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RETINYLIDENE PROTEINS: Structures and Functions from Archaea to Humans

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■ **Abstract** Retinylidene proteins, containing seven membrane-embedded α-helices that form an internal pocket in which the chromophore retinal is bound, are ubiquitous in photoreceptor cells in eyes throughout the animal kingdom. They are also present in a diverse range of other organisms and locations, such as archaeal prokaryotes, unicellular eukaryotic microbes, the dermal tissue of frogs, the pineal glands of lizards and birds, the hypothalamus of toads, and the human brain. Their functions include light-driven ion transport and phototaxis signaling in microorganisms, and retinal isomerization and various types of photosignal transduction in higher animals. The aims of this review are to examine this group of photoactive proteins as a whole, to summarize our current understanding of structure/function relationships in the best-studied examples, and to report recent new developments.

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INTRODUCTION AND OVERVIEW

Over three hundred photochemically reactive proteins that use vitamin-A aldehyde (retinal) as their chromophore (retinylidene proteins or commonly called rhodopsins) have been described in both prokaryotic and eukaryotic organisms. A common feature of these proteins is that they contain seven membrane-embedded α-helices that form an internal pocket in which the retinal is bound. Primary sequence alignments split retinylidene proteins into two clearly distinct families. One family (type 1) consists of the archaeal-type rhodopsins first observed in the archaeon *Halobacterium salinarum*, a halophilic prokaryote, and now also found in eukaryotic microbes. Type 1 rhodopsins function as light-driven ion transporters (bacteriorhodopsin and halorhodopsin), phototaxis receptors (sensory rhodopsins I and II), or yet undiscovered functions (e.g. fungal rhodopsins). The type 2 rhodopsins consist of the photosensitive receptor proteins in animal eyes, including human rod and cone visual pigments, receptor proteins in the pineal gland, hypothalamus, and other tissues of lower vertebrates. Also in this group are retinochrome (retinal photoisomerases first described in squid retina) ocular extraretinal retinylidene proteins (in ocular tissue but outside the retina) and others that are extra-ocular such as encephalopsin found in human and mouse brain. All type 2 rhodopsins so far reported are in higher eukaryotes.

Retinylidene proteins are among the few membrane proteins for which structural information is available from crystallography. Atomic resolution structures of bacteriorhodopsin have resulted from electron and X-ray crystallography (Grigorieff et al 1996, Kimura et al 1997, Pebay-Peyroula et al 1997, Luecke et al 1998, Essen et al 1998), and the 1.55 Å structure reported (Luecke et al 1999b), in which bound water molecules are visible, makes it one of the most highly resolved of any membrane protein. An atomic resolution structure (1.8 Å) of halorhodopsin has also been reported (Kolbe et al 2000), and very recently a 2.8 Å structure of bovine rod rhodopsin was obtained from X-ray diffraction data (Palczewski et al 2000). Near-atomic resolution information has been obtained from electron crystallography of others, namely the type 1 sensory rhodopsin II from an archaeal halophile (ERS Kunji, EN Spudich, R Grisshammer, R Henderson & JL Spudich, in preparation), and from three type 2 examples: bovine, frog, and squid visual pigments (Schertler & Hargrave 1995, Davies et al 1996, Krebs et al 1998, Schertler 1999). Despite the different primary structures of the two families, in a general sense the overall structural features are similar. In addition to the shared seven-helix architecture, spectroscopic and chemical analyses show that retinal is attached in a Schiff base linkage to a lysine residue in the seventh helix in all known cases. Moreover, the changes in chromophore and protein structure induced by similar photochemical reactions exhibit critical features shared by

the two families. On the other hand the crystallographic data reveal that the disposition of their seven helices differs between the two classes. In fact from the three type 1 and three type 2 pigments whose helices have been visualized, their assignment as to type 1 or 2 is immediately evident from their projection structures alone.

Functions also overlap between the two types, although notable differences exist. Visual pigments and pinopsins carry out photosensory signaling by light-induced protein-protein interactions, as do the archaeal sensory rhodopsins, which interact with phototaxis transducer proteins in haloarchaea. Retinochromes, homologous to visual pigments, are light-driven retinal isomerases rather than signaling proteins, and another type [Retinal G protein–coupled receptor (RGR) opsins] may have both isomer-regeneration and signaling functions. The archaeal family includes the sensory rhodopsins and transport rhodopsins that carry out light-driven ion pumping across the cytoplasmic membrane.

Several recent reviews deal with specific subsets of rhodopsin proteins: i.e the ion pumps bacteriorhodopsin and halorhodopsin (Oesterhelt 1998, Haupts et al 1999, Lanyi 1999), archaeal sensory rhodopsins (Hoff et al 1997, Spudich 1998), invertebrate visual pigments (Gärtner & Towner 1995), vertebrate rhodopsins (Helmreich & Hofmann 1996, Rao & Oprian 1996, Sakmar 1998, Tokunaga et al 1999), pinopsins (Okano & Fukada 1997), and retinochromes (Pepe & Cugnoli 1992). The purpose of this review is to bring together all of the known retinylidene proteins for a comparative analysis of their structures and functional mechanisms.

STRUCTURES OF RETINYLIDENE PROTEINS

Hydropathy plots of retinylidene apoproteins generally predict seven transmembrane helices, which have been confirmed by crystallography in six cases (discussed below). The seven transmembrane segments are arranged to form an internal pocket for the retinal prosthetic group. Another general property of rhodopsins, whether archaeal or animal, is the attachment of the retinal to the ε-amino group of a lysine in the seventh helix. Because this linkage is a protonated Schiff base, the result is a buried positive charge in the protein, and light-induced transfer of this proton within the binding pocket is important to function of both transport and sensory rhodopsins.

Although the features noted above are widely shared, three different aspects of their structure, (*a*) sequences of their apoproteins; (*b*) near-atomic to atomic crystallographic data showing the disposition of their seven helices; and (*c*) the isomeric configuration and ring/chain conformation of the retinal, divide the retinylidene proteins into the two distinct groups.

Primary Structure and Membrane Topology

The protein sequences of five type 1 rhodopsins illustrate the conservation of the retinal-binding pocket between prokaryotic pigments (the first four in Figure 1,

see color insert) and a eukaryotic member of the family. In the case of bacteriorhodopsin, the residues lining the cavity in which retinal is held are defined from its crystal structure (Henderson et al 1990, Grigorieff et al 1996, Luecke et al 1999b) and are shown in blue in the figure. Residues considered as in the pocket are as defined by Henderson et al (1990) except for Arg 82, which was included because it is H-bonded to the retinylidene Schiff base. These residues are 81% identical and 95% similar in sensory rhodopsin I, whereas the entire molecule exhibits only 26% identity with bacteriorhodopsin. Halorhodopsin and sensory rhodopsin II show similar conservation of the 22-residue pocket, but the chloride pump halorhodopsin stands out in not having the Asp residue in helix C (Asp85 in bacteriorhodopsin) that serves as a negative counterion to the protonated Schiff base in bacteriorhodopsin. Rather a chloride ion occupies nearly the exact position in halorhodopsin (Kolby et al 2000). This is a highly significant difference because mutagenic replacement of Asp85 in the proton pump bacteriorhodopsin with Thr is sufficient to enable it to pump chloride to a significant extent (Sasaki et al 1995). The corresponding Asp residues in the sensory rhodopsins are also functionally important, as discussed below. These four proteins are all from the same archaeal organism *H. salinarum*, but a retinylidene protein from an evolutionarily distant organism, the fungus *Neurospora crassa*, exhibits approximately the same degree of conservation in the pocket (Bieszke et al 1999a) (Figure 1).

Many additional residues in the *Neurospora* opsin NOP-1 are identical to those of two or more of the archaeal rhodopsins (indicated in green in Figure 1). Most of these lie within the predicted transmembrane domain of NOP-1 and include regions predicted to be outside of the chromophore contact residues. Especially conserved regions are in helix C, the extracellular side of helix D, the cytoplasmic side of helix F, and helix G. Mutant analysis and spectroscopic studies of light-induced conformational changes in bacteriorhodopsin (Subramaniam et al 1993, 1999; Vonck 1996; Luecke 1999a) indicate helices B, C, F, and G as conformationally active regions. The conservation, therefore, indicates conformational changes similar to those in bacteriorhodopsin may be a general feature of type 1 rhodopsins.

Two residues of known functional importance in both transporters and sensors, the lysine that forms a protonated Schiff base with retinal and the Schiff base primary counterion residue, are conserved in NOP-1. These are Lys216 in helix G (numbered according to bacteriorhodopsin; Lys263 in NOP-1) and Asp85 in Helix C (Asp131 in NOP-1). Four residues in bacteriorhodopsin involved in proton release (Glu194 and Glu204 near the extracellular surface) and in proton uptake (Thr46 and Asp96 near the cytoplasmic surface) are substituted non-conservatively in both SRI and SRII. Three of the four are conserved in NOP-1 (except for Glu194, which is a glycine residue). Therefore NOP-1 is more similar to bacteriorhodopsin than are SRI or SRII in its conservation of these residues; however, as addressed below, its photochemistry appears to be more similar to that of the sensory rhodopsins (Bieszke et al 1999b) (Figure 1, *top*).

Type 2 rhodopsins, such as visual pigments, exhibit different domain organization than the archaeal proteins. Whereas archaeal rhodopsins are 25–30 kDa

proteins with minimal interhelical loops (except for the BC loop of NOP-1), human rod rhodopsin is typical of visual pigments in that about half of its 38-kDa mass is buried in the membrane with the other half in hydrophilic loops protruding into the aqueous medium from both membrane surfaces (Figure 1, *back*). The differing domain organization reflects the mode of signaling by visual pigments, which bind heterotrimeric G proteins, receptor kinases, and other signaling proteins to their cytoplasmic loops (Khorana 1993, Helmreich & Hofmann 1996, Sakmar 1998, Hofmann 1999). The archaeal rhodopsins, on the other hand, function largely within the membrane to pump ions or to transmit signals to other integral membrane proteins (Spudich 1998, Zhang et al 1999).

Insight was gained from electron crystallography (Schertler 1999), modeling (Baldwin et al 1997), and mutagenesis (Shieh et al 1997), regarding the retinal-binding pockets of visual pigments, and very recently the atomic resolution structure of bovine rod rhodopsin (Palczewski et al 2000) provided a clear view of the pocket residues for this protein (Figure 1, *back*). It is notable that the most highly conserved sequences of the binding pocket in archaeal-type rhodopsins are not evident in visual pigments: namely the DXXXK motif that provides a negative charge one helix turn from the retinal-binding lysine in helix G; the sequence WXXYPXXW in helix F; and the sequence RYXDWXXTTP, containing the Schiff base counterion in most archaeal rhodopsins, in helix C, the most conserved helix in type 1 rhodopsins. Similar residues are present but not in the same positions nor the same sequence: the retinal-binding Lys in helix G, Trp, Tyr, or Phe residues in helices C and F, and in the two human pigments shown, a Glu in helix C (position 113 in rod rhodopsin). In the rod pigment, Glu113 serves as the Schiff base counterion forming a salt bridge between the carboxylate anion on helix C and the protonated Schiff base nitrogen on helix G (Rao & Oprian 1996). Analogous salt bridges are found in bacteriorhodopsin between Asp85 and the protonated Schiff base (Haupts et al 1999, Lanyi 1999) and in the homologous positions in sensory rhodopsin II (Engelhard et al 1996, Spudich et al 1997, Bergo et al 2000). As discussed below, the salt bridge and its disruption by light are important to function of each of these proteins.

Well-conserved positions among the four visual pigments shown, which are highly conserved in visual pigments in general, are found throughout helix C, on the cytoplasmic surface of helices A-C, the intradiscal side of helix D and the DE loop, and in clusters in helices F and G (shown in blue and green in Figure 1, *bottom*). Helix C contains the functionally important counterion as noted above, the cytoplasmic surfaces are involved in light-induced sequestering of signal transduction proteins to the receptor, and the intradiscal loops are indicated by deletion mutagenesis to play an important structural role in cellular sorting and processing and protein folding into the membrane (Khorana 1993). Another conserved feature are cysteine residues, which occasionally occur but are not conserved in the type 1 pigments. These are the two cysteine residues in the intradiscal loops (Cys110 and Cys187 in rod rhodopsin) that form a disulfide bond believed to stabilize the native folded structure. Palmitoyl groups attached to the cysteine pair at positions

322 and 323 form a hydrophobic extension believed to anchor this region in the membrane bilayer, creating a fourth loop on the cytoplasmic surface of the protein (Cai et al 1999, Sachs et al 2000).

Helix Positions from Crystallography

Crystal structures and electron density maps of sufficient resolution to visualize the arrangement of the seven α-helices are available for both transport and sensory archaeal rhodopsins, as well as for several visual pigments (Figure 2).

Bacteriorhodopsin was first visualized by Henderson & Unwin (1975) by electron crystallographic analysis of two-dimensional crystals, which are produced in vivo as densely packed pure protein in a lipid bilayer continuous with the cytoplasmic membrane of *H. salinarum*. Henderson and coworkers further developed the electron crystallographic refinement to produce a three-dimensional structure of bacteriorhodopsin at 3.5 Å (Grigorieff et al 1996).

A new crystallization procedure based on lipidic cubic phases yielded well-ordered three-dimensional crystals of bacteriorhodopsin suitable for synchrotron X-ray analysis (Landau & Rosenbusch 1996). The procedure produces three-dimensional crystals that are essentially highly ordered stacks of two-dimensional crystals like those used for electron crystallography. The electron crystallography-derived structures provide the phase information making possible atomic- resolution X-ray structure determination by molecular replacement. Bacteriorhodopsin structures ranging from 2.3 to 3.5 Å resolution based on either electron or X-ray crystallography were reported by various laboratories between 1997 and 1999 (summarized by Subramaniam 1999), and since then Leucke et al (1999) have produced an even higher resolution (1.55 Å) bacteriorhodopsin structure.

Recently Kolby et al (2000) grew halorhodopsin crystals in a lipidic cubic phase and accomplished the first X-ray structure determination of this anion pump. The best structure available to date [4 Å from electron crystallography (Kunji et al 2000)], was very similar to bacteriorhodopsin, and a striking feature of the 1.8-Å X-ray structure is the nearly identical positions of the helix backbones even at this new fine level of resolution.

A homolog of *H. salinarum* sensory rhodopsin II has been cloned from the alkalophilic halophile *Natronobacterium pharaonis* (Seidel et al 1995). Recently two-dimensional crystals of purified *N. pharaonis* sensory rhodopsin II in a halobacterial lipid bilayer were analyzed by electron crystallography and a 6-Å projection structure produced; the electron density map of sensory rhodopsin II is compared with those of the two transport rhodopsins at the same resolution in Figure 2. It is clear that the disposition of their seven helices is highly similar for both sensory and transport rhodopsins.

Helix-resolving electron density maps have also been determined for visual pigments from cow, frog, and squid by electron crystallography of two-dimensional crystalline arrays; their structures in projection are shown in Figure 2 (Schertler & Hargrave 1995, Davies et al 1996, Krebs et al 1998). The arrangements of the helices of these three visual pigments are in a highly similar pattern, which is a

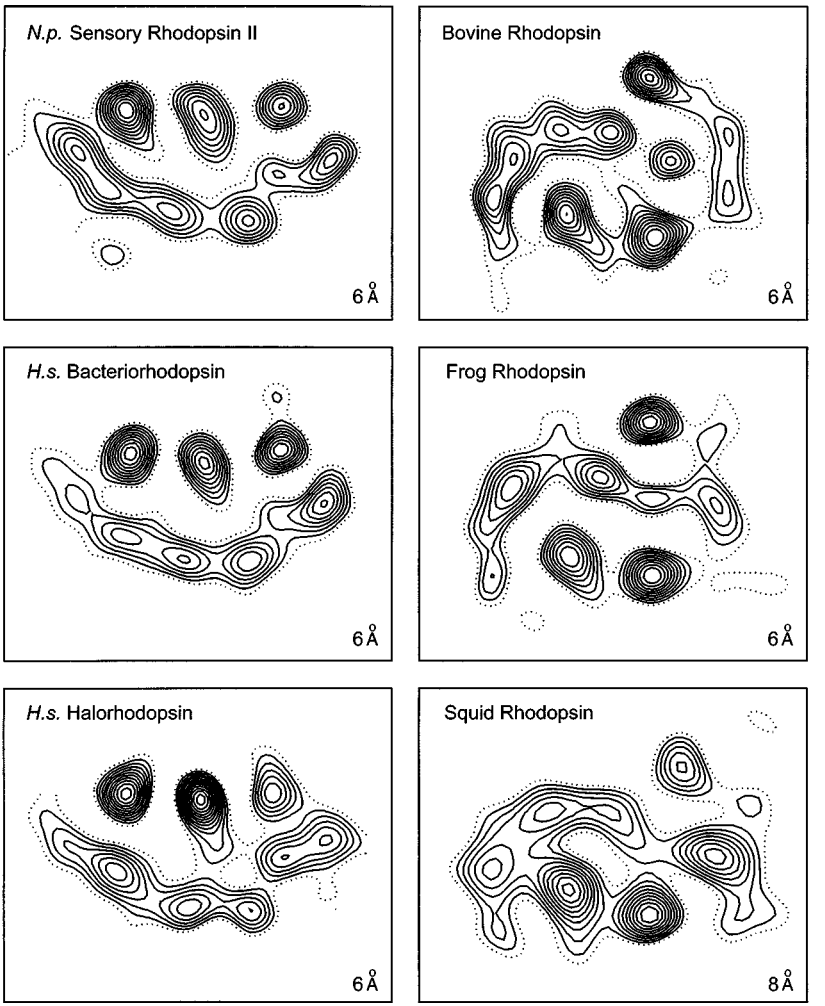

Figure 2 Projection structures of archaeal and visual rhodopsins. Electron density projection maps from electron cryocrystallography of two-dimensional lattices of ordered arrays of protein in lipid bilayers at the indicated resolution are *Natranobacterium pharaonis* SRII (ERS Kunji, EN Spudich, R Grisshammer, R Henderson, JL Spudich, in preparation), *H. salinarum* bacteriorhodopsin (Grigorieff et al 1996), and *H. salinarum* halorhodopsin (Kunji et al 2000), rod rhodopsin from cow (Krebs et al 1998), frog (Schertler & Hargrave 1995), and squid (Davies et al 1996).

very different pattern from that of the archaeal rhodopsins. By comparing the six projection maps it is clear that phylogeny transcends physiological function in the determination of structure in retinylidene proteins.

Retinal Configuration and Conformation

The primary phototransduction event in retinylidene proteins is the isomerization of the chromophore across one of the double bonds in the polyene chain. In all of the type 1 rhodopsins (proven for *H. salinarum* bacteriorhodopsin, halorhodopsin, and sensory rhodopsins I and II) the functional photoisomerization is from all-*trans* to 13-*cis* (Figure 3). Also the NOP-1 protein has been demonstrated to bind the all-*trans* isomeric configuration most efficiently (Bieszke et al 1999b). In mammalian rod rhodopsin and the other visual pigments the configuration of retinal in the dark is 11-*cis*, and photoisomerization converts it to all-*trans* (Figure 3). A number of other extra-retinal or extra-ocular retinylidene proteins (e.g. pinopsin of chicken pineal glands) have been shown to form pigments with the 11-*cis* isomer (Okano et al 1994). Exceptions to the rule that type 2 pigments undergo phototransformation from 11-*cis* to all-*trans* are retinochrome, which is found in the retina of cephalopods and gastropods, and RGR in mammalian retinal pigment epithelium (Pepe & Cugnoli 1992, Shen et al 1994). Retinochrome and RGR bind all-*trans* retinal and catalyze the stereospecific photoisomerization all-*transto* 11-*cis* (Hao & Fong 1999). A main function of these proteins appears to be to regenerate 11-*cis* retinal for reconstitution of bleached visual pigments.

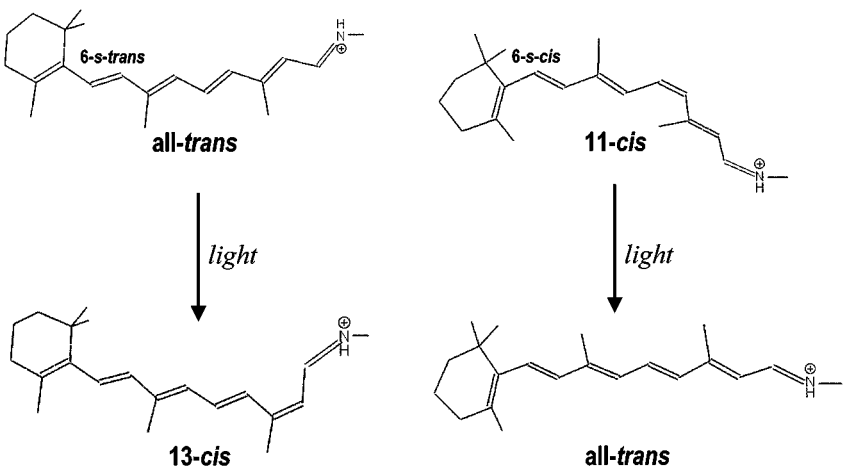

Figure 3 Retinal and its photoisomerization. Structure of the retinal moiety in type 1 (*left*, archaeal rhodopsin-like) and type 2 (*right*, visual pigment-like) retinylidene proteins and the photoisomerization catalyzed by the apoproteins.

The conformation across the C6-C7 single bond that links the retinal's polyene chain to its ionone ring differs in archaeal rhodopsins and in visual pigments (ring/chain 6-*s*-*trans* versus 6-*s*-*cis*, respectively) (Figure 3). The more planar ring/chain conformation (6-*s*-*trans*) provides an additional mechanism for regulating the absorption maximum of the archaeal pigments because the extended conjugated bond system shifts the absorption maximum to a higher wavelength. Current theories of spectral tuning in retinylidene proteins emphasize the interaction of polar or polarizable amino acid residues with the ground- and excited-state charge distributions of the chromophore as one of the most important mechanisms responsible for regulating the absorption maxima (Yan et al 1995, Kakitani et al 1999, Kochendoerfer et al 1999, Lin & Sakmar 1999).

Light-Induced Transformations: The Retinylidene Chromophore is an Endogenous Reporter Group

Photoisomerization of retinal in retinylidene proteins causes a sequence of photochemical events producing structural alterations in the proteins. The photochemical reactions are initiated in the retinal-binding pocket and subsequent changes in the protein outside of the pocket are usually tightly coupled to this photoactive site. Hence the absorption spectrum of the molecule is sensitive to conformational changes throughout the protein, and therefore the altered states are detected as spectrally distinct species (photointermediates). The absorption maxima of the photointermediates are shifted to either the red or blue of the unphotolyzed states of the pigments, which in the dark exhibit strong absorption bands in the visible range; the rod rhodopsin visible absorption maximum is 495 nm, bacteriorhodopsin 568 nm, halorhodopsin 578 nm, sensory rhodopsin I (SRI) 587 nm, and sensory rhodopsin II (SRII) 487 nm. In archaeal rhodopsins the photointermediates spontaneously (i.e. by thermal processes in the dark) return to the unphotolyzed state, completing a photochemical reaction cycle (photocycle). On the other hand, in vertebrate visual pigments the Schiff base attachment of the retinal is disrupted as a consequence of its photochemical reactions; that is, the pigment is bleached and all-*trans* retinal is released from the apoprotein. Metabolic processes in the retinal pigment epithelium regenerate 11-*cis* retinal which, after transport back to the retina, reconstitutes the apoprotein to complete the cycle. This system provides additional points of egulation important in visual information processing (Rando 1991).

In the best-studied cases–mammalian rhodopsin (from bovine rods), bacteriorhodopsin, and the archaeal sensory rhodopsins–the photointermediates produced have similar properties (Hofmann 1999, Spudich et al 1995). First, in picoseconds a red-shifted ("batho") intermediate is observed that undergoes thermal conversion via several steps in submillisecond times to a near-UV-absorbing state called Meta-II in rod rhodopsin and M in bacteriorhodopsin and SRs. These intermediates exhibit absorption maxima in the range of 360–412 nm, which are attributable to the deprotonation of the Schiff base in each of these four pigments. Meta-II is

a signaling state in rod rhodopsin in that high affinity for the G protein transducin occurs in this state (Hofmann 1999). Analogously, the M intermediates of SRI and SRII are signaling states that communicate with the Htr transducer subunits of the SR signaling complexes (Hoff et al 1997). In the proton pump bacteriorhodopsin, formation of the M intermediate is also a crucial step because the transfer of the Schiff base proton to Asp85 is a key part of its proton translocation pathway.

The deprotonated states are easily monitored because of their large spectral shifts, and the kinetics of M and Meta-II formation and decay are readily followed by flash spectroscopy even in whole-cell preparations. This feature and the important roles of Schiff base proton transfers in retinylidene proteins have made these intermediates extremely useful probes in the analysis of mutants and study of the correlation of biochemical properties with phototransformations. Recent work shows that other intermediates are also involved in signaling. Meta-I, the photointermediate that precedes Schiff base deprotonation in the rod pigment, exhibits high affinity for rhodopsin kinase, the first step in the deactivation pathway of the receptor (Hofmann 1999). SRII appears to continue to generate repellent signals to HtrII in the red-shifted O intermediate that occurs after reprotonation of the Schiff base in M decay (Hoff et al 1997). Halorhodopsin does not form M in its photochemical reactions. Protonation of its Schiff base appears to be stabilized by a chloride counterion, a feature of its chloride-transport function (Varo et al 1995). Moreover, certain mutants of both rod rhodopsin and SRII in which Schiff base deprotonation is blocked (and importantly the helix C-helix G salt bridge is eliminated or weakened) still exhibit formation of light-induced signaling states (see Visual Pigments).

RETINYLIDENE PROTEINS IN THE ARCHAEA AND HOMOLOGS IN EUKARYOTIC MICROBES

Transport and Sensory Rhodopsins in Halophilic Archaea

Four archaeal rhodopsins are found in *H. salinarum* membranes: bacteriorhodopsin, halorhodopsin, and sensory rhodopsins I and II (SRI and SRII) (Figure 4, see color insert), and related halophilic archaeons contain homologs of one or more. These four make up all the retinylidene proteins present in the organism according to its total genome sequence (Ng et al 2000). The bacteriorhodopsin and halorhodopsin proteins carry out light-driven electrogenic translocation of protons and chloride, respectively, across the cell membrane (Lanyi 1995, Haupts et al 1999). The resulting hyperpolarization of the membrane is used by the cell to synthesize ATP and to drive substrate active transport and other energy-requiring processes. The sensory rhodopsins are phototaxis receptors, subunits of a signaling complex including transducer proteins [HtrI (Yao & Spudich 1992) and HtrII (Seidel et al 1995, Zhang et al 1996b), respectively] that control a phosphorylation cascade modulating the flagellar motors (Hoff et al 1997) (Figure 4). SRI

is an attractant receptor that guides the cells to orange light able to photoactivate the transport rhodopsins. It also mediates strong repellent responses to potentially damaging near-UV light by a two-photon reaction based on a photochromic mechanism (Spudich & Bogomolni 1984). Bacteriorhodopsin, halorhodopsin, and SRI are produced in semi-anaerobic conditions, when respiration does not satisfy the cells' energy needs. SRII is a blue light-activated repellent receptor made by the cells when they are growing aerobically. SRII absorbs maximally in the spectral peak of solar radiation on the surface of the Earth and therefore is optimally tuned to efficiently detect the light and guide the cells to darkness under conditions in which photooxidative damage is a threat.

A common mechanism for the different functions of the archaeal rhodopsins is suggested by their similarity in structure and by experiments that convert one function to another. First of all, bacteriorhodopsin and halorhodopsin are able to switch functions. At acid pH the proton pump bacteriorhodopsin transports chloride (Der et al 1991), and in the presence of azide the chloride pump halorhodopsin transports protons (Bamberg et al 1993, Varo et al 1996). Moreover, a single mutation, D85T, converts bacteriorhodopsin into a chloride pump with halorhodopsin-like photochemical reactions (Sasaki et al 1995). The interconversion of proton and chloride pumping has been explained in terms of the alternating access of the Schiff base at the attachment site of the retinal (Der et al 1991). The key feature is that the molecules undergo a light-induced conformational change that switches the access of the Schiff base (in the middle of the protein) between the external and cytoplasmic sides of the membrane so that the proton or a mobile chloride counterion to the protonated Schiff base can be released or taken up on either side. The conformational change must make available half channels from the protein surface to the Schiff base at the appropriate times. Therefore, in wild-type bacteriorhodopsin, the reprotonation switch consists of the shift of Schiff base accessibility and the controlled opening of the cytoplasmic half channel in the latter half of the photocycle only after the proton has been committed to the outside (Lanyi 1995). In addition to the conformational change in the protein, a deprotonation-induced decrease in curvature of retinal has also been suggested to contribute to the switch in Schiff base accessibility (Subramaniam et al 1999).

Single mutations (D201N and several substitutions at H166) in SRI convert its attractant signaling form (SR_{587}) into a repellent receptor mediating SRII-like motility responses (Olson et al 1995, Zhang & Spudich 1997), but the most dramatic switch in function of a sensory rhodopsin is its conversion into a light-driven pump. Without mutations SRI is converted from a sensory to a transport rhodopsin simply by liberating it from the tightly bound transducer HtrI subunit. SRI expressed in cells devoid of HtrI pumps protons across the membrane in the same direction as does bacteriorhodopsin (Bogomolni et al 1994, Haupts et al 1995). The electrogenic transport function of transducer-free SRI demonstrates that the essential features of the bacteriorhodopsin pumping mechanism have been conserved in the evolution of the sensor. HtrI interacts with SRI by transmembrane helix-helix contacts (Zhang et al 1999) and inhibits the pump by closing SRI's

cytoplasmic channel (Spudich 1994). This conservation of the pump mechanism and its inhibition by HtrI interaction indicates that the pump machinery, but not the transport activity itself, is functionally relevant for signaling. Recently HtrII-free SRII from the halobacterial species *N. pharaonis* has also been demonstrated to exhibit light-induced proton transfers similar to those of bacteriorhodopsin and carry out light-driven proton translocation (Iwamoto et al 1999, Schmies et al 2000).

Because the transport mechanism of bacteriorhodopsin is conserved in sensory rhodopsins, it seems likely that the light-induced conformational change that switches Schiff base accessibility in bacteriorhodopsin is also conserved. Indeed, a bacteriorhodopsin-like conformational switch is a key component in a current model of signal relay from SR to Htr proteins (Spudich 1998). The light-induced change in the bacteriorhodopsin conformation has been detected directly by electron two-dimensional crystallography of an intermediate in the bacteriorhodopsin photocycle trapped by rapid freezing after illumination (Subramaniam et al 1993, Subramaniam & Henderson 1999) and by analysis of two-dimensional crystals of a mutant containing three mutations that favor the normally light-induced conformation in the dark (Subramaniam et al 1999). The change involves a tilting of helices outward on the cytoplasmic side of the protein, especially Helix F, which pivots from residues Tyr185 and Pro186 outward displacing its cytoplasmic end $\sim$3 Å (Subramaniam et al 1993, Vonck 1996, Subramaniam & Henderson 1999, Luecke et al 1999a,b). An X-ray crystallography study of three-dimensional crystals further characterizes the conformational change occurring in the region identified by electron crystallography (Luecke et al 1999a). More detailed atomic information awaits overcoming the limitation as phototransformation of the protein appears to disorder the three-dimensional crystal lattice in this most conformationally active region of the protein.

Conformations of light-induced intermediates have not been directly observed in the sensory rhodopsins by crystallographic methods, but the following findings taken together argue strongly for the view that a similar change as in bacteriorhodopsin occurs and that the Htr transducers couple to the conformationally active helices on the cytoplasmic side: (*a*) Transducer-free SRI pumps protons and its proton-pumping activity is abolished by interaction with its transducer HtrI (Bogomolni et al 1994, Sasaki & Spudich 1999). (*b*) HtrI blocks or prevents the light-induced movement of protons through the cytoplasmic channel that occurs in HtrI-free SRI (Spudich & Spudich 1993, Olson & Spudich 1993). (*c*) A mutation in SRII (D73N), which is homologous to a mutation in bacteriorhodopsin (D85N) that induces the open cytoplasmic channel conformation of bacteriorhodopsin in the dark, constitutively activates SRII in the dark (Spudich et al 1997). (*d*) The light-minus-dark difference FTIR spectrum of SRII indicates that a protein conformational change similar to that in bacteriorhodopsin occurs in the second half of the SRII photocycle (Bergo et al 2000). (*e*) Htr transducer deletions (Perazzona et al 1996) and chimeras (Zhang et al 1999) show that the signal relay from SRs to their cognate Htrs is via lateral interactions of their respective transmembrane

helices, as would be expected if helix displacements of the SRs similar to those that occur in bacteriorhodopsin induce displacements in Htr helices. (*f*) Mutations in the cytoplasmic side of the HtrI second transmembrane helix (TM2) modulate SRI photochemical reactions, indicating conformational coupling in this region (Jung & Spudich 1996), and a suppressor selection scheme designed to obtain HtrI mutations restoring SRI mutant defects produced mutations clustered in the cytoplasmic side of TM2 of HtrI (Jung & Spudich 1998).

Based on these observations, a model has been proposed that relates sensory rhodopsin signal transmission to the opening of the cytoplasmic channel in the bacteriorhodopsin pumping cycle (Spudich 1998). Further discussion of the effects of Htr transducers on their cognate sensory rhodopsins' photochemistry can be found in Spudich (1994) and Hoff et al (1997) and on their light-induced proton transfers in Sasaki & Spudich (2000).

Rhodopsins in Fungi and Homologs in Fungi and Yeast

Up until last year, ~30 members of the archaeal rhodopsin family had been described, all in archaeal halophilic prokaryotes (Mukohata et al 1999). The recent reports of members of this family in eukaryotes open the possibility for a much broader distribution than previously realized. A sequencing project on the genome of the filamentous fungus *N. crassa* revealed the first of the eukaryotic homologs, designated NOP-1 (Bieszke et al 1999a). The *nop-1* gene was heterologously expressed in the yeast *Pichia pastoris*, and the results demonstrated that it encodes a membrane protein that forms with all-*trans* retinal a green light-absorbing pigment (λ_{max} 534 nm) with a spectral shape and bandwidth typical of rhodopsins (Bieszke et al 1999b). Laser flash kinetic spectroscopy of the retinal-reconstituted NOP-1 pigment reveals that it undergoes a slow photocycle 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 bacteriorhodopsin and sensory rhodopsins I and II. A red-shifted intermediate late in the photocycle similar to the O intermediate in bacteriorhodopsin and SRII was also detected. The presence of both M-like and O-like species in a seconds-long photocycle is most closely similar to that of the phototaxis receptor SRII (Sasaki & Spudich 1998).

The function of NOP-1 cannot be assigned based on its primary sequence because phylogenetic analysis places NOP-1 equally distant from archaeal transport and archaeal sensory rhodopsins (Bieszke et al 1999a). The flash photolysis analysis of the Neurospora rhodopsin formed from NOP-1 and retinal, however, perhaps provides a clue. The transport rhodopsins are characterized by photocycles of ~10 ms, whereas sensory rhodopsins are slow-cycling pigments with lifetimes in the 100 ms to seconds range (Spudich et al 1995). This kinetic difference is functionally important because a rapid photocycling rate is advantageous for efficient pumping for a transporter, whereas a slower cycle provides more efficient light detection to a sensor since signaling states persist for longer times (Yan et al 1991b). The long lifetime of the photo intermediates of Neurospora rhodopsin therefore

argues for a sensory function. One caveat is that the Neurospora rhodopsin kinetics may be altered by heterologous expression in the non-native membrane environment.

A search of genome databases currently in progress indicates the presence of archaeal rhodopsin homologs in various filamentous fungi. Two genes, one in *Fusarium sporotrichioides* and the other in *Leptosphaeria maculans*, predict proteins with high identity in the retinal-binding pocket. Generalizing from the NOP-1 results, these genes are likely to encode photoactive retinylidene proteins. Several other genes in *N. crassa*, *Aspergillus nidulans*, and *Saccharomyces cerevisiae* predict proteins that exhibit significant homology to archaeal rhodopsins but are missing critical residues in the retinal-binding pocket.

Comparison of the sequences of these archaeal-opsin-related proteins reveals patches of conserved residues. The 22-residue retinal-binding pocket of the archaeal rhodopsin family is shown in blue in the NOP-1 sequence, and the residues shared with the archaeal rhodopsins outside of the pocket are shown in green (Figure 5, *top*; see color insert). Also shown in the figure are two representative opsin-related protein sequences, one from the filamentous fungus *A. nidulans* (*middle*) and the other from the yeast *S. cerevisiae* (*bottom*). Eight and 12 residues, respectively, of the retinal-binding pocket are missing in these two proteins, including the critical lysine in the seventh helix to which retinal attaches covalently. The archaeal-opsin-related proteins are therefore not likely to form a photoactive pigment with retinal. The strongest region of conservation in these proteins is along helix C, the extracellular part of helix E, and the middle of helix F. Given the transport function of archaeal rhodopsins, it is intriguing that one of the yeast opsin-related proteins, HSP30 (30 kDa heat shock protein), is implicated as interacting with two transport proteins. First, HSP30 has a known function of down-regulating stress-stimulation of H^+ATPase activity under heat shock conditions (Piper et al 1997). More recently, it has been proposed to interact with the preprotein translocase of the mitochondrial outer membrane (Plesofsky et al 1999). It is tempting to speculate that the conformational switching properties of the archaeal rhodopsins have been preserved in these opsin-related proteins, while the photoactive site has been lost and replaced, presumably by another input module such as a protein-protein interaction domain.

Evidence for Archaeal Rhodopsin Homologs as Phototaxis Receptors in Algae and Fungi

Restoration of phototaxis by retinal addition to a pigment-deficient mutant of *Chlamydomonas reinhardtii* first indicated a retinal-containing photoreceptor in a eukaryotic microorganism (Foster et al 1984). Reconstitution with various retinal analogs was shown to shift the action spectrum for phototaxis, which is strong evidence that the added retinal enters the photoreceptor as opposed to stimulating phototaxis in an indirect manner. Similar evidence has been reported for phototaxis by zoospores of the fungus *Allomyces reticulatus* (Saranak & Foster 1997). Several

laboratories have subsequently studied motility behavior of pigment-deficient *C. reinhardtii* cells reconstituted with retinal isomers and analogs. These studies confirmed the retinylidene chromophore in *C. reinhardtii* phototaxis and, moreover, demonstrated an archaeal-like retinal configuration (all-*trans*) and ring/chain conformation (6-*s*-*trans*) in the *C. reinhardtii* photoreceptor (Hegemann et al 1991, Lawson et al 1991, Sakamoto et al 1998). Moreover, preventing isomerization across the C13-C14 double bond by using "isomer-locked" retinals prevented behavioral photoresponses, indicating all-*trans* to 13-*cis* isomerization is required for signaling, as in archaeal sensory rhodopsins.

Euglena gracilis is another motile, unicellular green alga that exhibits phototaxis. The receptors responsible for its photoresponses have been the focus of several studies, and much of the evidence points to a flavin and/or pterin chromophore (Schmidt et al 1990). However, Gualtieri and coworkers (Gualtieri et al 1992) extracted all-*trans* retinal from *Euglena* cells and suggested that this chromophore derives from a rhodopsin-like photoreceptor.

Kreimer and coworkers reported the green alga *Spermatozopsis similis* exhibits photobleached absorption peaks by difference absorption spectroscopy of enriched eyespots and associated membranes (Kreimer et al 1991a,b). Reconstitution of bleached material with retinal generated an absorption band at 540 nm, and retinal was extractable from unbleached material.

Chlamyrhodopsin and Volvoxrhodopsin A 26-kDa membrane protein given the name chlamyrhodopsin was isolated from eyespot preparations from *C. reinhardtii* and proposed to be a retinylidene receptor for phototaxis (Deininger et al 1995). The same group cloned a gene encoding a homologous protein named by the authors volvoxrhodopsin from the multicellular alga *Volvox carteri* (Ebnet et al 1999). These proteins do not exhibit the characteristic seven-helix motif nor extensive regions of identity with either archaeal or visual rhodopsins and therefore have been proposed to represent a new type of photosensory receptor. The main evidence for the proposed function of these proteins as retinylidene phototaxis receptors is as follows: (*a*) The chlamyrhodopsin protein forms a covalent attachment with radioactive retinal upon treatment with a reducing agent, indicating that retinal forms a Schiff base linkage with one or more of the protein's lysines (Deininger et al 1995). (*b*) The protein is located in the eyespot region of the cell, which is where the phototaxis machinery is known to be located. (*c*) In eyespot preparations similar to those used to isolate the protein, hydroxylamine-bleaching and retinal addition indicate the presence of a pigment with the appropriate absorption spectrum (Beckmann & Hegemann 1991). (*d*) The volvoxrhodopsin primary sequence (although not that of chlamyrhodopsin) matches 10 out of 27 residues in a consensus sequence in the retinal attachment site in a group of invertebrate rhodopsins, including an alanine-lysine-alanine containing the retinal-binding lysine in the invertebrate rhodopsins. (*e*) An antisense transformant with $\sim$10% of the volvoxrhodopsin protein exhibits phototaxis sensitivity reduced 10-fold (Ebnet et al 1999).

The data are suggestive, although further evidence is needed before concluding definitively that the proteins are photoactive retinylidene proteins. It would be important to establish that the reductive-labeling with radioactive retinal is specifically with one lysine since retinal readily forms Schiff base linkages with lysine ε-amino groups, and the proteins proposed to be the receptors have relatively high lysine contents (15% of the residues in chlamyrhodopsin protein and 17% of those in volvoxrhodopsin protein). The detection of color and/or photoactivity directly attributable to the proteins would be valuable. Also gene knockout and rescue experiments might provide definitive information.

If the identified proteins are retinylidene photoreceptors, they represent an interesting new class of such proteins, as the authors point out (Deininger et al 1995). The lysine-rich sequences interspersed with hydrophobic residues are similar to the S4 stretches of voltage-gated or cGMP-gated channels (Ebnet et al 1999). This feature would be consistent with the rapidly induced photoreceptor currents suggesting that the receptor controls a pre-bound ion channel or that it has a light-regulated conductance (Braun & Hegemann 1999). On the other hand, the recent findings of archaeal-type rhodopsins in eukaryotic microbes and the archaeal-type retinylidene chromophore deduced from the in vivo retinal analog reconstitution studies suggest type 1 rhodopsins may be present in *C. reinhardtii*. It is possible that the protein called chlamyrhodopsin is an ion channel-containing component that interacts with an undiscovered retinylidene photoreceptor.

HIGHER ANIMAL RETINYLIDENE PROTEINS

Visual Pigments

Whereas type 1 sensory rhodopsins signal to integral membrane transducers by transmembrane helix interactions, signaling by mammalian rod rhodopsin involves its cytoplasmic loops.

Light-activation of rod rhodopsin recruits at least three proteins to its cytoplasmic surface: the G protein transducin, rhodopsin kinase, and arrestin. How structural changes initiated at the photoactive center of the protein are propagated to the loops at the surface has been investigated by a variety of mutagenesis and biophysical studies (Helmreich & Hofmann 1996, Rao & Oprian 1996). Spin-labeling studies of cysteine-substituted mutants detect light-induced outward movements of helices C and F and a rotation of helix F relative to helix C (Farahbakhsh et al 1995, Farrens et al 1996). The introduction of a zinc-binding site engineered at the cytoplasmic ends of transmembrane helices C and F creates Zn^{2+}-dependent inhibition of transducin activation, which indicated that the helices C and F are in close proximity and that their movement relative to one another is required for transducin activation (Sheikh et al 1996). A recent cross-linking study (Borhan et al 2000) found that in rod rhodopsin the retinal β-ionone ring moves from the vicinity of Trp265 in helix F to that of Alal69 in helix D. This result suggests

a substantial light-induced conformational change in the protein because Alal69 does not appear to be accessible to the ionone ring in the dark crystal structure (Palczewski et al 2000), even after considering possible changes in the retinal position owing to isomerization (Bourne & Meng 2000). These results, the locations of activating mutations, and fluorescent reporter group studies of other G protein–coupled receptors suggest models in which helices C and F rotation and translation propagate conformational changes through the membrane-embedded structure of the receptor to the loops (Hulme et al 1999).

It is interesting to compare the molecular events leading to rhodopsin activation with those of archaeal sensory rhodopsins. The outcome of activation in both cases is altered protein-protein interaction but with different proteins, membrane-embedded in the case of the SRI and SRII and soluble in the case of rod rhodopsin. Hence the altered receptor surfaces are expected to be different, but the initial events occurring in the photoactive site are strikingly similar: (*a*) Both types of sensory receptors are activated by photoisomerization of the retinal. (*b*) Both require a similar steric trigger to generate its signaling state. SRI requires steric interaction of a methyl group on the retinal polyene chain (the 13-methyl) with a protein residue to generate its signaling state M (Yan et al 1991a), and rod rhodopsin requires interaction of the other methyl group on the retinal polyene chain (the 9-methyl) with the protein to generate its signaling state Meta-II (Ganter et al 1989). (*c*) Formation of the respective signaling states SRII M (λ_{max} 350 nm) and rod rhodopsin Meta-II (λ_{max} 380 nm) entail transfer of the Schiff base proton from helix G to the proton acceptor on helix C [Asp73 in SRII (Bergo et al 2000); Glu113 in rod rhodopsin (Khorana 1993)]. (*d*) Mutagenic disruption of the helix G-helix C interhelical salt bridge formed by the protonated Schiff base with Asp73 and Glu113 (by mutations D73N and E113Q) constitutively activate rod rhodopsin and SRII, respectively. The disruption of the Lys216 protonated retinylidene Schiff base salt bridge with Asp85 in bacteriorhodopsin (Figure 6, *left*; see color insert) is a major contributor to the conformational change in its pumping cycle (Lanyi 1995). Similarly, disruption of the corresponding helix G-helix C salt bridges in SRII (Spudich et al 1997) and in rod rhodopsin (Robinson et al 1992) induces their signaling conformations, although retinal must be isomerized or removed for activation in the latter but not in the former. (*e*) As discussed above, helices C and F are implicated in the conformational switching in SRs, and analogously helices C and F are implicated in the activation of rod rhodopsin. (*f*) Finally, in both cases helix F appears to be the most mobile helix in its light-induced conformational change.

The double mutant of rod rhodopsin E113A/A117E, which provides an alternative counterion for the protonated Schiff base on helix C, exhibits light-induced activation of the receptor without Schiff base deprotonation (Fahmy et al 1994). This result indicates that the primary role of the deprotonation of the Schiff base is to neutralize E113, but that if it is already neutral, other consequences of photoisomerization are still sufficient to drive the protein to some extent into its signaling state. The same is true of SRII, in that the SRII mutant D73A, which has a Schiff base that remains protonated throughout the photocycle (probably stabilized by

a chloride), is partly constitutively active but still generates strong light-induced repellent signals to HtrII (EN Spudich. J Sasaki & JL Spudich, unpublished data).

Octopus rhodopsin is similar to the neutralized counterion mutants of rhodopsin and SRII in that it lacks an anionic counterion to the Schiff base, and its signaling conformation is formed without Schiff base deprotonation (Nakagawa et al 1999). The authors of this study point out that invertebrate rhodopsin activation may be simpler than that of vertebrates because the proton transfer step does not need to occur owing to the fact that there is no Schiff base/counterion salt bridge constraining the protein in an inactive state.

Work on other G protein-coupled receptors raises the possibility that visual pigments, like the archaeal SRs, may exhibit lateral helix-helix interactions in the membrane (Bockaert & Pin 1999). The angiotensin II receptor and mutant chimeric muscarinic/adrenergic receptors form dimers (Maggio et al 1993, Monnot et al 1996), and a peptide corresponding to helix F of the β2-adrenergic receptor was found to inhibit dimerization, indicating helix-helix interaction (Hebert et al 1996). The GABA-B receptor is a heterodimer of two similar seven-helix subunits that may make contact in the transmembrane regions (Jones et al 1998, Kaupmann et al 1998, White et al 1998). Also single transmembrane helix proteins (RAMPs) associate with the calcitonin- receptor-like receptor (CRLR) to regulate its transport and ligand specificity (McLatchie et al 1998).

Pinopsins

Genes expressing extra-ocular photoreceptor pineal-specific opsins (pinopsins) have been cloned from chicken (*Gallus gallus*) (Okano et al 1994, Max et al 1995), pigeon (*Columba livia*) (Kawamura & Yokoyama 1996), and chameleon (*Anolis carolinensis*) (Kawamura & Yokoyama 1997). Similar extra-ocular receptor genes have been cloned from the brain hypothalamus of toads (*Bufo japonicus*) (Yoshikawa et al 1998) and marine lamprey (*Petromyzon marinus*) (Yokoyama & Zhang 1997).

There is indirect evidence that pinopsin is a circadian rhythm photoreceptor. In avian pineal glands, a light signal resets the phase of the endogenous circadian oscillator that controls serotonin *N*-acetyltransferase activity, which is responsible for the melatonin rhythm and triggers a light-induced phase-shift of the circadian pacemaker (Okano & Fukada 1997). Pinopsin is the most abundant photoreceptive molecule in the pineal gland and is not detectable in retina. Also, toad pinopsin may play a role in breeding behavior such as the organism's photoperiodic gonadal response.

The pinopsin proteins are 43–48% identical in amino acid sequence to vertebrate retinal opsins. The apoprotein binds 11-*cis*-retinal and is sensitive to blue-green light ($\lambda_{max} = 470$ nm in chicken, 481 nm in pigeon). The Schiff base lysine in helix G, corresponding to Lys296 in bovine rod rhodopsin, is conserved, as is the glutamate counterion in helix C, the retinal pocket implicated in spectral tuning, and most of the residues in the cytoplasmic domains that are functionally coupled with G-proteins.

Retinochromes and RGR Opsins

Retinochrome (retinal photoisomerase) is a photosensitive pigment found in the retina of squid (*Todarodes pacificus*) and octopus (*Octopus bimaculoides*) (Hara & Hara 1982). Retinochrome facilitates the photoregeneration of visual pigments in visual cells of these organisms during daylight by catalyzing photoisomerization of all-*trans* retinal to the 11-*cis* configuration used by visual rhodopsins (Pepe & Cugnoli 1992). The retinochrome apoprotein forms with the all-*trans* isomer a photoreactive retinylidene pigment that is photoconverted to metaretinochrome, which contains 11-*cis* retinal. The visible absorption maximum of *Todarodes* retinochrome is 495 nm and that of its metaretinochrome photointermediate is near 470 nm. The visible absorption maximum of retinochrome varies from species to species, but it is generally 15–25 nm longer than that of the corresponding visual rhodopsin (Hara & Hara 1982). The hydropathy profile indicates that retinochrome contains seven transmembrane helices and that there is recognizable homology to bovine rod rhodopsin (Lomize et al 1999). The protein at 33 kDa is smaller than visual pigments, and it lacks the C-terminal region that contains phosphorylation sites involved in arrestin binding. Retinochrome may have only a catalytic as opposed to signaling function.

A retinal photoisomerase that catalyzes all-*trans* to 11-*cis* isomerization is also found in mammalian retinal pigment epithelium (RPE) in the eye. The RPE is a specialized cell monolayer that lies adjacent to the photoreceptors and performs the function of restoring the 11-*cis* retinal from its all-*trans* configuration released from the visual pigments, thereby allowing their regeneration after bleaching. The RPE and Muller cells contain a blue and UV-absorbing pigment (absorption maxima at 469 nm and 370 nm), called the RPE retinal RGRor RGR opsin). RGR has been identified in human and mouse and it binds preferentially all-*trans*-retinal rather than the 11-*cis* isomer (Shen et al 1994). Photoexcitation of either of its absorption bands results in stereospecific isomerization of the bound all-*trans*-retinal to an 11-*cis* configuration (Hao & Fong 1996, 1999). Because RGR appears to contain a G protein–binding domain, a signal transduction function seems likely in addition to an 11-*cis* retinal regeneration function.

Other Retinylidene Receptor Proteins (Parapinopsin, VA opsin, Melanopsin, Peropsin, and Encephalopsin)

There are green, blue, violet, and red-absorbing rhodopsins in retina for visual reception in vertebrates. Additional retinal, extra-retinal, and extra-ocular photoreceptors may play important roles in circadian rhythmicity, camouflage and body color change, detection of ambient light conditions and seasonal changes in the photoperiod, and, especially in juvenile stages, negative phototaxis. Parapinopsin is present in the parapineal gland of a cold-blooded vertebrate, the channel catfish (*Ictalurus punctatus*) (Blackshaw & Snyder 1997). Parapinopsin is predominantly localized to the parapineal organ and is expressed in relatively small amounts in the pineal gland in catfish. In Atlantic salmon (*Salmo salaar*) a retinylidene

pigment called vertebrate ancient opsin (VA opsin) is expressed not only in retinal horizontal and amacrine cells of the eye but also in cells of the pineal and sub-habeular areas of the brain (Soni & Foster 1997, Soni et al 1998). Therefore, retinal photoreception is not restricted to just rod and cone cells. Both areas of the pineal complex and eyes are involved in circadian rhythms and the production of melatonin; hence VA receptors may mediate effects of light upon the circadian clock and/or melatonin synthesis.

A variety of novel opsins in non-mammalian vertebrates have been identified including melanopsin, which is found in photosensitive dermal melanophores and retinal horizontal cells in *Xenopus laevis* (Provencio et al 1998). The primary amino acid sequence shows greatest homology with cephalopod opsins. A predicted secondary structure indicates the presence of a G protein–binding domain and an exceptionally long C-terminal tail with multiple putative phosphorylation sites. Melanopsin mRNA is expressed in hypothalamic sites and in the iris. Its expression in both retinal and nonretinal tissues suggests a role in vision and nonvisual photoreceptive functions such as photic control of skin pigmentation, pupillary aperture, and circadian and photoperiodic physiology. A human melanopsin was described recently in the inner retina of the eye (Provencio et al 2000). Expression was restricted to cells within the ganglion and amacrine cell layers of primate and murine retinas, and notably, was not observed in the visual pigment-containing cells of the outer retina, suggesting that melanopsin mediates only nonvisual photoreceptive tasks.

Another visual pigment-like protein, peropsin, has been identified in cDNA derived from human ocular tissue (Sun et al 1997). The corresponding mRNA was found only in the eye, where it is localized to the apical face of RPE and most prominently to the microvilli that surround the photoreceptor outer segments. The authors suggest that peropsin may play a role in RPE physiology, either by detecting light directly or by monitoring the concentration of retinoids or other photoreceptor-derived compounds.

Encephalopsin is the first putative extraocular opsin identified in mammals (human and mouse). It shows strong and specific expression in the brain in the preoptic area and paraventricular nucleus of the hypothalamus and may play a modulatory role in circadian entrainment (Blackshaw & Snyder 1999).

These retinal, extra-retinal and extra-ocular pigments fulfill the criteria expected of functional opsins. The Schiff base lysine corresponding to Lys296 in bovine rod rhodopsin is conserved, as is the acidic residue corresponding to the counterion Glu113 (Glu or Asp). Many residues involved in G protein–binding and spectral tuning in vertebrate opsins are conserved as well.

CONCLUSIONS AND PROSPECTIVES

The evolutionary relationship between type 1 and type 2 rhodopsins cannot be decided with certainty. Did nature discover retinal just once and evolve two distinct families? Their common origin may be obscured in the examples that we

know, which are after all from evolutionarily distant creatures, since all type 1 rhodopsins so for identified are found in microorganisms and all type 2 are found in higher animals. The single- progenitor hypothesis will be tested in future genome projects and would be confirmed if a missing link were to be found, i.e. a gene encoding a retinylidene protein with both type 1 and type 2 sequence identity.

On the other hand, the genome sequence data presently available and the three-dimensional structures of the molecules themselves argue that nature discovered retinal twice, and both times found it useful, when solvated with seven helices, for photosensory signaling as well as other phototransduction functions. The two-progenitor hypothesis would require that archaeal sensory rhodopsins and mammalian rhodopsins have converged on remarkably similar mechanisms of receptor photoactivation. Such closely similar mechanisms could result from "likely reinvention" determined by the inherent properties of retinal as a chromophore.

Retinylidene proteins are likely to continue to be on the cutting edge in research on atomic-resolution structure/function of membrane proteins. The inherent advantage of using light to produce conformational change is being exploited to trap photointermediate conformations of bacteriorhodopsin in two- and three-dimensional crystals. This approach and time-resolved molecular spectroscopy can be expected to fill out our now mostly static views in the dark with a moving picture of the light-induced development of functional protein conformational changes from the initial photoisomerization of retinal. Similar approaches to the archaeal sensory rhodopsin signaling complexes are likely to contribute greatly to our understanding of protein-protein interaction within the membrane, and to visual rhodopsins the recruitment of signal transducing proteins to the membrane.

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NOTE ADDED IN PROOF

A new rhodopsin with sequence homology to the previously described type 1 rhodopsins in *Archaea* and *Eucarya* has recently been discovered in marine bacterioplankton proteobacteria, extending the existence of this family to the third domain, *Bacteria*. The bacterial pigment, named proteorhodopsin, functions as a light-driven proton pump when heterologously expressed in *E. coli*.
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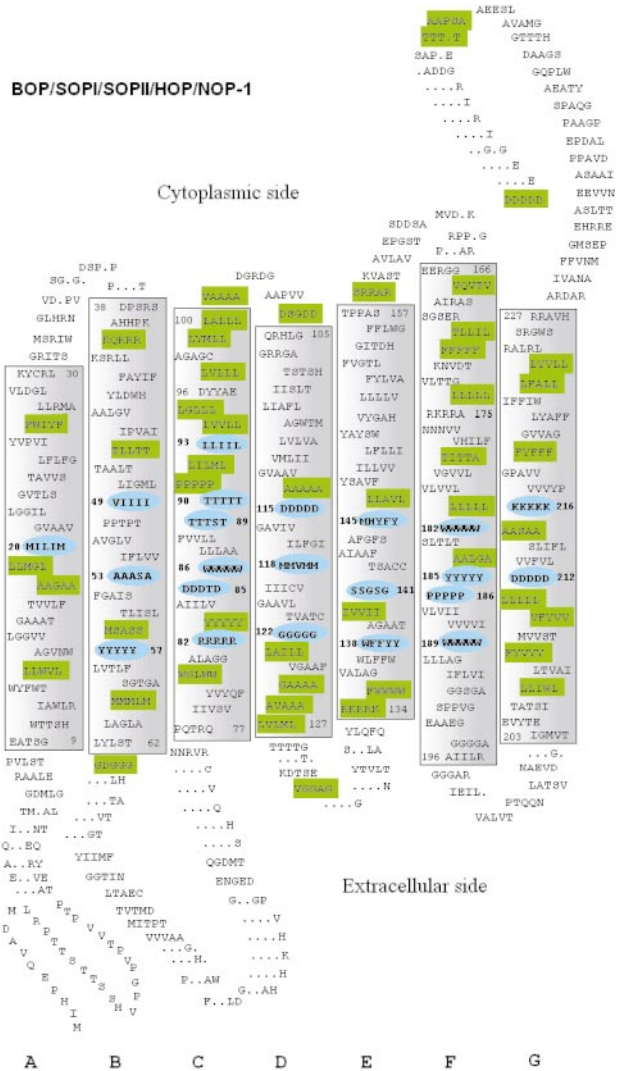

Figure 1 Seven-helix transmembrane helix topology of representatives of the type 1 and type 2 retinylidene protein families. Alignment of five archaeal-type retinylidene apoproteins (opsins) of bacteriorhodopsin (BOP), sensory rhodopsins I and II (SOPI, SOPII), halorhodopsin (HOP) from *Halobacterium salinarum*, and NOP-1 protein from *Neurospora crassa.* The helix boundaries are based on those of bacteriorhodopsin, as are the residue numbers. Residues corresponding to those of bacteriorhodopsin forming the retinal-binding pocket are indicated in blue, and residues in NOP-1 identical to at least two of the four archaeal rhodopsin residues at the corresponding position are indicated in green. Dots represent gaps in the corresponding sequence.

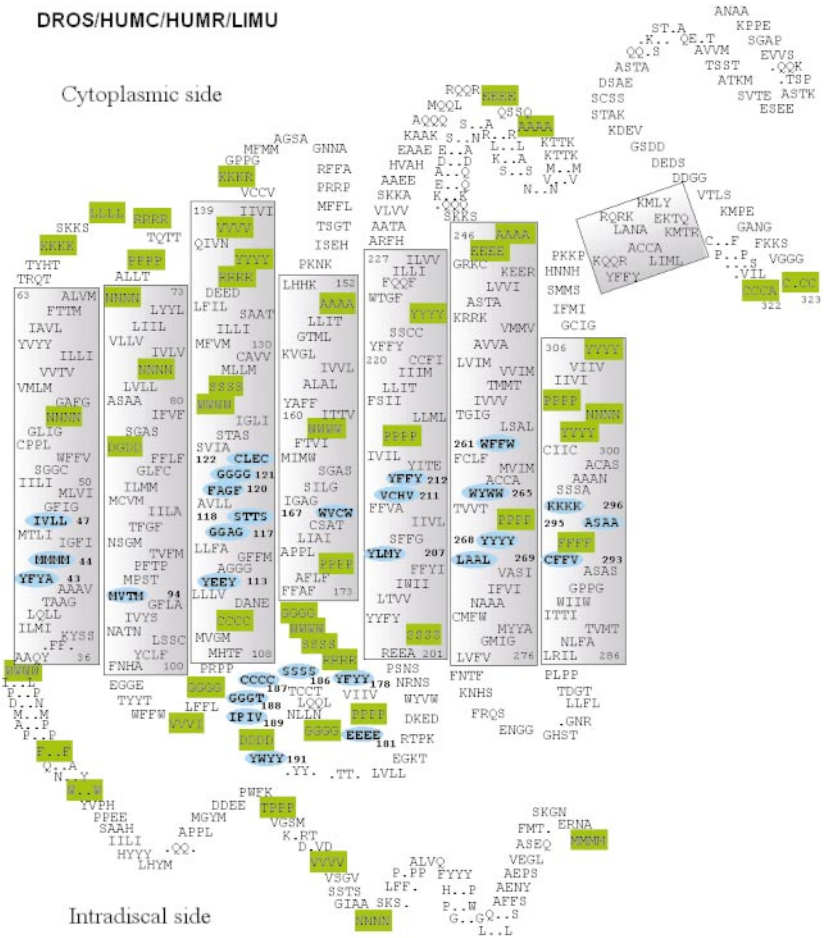

Figure 1 (back) Alignment and partial retinal-binding pocket of four visual pigment apoproteins, from *Drosophila melanogaster* (DROS), human red cones (HUMC), human rod rhodopsin (HUMR), and *Limulus polyphemus* lateral eye opsin (LIMU). The helix boundaries and residue numbers are based on those of bovine rod rhodopsin (Palczewski et al 2000). Residues corresponding to those of bovine rod rhodopsin forming the retinal-binding pocket are indicated in blue. Residues at positions 186 through 189 form a $-sheet that covers the intradiscal side of the retinal polyene chain. Positions at which identical residues occur are indicated in green. Also indicated in green are residues at positions 23, 83, 104, and 141 (numbers correspond to human rod rhodopsin) that have been shown to be conserved in previous alignments with a large number of visual pigments (Applebury & Hargrave 1986).

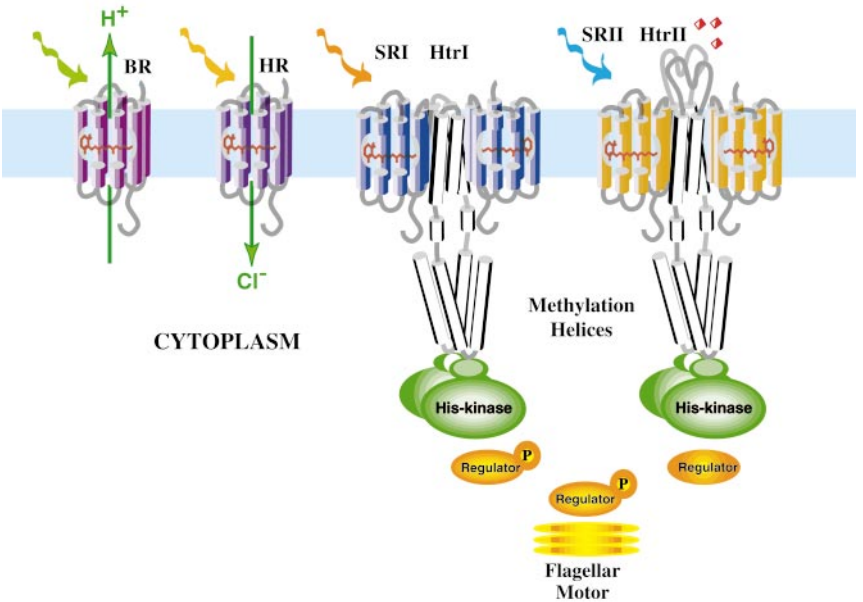

Figure 4 The four archaeal rhodopsins in *H. salinarum.* The transport rhodopsins bacteriorhodopsin (BR, a proton pump) and halorhodopsin (HR, a chloride pump) and the phototaxis receptors sensory rhodopsins I and II (SRI and SRII) with components in their signal transduction chains are shown. The colors of the four rhodopsins are the approximate colors of the pigments. The sensory rhodopsins are complexed to their cognate transducer proteins HtrI and HtrII, which have adaptative methylation and histidine kinase-binding domains conserved in the large family of prokaryotic taxis transducers (Yao & Spudich 1992, Zhang et al 1996). The Htr proteins modulate kinase activity, which in turn controls flagellar motor switching through a cytoplasmic phosphoregulator (Rudolph et al 1995). HtrII also functions as a chemotaxis receptor for serine via its external domain (Hou et al 1998). The structures drawn for the Htr transducers are only approximate, based on secondary structure predictions (Surette & Stock 1996) and a crystal structure of a eubacterial member of the transducer family, the serine chemotaxis receptor of *E. coli* (Kim et al 1999). The transducers are represented as dimers based on the rapid quantitative disulfide crosslinking into dimers observed following oxidation of HtrI engineered to contain cysteine residues (Zhang & Spudich 1998). It is not certain whether the stoichiometry is one or two SR molecules per Htr dimer. The relative positions of Htr and SR helices are chosen for illustration only.

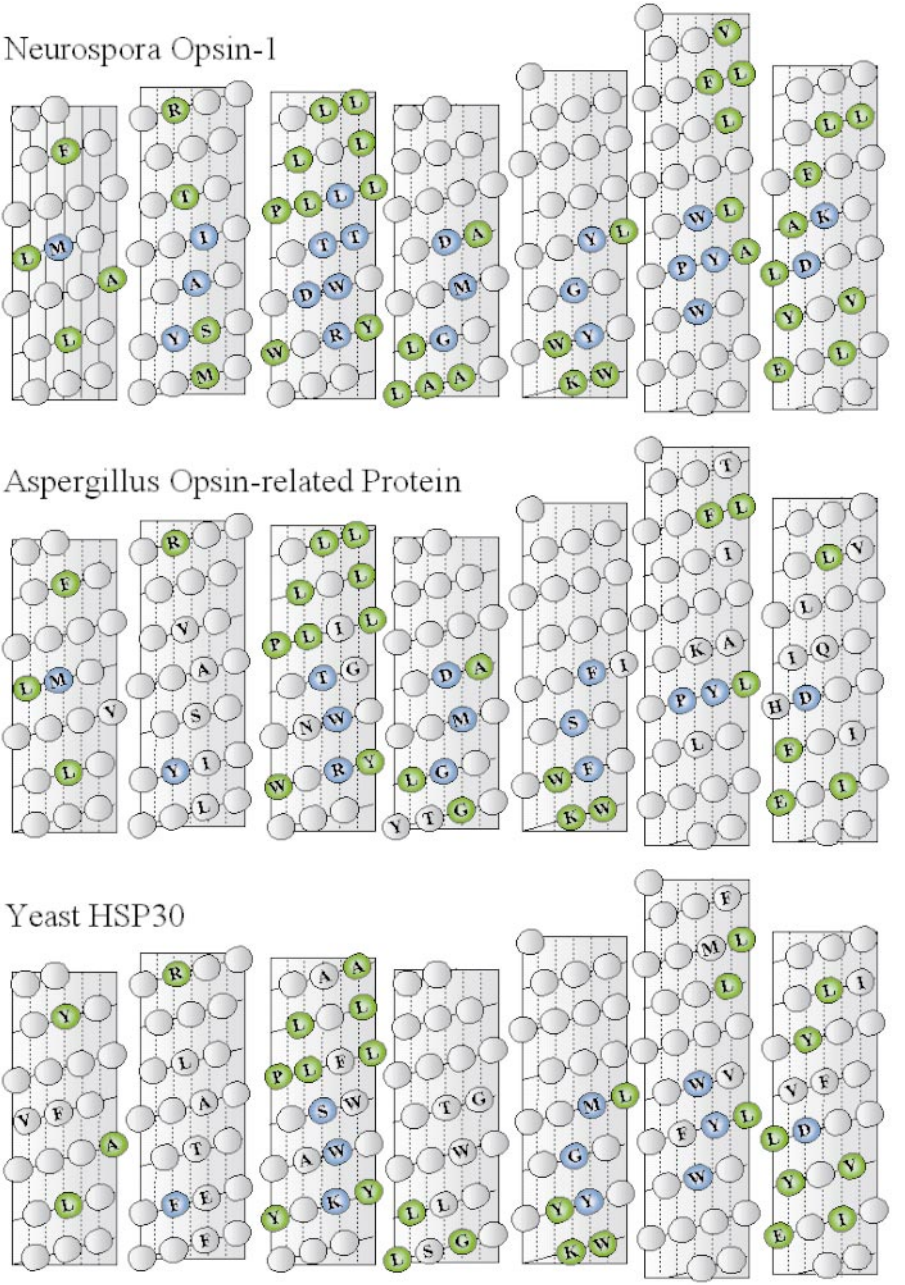

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Figure 5 Progressive disappearance of the archaeal retinal-binding pocket (blue) and other conserved residues (green) in eukaryotic opsins and opsin-related proteins. *Top:* NOP-1 from *N. crassa; Middle:* an opsin-related protein from the filamentous fungus *Aspergillus nidulans; Bottom:* (C) HSP30 (a 30-kDa heat shock protein) from *Saccharomyces cerevisiae.* The 22-residue retinal-binding pocket of the type 1 rhodopsin family (Figure 1) is indicated in blue in the NOP-1 sequence, and the residues shared with the archaeal rhodopsins outside of the pocket are indicated in green. These positions are labeled in the Aspergillus and yeast proteins even if not conserved and colored only if they match one or more of the archaeal rhodopsins. For the purpose of assessing conservation, Lys and Arg, Ser and Thr, and Trp, Tyr and Phe were considered as conservative substitutions.

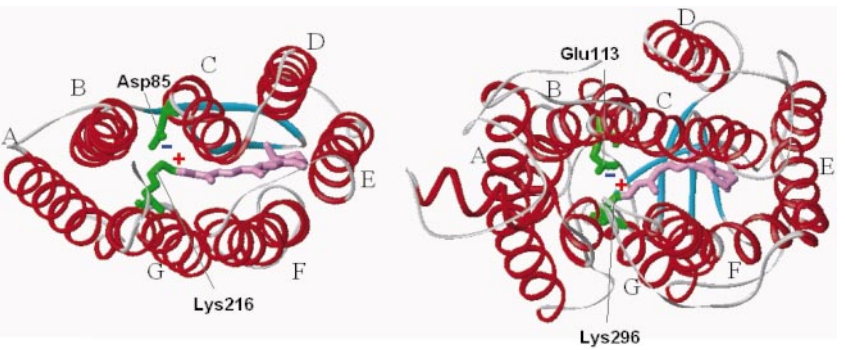

Figure 6 Retinal and the interhelical salt-bridge in archaeal and visual rhodopsins. Projection views of bacteriorhodopsin (*left*) and bovine rod rhodopsin (*right*). The coordinates for bacteriorhodopsin and bovine rod rhodopsin are derived from their crystal structures (Luecke et al 1999b and Palczewski et al 2000, respectively). The molecules have been rotated to best show the retinal (purple), the Schiff base lysine (green), and the third helix counterion residues (green), Asp85 in bacteriorhodopsin and Glu113 in bovine rod rhodopsin, that form a salt-bridge with their respective protonated Schiff base nitrogens.