellular → cytoplasmic direction [18]. An aspartate at the position of Asp85 in BR is replaced by threonine in HR (Fig. 1B). This aspartate is a member of the electric quadruple in other rhodopsins, and the quadruple structure is maintained by the binding of an anion in HR [19]. In 1995, Dr. Sasaki and coworkers reported that the replacement of a single amino acid residue converts BR from a proton to a chloride pump, which implies that the essential