Research Focus

Crossing the borders: archaeal rhodopsins go bacterial☆

Wolfgang Gärtner[1] and Aba Losi[2]

[1]Max-Planck-Institut für Strahlenchemie, D-45470 Mülheim an der Ruhr, Germany
[2]Department of Physics, University of Parma and Istituto Nazionale per la Fisica della Materia, Parco Area delle Scienze 7/A, 43100 Parma, Italy

***All-trans*-retinal based, light-driven ion pumping and light sensing are no longer an exclusive archaeal enterprise after the exciting discovery of archaeal-type rhodopsins in bacteria and eukarya. Following the discovery of proton-pumping rhodopsins in marine bacteria (proteorhodopsins), an archaetypal system, consisting of a membrane-intrinsic sensory rhodopsin and a soluble interacting transducer, was recently identified in the cyanobacterium *Anabaena*. The powerful approach that combines genome 'digging' and protein expression is rapidly changing our understanding of light responses in lower organisms.**

Archaeal rhodopsins are light-responsive seven-helix transmembrane proteins that bind *all-trans* retinal as chromophore. They are type I rhodopsins, in contrast to 11-*cis*-retinal-based type II rhodopsins, such as the animal visual pigments [1]. Bacteriorhodopsin (BR) and halorhodopsin (HR) are light-driven proton and chloride pumps, respectively, absorbing maximally around 570 nm. They enable *Halobacterium salinarum* to grow phototrophically by establishing electrochemical gradients across the cell membrane to provide cellular energy and pH balance in the extreme ionic environmental conditions. Sensory rhodopsins (SRs) I and II, however, act as attractants for orange (SRI) and repellent photoreceptors for UV-blue (SRI) and green light (SRII), respectively (Fig. 1). The covalent attachment of the chromophore (via a protonated Schiff base to a lysine residue) and interactions with selected amino acids within the binding pocket allow a tuning of the absorption wavelength over a wide spectral range. Light-induced *trans-cis* isomerization of the chromophore triggers a photocycle that includes Schiff base deprotonation (in BR and SRs) and protein conformational changes, the former evident from a strong hypsochromic shift of this intermediate species (called M in BR [1]). In BR the reprotonation of the Schiff base occurs from the side opposite to the deprotonation direction, yielding a vectorial proton transport. By contrast, reprotonation in the SRs takes place from the same side and the proton transfer cycle is non-electrogenic. It should be noted, however, that a moderate, light-induced proton-transport capability can be found in SRII when its cognate transducer is removed [2]. In HR there is no Schiff base deprotonation in accordance with its chloride transporter role. BR and HR, as ion pumps, are self-standing proteins, whereas SRs perform their activity by interacting with a second protein, a 'transducer', thus being integral parts of light-to-signal transduction chains [1]. The ion pumps differ from the photosensors also in their photocycle kinetics: the former complete their photocycle in less than 20 ms at ambient temperature, thus performing efficient charge transport across the membrane. The photosensors cycle slowly, and typically the signalling state has a half-life of hundreds of ms; this allows its accumulation for efficient inter-protein communication in the signal-transduction chain. Furthermore, the long-lived signalling M-state of SRI (S_{373}), can absorb a second photon and acts in a two-photon-activated cycle as a repellent sensor for UVA-blue light [3].

In recent years, evidence has emerged that the occurrence of type I rhodopsins has spread beyond the borders of the Archaea domain. Two archaeal-type rhodopsins were found to act as phototaxis receptors in the green alga *Chlamydomonas reinhardtii* [4,5], and to be responsible for light-gated proton channel activity, being called accordingly 'channelrhodopsin' [5]. However, this atypical membrane protein that couples light-sensing to a channel activity, awaits more detailed functional characterization and an unambiguous

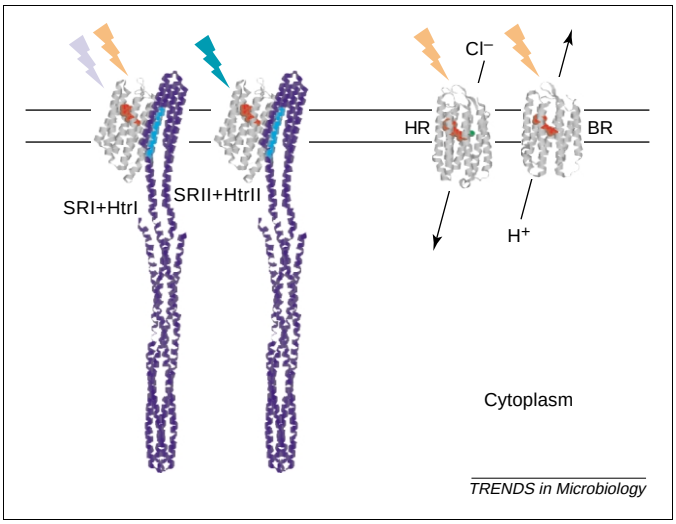

Fig. 1. Scheme of the four archaeal rhodopsins and of their arrangement in the cell membrane. Depicted are: the retinal chromophore (red); the association of sensory rhodopsins, SRI and SRII, to their methyl-accepting transducer proteins HtrI and HtrII (purple); the light-driven proton pump bacteriorhodopsin (BR) and the chloride pump halorhodopsin (HR) together with the direction of ion translocation across the membrane; the attracting (SRI) and the repellent (UVA-blue, SRI; green, SRII) light qualities. These structures have been drawn with Rasmol 2.7 using the coordinates from the PDB Protein Databank (entries: 1FBB, BR; 1E12, HR; 1H2S, SRII). For SRI no structure is available and therefore a duplicate of the SRII template is shown. The cytoplasmic part of Htr's was taken from the serine chemotaxis transducer of *Escherichia coli* (1QU7).

Corresponding author: Wolfgang Gärtner (gaertner@mpi-muelheim.mpg.de).

assignment to a type-I rhodopsin. Fungal rhodopsins, first identified in *Neurospora crassa*, have also been generated as recombinant chromoproteins, but their function as ion transporters or photosensors still awaits elucidation [1,6].

The first bacterial rhodopsin

Despite its classical name, BR is not a 'bacterial', but rather an archaeal rhodopsin; the first bacterial rhodopsin, proteorhodopsin, was serendipitously identified in the genome of an uncultivated member of the marine γ-*Proteobacteria* (the SAR86 group) of the Monterey bay bacterioplankton. It was also subsequently found in bacteria from many other ocean sites [7–9]. The retinal-reconstituted recombinant proteorhodopsin (expressed in *Escherichia coli*) showed an absorption maximum around 520 nm, approximately 50 nm blue-shifted to BR [7,8]. *E. coli* membranes and proteoliposomes containing proteorhodopsin show light-driven proton translocation activity and a photocycle including an M-like intermediate [7,10]. Proteorhodopsins in native membranes from Pacific Ocean plankton, because of their efficient photocycle, add significantly to the photosynthetic processes in the oceans [8], thus probably demanding a re-estimation of the extent of light-induced biomass generation.

A bacterial sensory rhodopsin in a cyanobacterium

The same method that was recently employed by the Spudich group for the *Neurospora* rhodopsin, the 'genome digging' [6], enabled them to identify the first eubacterial sensory rhodopsin [11]. An open reading frame that encodes a putative type I rhodopsin was identified in the genome of the cyanobacterium *Anabaena sp.* PCC7120, which made it the first representative of bacterial rhodopsins in chlorophyll-based photosynthetic organisms. The sequence of the encoded protein, referred to as the *Anabaena* sensory opsin (ASO), contained all the significant amino acid residues essential for retinal binding. Additionally, the recombinant protein, when expressed in *E. coli* and incubated with *all-trans* retinal, absorbed maximally at 543 nm. Interestingly, this wavelength falls in a trough within the strong absorption range of the photosynthetic pigments (Fig. 2a). ASO undergoes a photocycle similar to that of archaeal SRII, completed in a timescale of hundreds of ms [11]. The flash-induced difference spectrum at 10 ms after the laser flash (Fig. 2b) shows the absorption changes characteristic of a bacterial rhodopsin: the formation of a short wavelength-absorbing, deprotonated M-, and a long lived (K- or O-) intermediate. The length of the photocycle, as well as the absence of indicative amino acid residues in the sequence (the proton donor in BR, Asp96, is changed to a serine in ASO), led to the suggestion that this protein is most probably a sensor rather than an ion pump. An additional experiment provided proof for an internal rather than external reprotonation of the Schiff base: no net proton transport was observed upon illumination [11]. The most convincing, yet surprising evidence for ASO's function as a light sensor is the presence of a second open reading frame on the same operon, encoding a small (14 kD) protein that, as shown by plasmon surface resonance spectrometry and affinity purification, physically interacts *in vitro* with

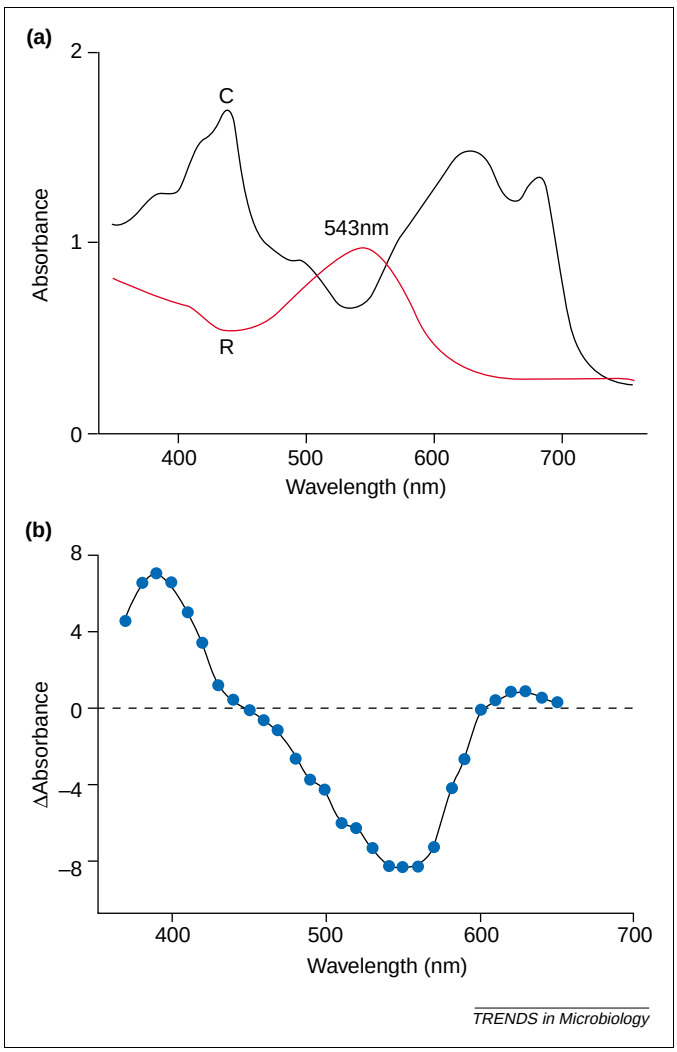

Fig. 2. (a) Absorption spectra of recombinant *Anabaena* rhodopsin (R) and an *Anabaena* cell culture (C). (b) Flash-induced difference spectrum of *Escherichia coli* membranes containing *Anabaena* rhodopsin, recorded at 10 ms after the 532 nm laser flash [11]. Reproduced, with permission from, Blackwell Publishing Ltd.

Anabaena rhodopsin [11]. Such interaction is a typical property of photosensors [1]. Incubation of ASO with this protein produces an unquestionable acceleration of the *Anabaena* rhodopsin photocycle [11], an effect similar to that found for SRI and SRII when associated with their respective transducers [summarized in [1]). Unlike the case of archaeal sensory rhodopsins, this putative transducer is not predicted to be a membrane protein, neither is it a member of any known prokaryotic taxis-transducer family. Nevertheless, if the role of the 14 kD protein as a transducer is confirmed, this will establish a new light-to-signal transduction process, based on membrane-intrinsic photosensors and soluble transducers in bacterial light-sensing.

The physiological role of *Anabaena* rhodopsin still awaits characterization. Phototrophic bacteria need to adapt to changing environmental conditions to optimize their use of light, and indeed, cyanobacteria exhibit diverse responses to light, such as photo-entrainment of circadian rhythms, chromatic adaptation, regulation of photosynthesis activity, hormogonium differentation and phototaxis [12]. Spudich and co-workers suggest

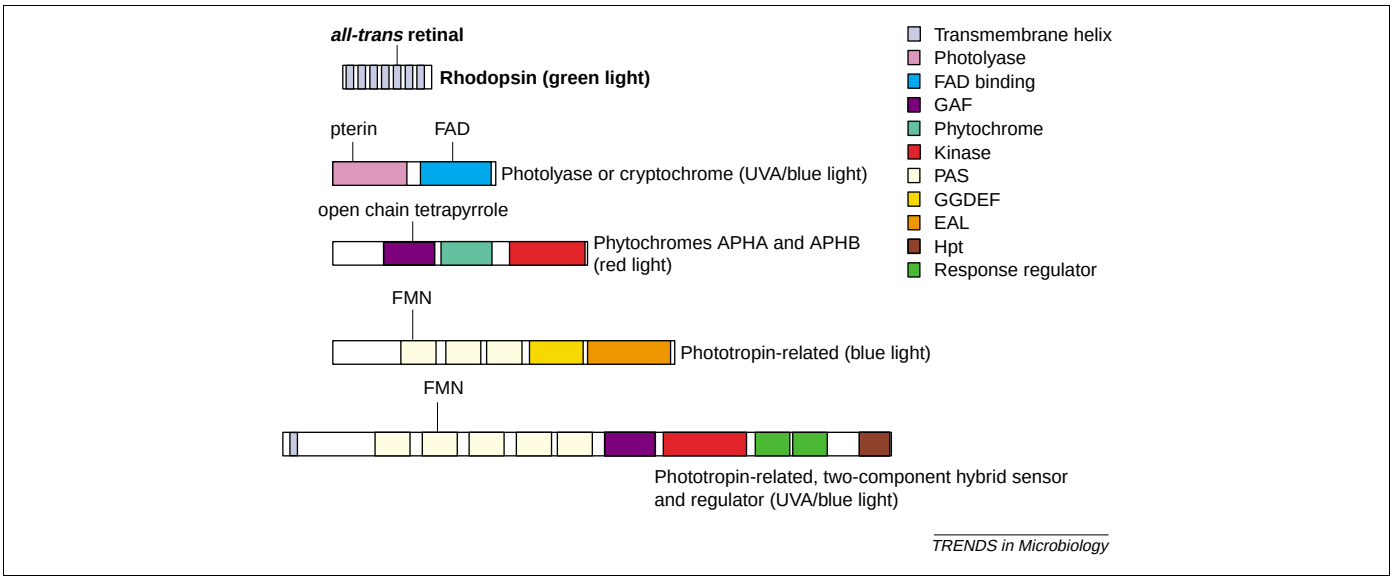

Fig. 3. Domain architecture of photoreceptor candidates in *Anabaena sp.* PCC7120 and the putative light quality to which they respond. For proteins other than *Anabaena* rhodopsin, the putative chromophore type and binding depicted are inferred from homology to known photoreceptor sequence data. FAD, flavin-adenine dinucleotide; FMN, flavin-mononucleotide; GAF, domain present in phytochromes and cGMP-specific phosphodiesterases; GGDEF and EAL, named after their conserved sequences; Hpt, histidine phosphotransfer domain; PAS, sensor domain with ligand binding sites. Sequence and domain analysis by applying BLAST network service [16] and PROSITE protein domain databank [17].

[11] that *Anabaena* rhodopsin may be a long-proposed green-light-sensor, which promotes phycoerythrin synthesis in chromatic adaptation. A genetic approach combined with photophysiological studies is expected to clarify this point. *Anabaena* carries, besides genes that encode the sensory rhodopsin, three phytochrome homologs, two photolyases (or cryptochromes) and genes for two proteins related to the recently identified plant blue-light receptors, phototropins [13]. This indicates that *Anabaena* potentially uses a more sophisticated method of sensing external stimuli than has been assumed to date (Fig. 3).

The work by Spudich and co-authors [11] also supports the idea of a rhodopsin being the green-light photoreceptor in *Calothrix* [14] (closely related to *Anabaena*), and that the pigment in the apical cell of the filamentous cyanobacterium *Leptolyngbya* might also be of rhodopsin-type [15].

Concluding remarks

Bacteria living in a changing environment experience variable light quality and intensity. Genome sequencing and sequence comparison tools are revealing that genes encoding similar photoreceptors are shared among distant taxa. Protein expression and functional analysis are demonstrating that the predicted light-induced reactions do occur in these putative photoreceptors. Bacteria appear to be fully equipped with an ensemble of various photoreceptors, formerly believed to be exclusively present in plants, cyanobacteria or other phototrophic organisms, to use light as a source of energy and information. The deciphering of the possible interplay between these novel photosensors, their mutual control and regulation, and their signaling activity that might induce a manifold of physiological responses, is still a most challenging and fascinating task.

References

1 Spudich, J.L. *et al.* (2000) Retinylidene proteins: structures and functions from archaea to humans. *Annu. Rev. Cell Dev. Biol.* 16, 365–392
2 Kamo, N. *et al.* (2001) Photochemistry and photoinduced proton-transfer by pharaonis phoborhodopsin. *Biochemistry (Mosc.)* 66, 1277–1282
3 Spudich, J.L. and Bogomolni, R. (1984) Mechanism of colour discrimination by a bacterial sensory rhodopsin. *Nature* 312, 509–513
4 Sineshchekov, O.A. *et al.* (2002) Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8689–8694
5 Nagel, G. *et al.* (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* 296, 2395–2398
6 Bieszke, J.A. *et al.* (1999) A eukaryotic protein, NOP-1, binds retinal to form an archaeal rhodopsin-like photochemically reactive pigment. *Biochemistry* 38, 14138–14145
7 Béjà, O. *et al.* (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* 289, 1902–1906
8 Béjà, O. *et al.* (2001) Proteorhodopsin phototrophy in the ocean. *Nature* 411, 786–789
9 Man, D. *et al.* (2003) Diversification and spectral tuning in marine proteorhodopsins. *EMBO J.* 22, 1725–1731
10 Friedrich, T. *et al.* (2002) Proteorhodopsin is a light-driven proton pump with variable vectoriality. *J. Mol. Biol.* 321, 821–838
11 Jung, K.H. *et al.* (2003) Demonstration of a sensory rhodopsin in eubacteria. *Mol. Microbiol.* 47, 1513–1522
12 Mullineaux, C.W. (2001) How do cyanobacteria sense and respond to light? *Mol. Microbiol.* 41, 965–971
13 Briggs, W.R. and Christie, J.M. (2002) Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci.* 7, 204–210
14 Hoff, W.D. *et al.* (1995) Rhodopsin(s) in eubacteria. *Biophys. Chem.* 56, 193–199
15 Albertano, P. *et al.* (2000) A complex photoreceptive structure in the cyanobacterium *Leptolyngbya sp*. *Micron* 31, 27–34
16 Altschul, S.F. *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402
17 Sigrist, C.J. *et al.* (2002) PROSITE: a documented database using patterns and profiles as motif descriptors. *Brief. Bioinform.* 3, 265–274

0966-842X/$ - see front matter
doi:10.1016/S0966-842X(03)00203-8