Journal of Photoscience (2002), Vol. 9(3), pp. 45–48

Microbial Rhodopsins: Genome-mining, Diversity, and Structure/Function

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Microbial rhodopsins, photoactive 7-transmembrane helix proteins that use retinal as their chromophore, were observed initially in the *Archaea* and appeared to be restricted to extreme halophilic environments. Our understanding of the abundance and diversity of this family has been radically transformed by findings over the past three years. Genome sequencing of cultivated microbes as well as environmental genomics have unexpectedly revealed archaeal rhodopsin homologs in the other two domains of life as well, namely *Bacteria* and *Eucarya*. Organisms containing these homologs inhabit such diverse environments as salt flats, soil, freshwater, and surface and deep ocean waters, and they comprise a broad phylogenetic range of microbial life, including haloarchaea, proteobacteria, cyanobacteria, fungi, and algae. Analysis of the new microbial rhodopsins and their expression and structural and functional characterization reveal that they fulfill both ion transport and sensory functions in various organisms, and use a variety of signaling mechanisms. We have obtained the first crystallographic structure for a photosensory member of this family, the phototaxis receptor sensory rhodopsin II (SRII, also known as phoborhodopsin) that mediates blue-light avoidance by the haloarchaeon *Natronobacterium pharaonis*. The structure obtained from x-ray diffraction of 3D crystals prepared in a cubic lipid phase reveals key features responsible for its spectral tuning and its sensory function. The mechanism of SRII signaling fits a unified model for transport and signaling in this widespread family of phototransducers.

key words: microbial rhodopsins, retinal, phototaxis, photosensory reception

INTRODUCTION

Two of the most fundamental functions of integral membrane proteins are selective transport of materials across the membrane and the sensing and transmission of information from the extracellular environment to the cell interior. The family of four archaeal rhodopsins in *Halobacterium salinarum* stands out in that active transport and sensory signaling are accomplished by homologous proteins which are modifications of a common design: seven membrane-embedded helices with a conserved interior binding pocket surrounding a retinal prosthetic group [1,2]. Similar photochemical reaction cycles initiated by retinal photoisomerization in each of the four proteins have distinctly different outcomes: bacteriorhodopsin (BR) and halorhodopsin (HR) are light-driven ion pumps for protons and chloride ions, respectively. Sensory rhodopsins I and II (SRI and SRII, the latter also called phoborhodopsin) are phototaxis receptors, each of which couples to its cognate membrane-embedded transducer protein (HtrI and HtrII) to control a phosphorylation cascade that modulates the cell's motility [3-5]. SRI is an attractant receptor that guides the cells to green/orange light used by the transport rhodopsins. SRII is a blue light-activated repellent receptor made under highly aerobic conditions when danger of photooxidation is greatest and when the transport rhodopsins and SRI are not made.

SRI, SRII, BR and HR were discovered in *H. salinarum*, and ~30 homologous proteins with the same functions have been found in related haloarchaeal species [2,6]. A far greater abundance and diversity of this family has been discovered over the past three years. Photoactive *Archaeal* rhodopsin homologs have been demonstrated in marine plankton proteobacteria [7,8], cyanobacteria (*Anabaena* sp. PCC7120; K.-H. Jung, V. Trivedi, & J. Spudich, in preparation) and eucaryotic microbes, namely fungi [9,10] and green algae [11]. In this communication, we describe briefly the characterization in our laboratory of new members of the microbial rhodopsin family.

RESULTS & DISCUSSION

Proteorhodopsin.

A new rhodopsin derived from eubacteria was discovered by genomic analyses of naturally-occuring marine bacterioplankton from Monterey Bay [7]. The eubacterial rhodopsin was encoded in the genome of an uncultivated γ-proteobacterium, and shares amino acid sequence similarity with

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Received July 17, 2002; accepted October 22, 2002

archaeal rhodopsins, especially in the retinal-binding residues. When the new protein was expressed in *E. coli,* it bound retinal to form a light-driven proton pump, named proteorhodopsin for its proteobacterial origin. Proteorhodopsin exhibits a photochemical reaction cycle with intermediates and kinetics characteristic of proton-pumping rhodopsins. The study demonstrated that archaeal-like transport rhodopsins are found outside of haloarchaea and that they provide a previously unsuspected mode of phototrophic light energy harvesting in the sea. By flash photolysis we directly demonstrated the protein's photoactivity in ocean plankton, which allowed calculation of its concentration, which is sufficiently high to permit phototrophic growth in principle, although the energy metabolism of the bacterium and the relative extent of light's contribution are not known [8]. Moreover, sampling different ocean genomes established that a diverse family of proteorhodopsins, spectrally tuned to the light available in their environment, commonly occurs in oceanic surface waters worldwide, including Antarctic seawater and Hawaiian surface and deep ocean water [8]. Proteorhodopsin phototrophy is therefore likely to be a globally significant oceanic microbial process.

Anabaena rhodopsin

The proteorhodopsins established that transport rhodopsins exist in bacteria. A pigment in a fresh-water cyanobacterium establishes that sensory rhodopsins also exist in bacteria. A gene with homology to the archaeal rhodopsin apoprotein genes was identified in a genome-sequencing project of heterocystous *Anabaena sp.* PCC7120 at Kazusa DNA Research Institute (http://www.kazusa.or.jp). The genome contains a DNA segment containing two open reading frames separated by 16 base pairs under control of a single promoter. This operon is predicted to encode a 261-residue archaeal type rhodopsin apoprotein (opsin) and a 125-residue (14 kDa) protein.

The *Anabaena* opsin protein exhibits high identity in the most conserved regions in archaeal rhodopsins, namely helices C, F, and G. These regions are also conserved in proteorhodopsin, *Neurospora* rhodopsin, and *Chlamydomonas reinhardtii* rhodopsin sequences. In particular, residues in contact with the retinal chromophore according to the crystal structures of BR [12] and SRII [13,14] are highly conserved. The Schiff base proton acceptor found in proton pumps and SRs is conserved (Asp85 in BR), but the proton donor specific to proton pumps (Asp96 in BR) is replaced with a serine residue (Ser86 in *Anabaena* rhodopsin), a non-ionizable residue, as in the haloarchaeal sensory rhodopsins. The *Anabaena* opsin gene product in *E. coli* binds *all-trans* retinal to produce a visible light-absorbing pigment (λmax=543 nm) (K.-H. Jung, V. Trivedi, and J. L. Spudich, in preparation). The pigment did not exhibit detectable light-driven proton ejection activity in *E. coli* membranes.

The rate of the *Anabaena* rhodopsin photocycle was increased 20% when the pigment and the 14 kDa protein were co-expressed in *E. coli*, indicating physical interaction between the two proteins. We confirmed binding of the 14-kDa protein to *Anabaena* rhodopsin by affinity enrichment measurements and Biacore interaction analysis. We interpret the participation in protein-protein interaction, the cotranscription with the 14-kDa protein, and primary sequence similarity to haloarchael sensory rhodopsins, as compelling evidence that *Anabaena* rhodopsin functions as a photosensory receptor in its natural environment. The results strongly suggest that the soluble 14-kDa protein transduces a signal from the receptor. Therefore, unlike the archaeal sensory rhodopsins, which transmit signals by transmembrane helix-helix interactions with membrane-embedded transducers, the *Anabaena* sensory rhodopsin may signal through a soluble cytoplasmic protein, analogously to higher animal visual pigments. These results, considered together with the finding of proteorhodopsins, make it clear that both transport and sensory rhodopsins exist in the bacterial domain, as previously found for the archaeal domain.

Eukaryotic microbial rhodopsins

Search of genome databases currently completed or in progress indicates the presence of archaeal rhodopsin homologs in a number of fungi: *Neurospora crassa, Botrytis cinerea, Fusarium sporotrichioides, Leptosphaeria maculans*, and the pathogenic fungus *Cryphonectria parasitica* (a plant pathogen; D. Nuss, UMBI, MD, USA, personal communication) and the human pathogen *Cryptococcus neoformans*. One homolog is also evident in the alga *Guillardia theta* and two in *Chlamydomonas reinhardtii.*

We expressed the gene from *N. crassa* heterologously in the yeast *Pichia pastoris* [9]. The expressed protein forms with all-*trans* retinal a visible light-absorbing pigment with a 534 nm maximal absorption. Laser flash kinetic spectroscopy demonstrates that the retinal-reconstituted pigment undergoes a photochemical reaction cycle with a near UV-absorbing intermediate that is similar to the M intermediates produced by transient Schiff base deprotonation of the chromophore in the photocycles of BR and sensory rhodopsins I and II. The slow photocycle (seconds) and long-lived intermediates (M and O) are most similar to those of the phototaxis receptor sensory rhodopsin II. The results were the first to demonstrate a photochemically reactive member of the archaeal rhodopsin family in a eukaryotic cell, and its slow photocycle argues for a sensory function. However the pigment's physiological role and its properties in the *N. crassa* membrane have not yet been elucidated.

The first archaeal-type rhodopsins reported in eukaryotic organisms for which physiological functions are demonstrated are CSRA and CSRB in the green alga *Chlamydomonas reinhardtii* [11]. Recently we have found by RNA*i* suppression of gene expression that the *Chlamydomonas* rhodopsins CSRA and CSRB mediate photomotility responses (phototaxis and photophobic responses) to low and high intensity light, respectively, in *C. reinhardtii.* Each of the receptors is predicted to encode a 300-residue seven-transmembrane helix domain

with a retinal-binding pocket homologous to that of archaeal rhodopsins, followed by 400 residues encoding an additional membrane-associated portion. The function of the two rhodopsins as phototaxis receptors is demonstrated by analysis of photoreceptor electrical currents and motility responses in transformants with RNA*i* directed against each of the rhodopsin genes. CSRA has an absorption maximum near 510 nm and mediates a fast photoreceptor current that saturates at high light intensity. In contrast, CSRB absorbs maximally at 470 nm and generates a slow photoreceptor current saturating at low light intensity. The saturation at different light fluence levels extends the range of light intensity to which the organism can respond. Further, at intensities where both operate, their light signals are integrated at the level of membrane depolarization caused by the two photoreceptor currents.

Therefore, the new results reveal three different modes of signaling used by microbial sensory rhodopsins:

(i) Halobacterial SRI and SRII interact with their cognate transducers by contacts between the two integral membrane proteins via transmembrane helix interactions [15-17].

(ii) The *Anabaena* sensory rhodopsin interacts with the 14-kDa protein, presumably a transducer, which is a soluble protein, and therefore the hydrophilic surfaces of the receptor facing the aqueous medium are involved (K.-H. Jung, V. Trivedi, and John Spudich, in preparation).

(iii) The *Chlamydomonas* rhodopsins CSRA and CSRB function as the photoactive domains of phototaxis receptors that control transmembrane ion currents and membrane potential in this unicellular eukaryote [11]. In these proteins the 7-helix retinal-binding domains are fused to larger putative signal-transducing domains, which control ion permeability of the membrane.

CONCLUSIONS

A unified molecular mechanism for transport and signaling has emerged from studies of the haloarchaeal rhodopsins [4,18], in which the same conformational change, a tilting of helices (primarily helix F) which opens the structure on the cytoplasmic side of the protein, is proposed to be responsible for key steps in transport and signaling. In the proton pump BR the conformational change opens a cytoplasmic channel for proton uptake during the pumping cycle. In SRI and SRII, the same conformational change is proposed to activate the receptor protein, producing a "signaling state". The altered conformation triggers changes in the transducer by trans-membrane helix-helix inter-actions, thereby transmitting the signal. The regions of conservation in the newly found rhodopsins and the overall similarities in photochemistry suggest that Nature has found that variations of this same basic mechanism could be used for alternative modes of signalling as well. Recent progress on x-ray crystallography of the microbial rhodopsins, in particular the high-resolution structures of SRII from *Natronobacterium pharaonis* [13,14] are promising signs that soon these variations on a theme will be elucidated at atomic-resolution.

REFERENCES

1. Oesterhelt, D. (1998) The structure and mechanism of the family of retinal proteins from halophilic archaea. *Curr. Opin. Struct. Biol.* **8**, 489-500.
2. Spudich, J. L., C.-H. Yang, K.-H. Jung and E. N. Spudich (2000) Retinylidene Proteins: Structures and Functions from Archaea to Humans. *Annu. Rev. Cell. & Dev. Biol.* **16**, 365-392.
3. Hoff, W. D., K.-H. Jung and J. L. Spudich (1997) Molecular Mechanism of Photosignaling by Archaeal Sensory Rhodopsins. *Annu. Rev. Biophys. Biomole.c Struct.* **26**, 223-258.
4. Sasaki, J. and J. L. Spudich (2000) Proton transport by sensory rhodopsins and its modulation by transducer-binding. *Biochim. Biophys. Acta.* **1460**, 230-239.
5. Kamo, N., K. Shimono, M. Iwamoto and Y. Sudo (2001) Photochemistry and photoinduced proton-transfer by *pharaonis* phoborhodopsin. *Biochemistry (Mosc)* **66**, 1277-1282.
6. Mukohata, Y., Ihara, K., Tamura, T. and Y. Sugiyama (1999) Halobacterial rhodopsins. *J. Biochemistry (Tokyo)*, **125**, 649-657.
7. Béjà, O., L. Aravind, E. V. Koonin, M. T. Suzuki, A. Hadd, L. P. Nguyen, S. B. Jovanovich, C. M. Gates, R. A. Feldman, J. L. Spudich, E. N. Spudich and E. F. DeLong (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science*, **289**, 1902-1906.
8. Béjà, O., E. N. Spudich, J. L. Spudich, M. Leclerc and E. F. DeLong (2001) Proteorhodopsin photo-trophy in the ocean. *Nature*, **411**, 786-789 {*1st two authors equal contributors*}.
9. Bieszke, J. A., E. N. Spudich, K. L. Scott, K. A. Borkovich and J. L. Spudich (1999). A eukaryotic protein, NOP-1, binds retinal to form an archaeal rhodopsin-like photochemically reactive pigment. *Biochemistry*, **38**, 14138-14145.
10. Brown, L. S., A. K. Dioumaev, J. K. Lanyi, E. N. Spudich and J. L. Spudich (2001) Photochemical reaction cycle and proton transfers in *Neurospora* rhodopsin. *J. Biol. Chem.* **276**, 32495-32505.
11. Sineshchekov, O. A., K.-H. Jung and J. L. Spudich (2002) Two rhodopsins mediate phototaxis to low and high intensity light in *Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. USA*, **99**, 8689-8694.
12. Luecke, H., B. Schobert, H. T. Richter, J.-P. Cartailler, and J. K. Lanyi (1999) Structure of bacteriorhodopsin at 1.55 Angstrom resolution. *J. Mol. Biol.* **291**, 899-911.
13. Luecke, H., B. Schobert, J. K. Lanyi, E. N. Spudich and J. L. Spudich (2001) Crystal Structure of Sensory Rhodopsin II at 2.4 Å: Insights into Color Tuning and Transducer Interaction. *Science*, **293**, 1499-1503.
14. Royant, A., P. Nollert, K. Edman, R. Neutze, E. M. Landau, E. Pebay-Peyroula and J. Navarro (2001) X-ray structure of sensory rhodopsin II at 2.1-A resolution. *Proc. Natl. Acad.*

Sci. USA, **98**, 10131-10136.

15. Zhang, X.-N., J. Zhu and J. L. Spudich (1999) The specificity of interaction of archaeal transducers with their cognate sensory rhodopsins is determined by their transmembrane helices. *Proc. Natl. Acad. Sci. USA,* **273**, 857-862.
16. Jung, K.-H., E. N. Spudich, V. D. Trivedi and J. L. Spudich (2001) An archaeal photosignal-transducing module mediates phototaxis in *Escherichia coli. J. Bacteriol.* **183**, 6365-6371.
17. Yang, C. S. and J. L. Spudich (2001) Light-induced structural changes occur in the transmembrane helices of the *Natronobacterium pharaonis* HtrII transducer. *Biochemistry,* **40**, 14207-14214.
18. Spudich, J. L. (1998) Variations on a molecular switch: transport and sensory signaling by archaeal rhodopsins. *Molecular Microbiology,* **28**, 1051-1058.