*Journal of Bioenergetics and Biomembranes, Vol. 24, No. 2, 1992*

*Minireview*

# Sensory Rhodopsin I: Receptor Activation and Signal Relay

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*Received October 22, 1991; accepted November 21, 1991*

Recent progress is summarized on the mechanism of phototransduction by sensory rhodopsin I (SR-I), a phototaxis receptor in *Halobacterium halobium*. Two aspects are emphasized: (i) *The coupling of retinal isomerization to protein conformational changes.* Retinal analogs have been used to probe chromophore-apoprotein interactions during the receptor activation process. One of the most important results is the finding of a steric trigger deriving from the interaction of residues on the protein with a methyl group near the isomerizing bond of the retinal (at carbon 13). Recent work on molecular genetic methods to further probe structure/function includes the synthesis and expression of an SR-I apoprotein gene designed for residue replacements by cassette mutagenesis, and transformation of an *H. halobium* mutant lacking all retinylidene proteins known in this species to $SR\text{-}I^+$ and $(BR)^+$. (ii) *The relay of the SR-I signal to a post-receptor component.* A carboxylmethylated protein ("MPP-I") associated with SR-I and found in the *H. halobium* membrane exhibits homology with the signaling domain of eubacterial chemotaxis transducers (e.g., *Escherichia coli* Tar, Tsr, and Trg proteins), suggesting a model based on SR-I → MPP-I signal relay.

**KEY WORDS:** Phototaxis; photoreception; signal transduction.

## PERSPECTIVES AND OVERVIEW

Modulation of cell behavior by the environment requires two sequential processes: (1) sensory receptor activation, the mechanism by which ligand binding by chemoreceptors or photon absorption by photoreceptors initiates a message to the cell, and (2) signal transduction, the relay of this message to a response organelle. Post-receptor signal transduction pathways have been elucidated in a number of systems, e.g., phosphotransferase cascades in prokaryotes (Ninfa and Magasanik, 1986; Bourret *et al.*, 1989; Stock *et al.*, 1990), and G-protein and phosphoinositol pathways in eukaryotes (Stryer and Bourne, 1986; Berridge and Irvine, 1984). However, the first step in sensory signaling, the conversion of the extracellular stimulus into an intracellular signal by the receptor, is largely a black box. Stimuli "activate" the receptor protein by inducing structural alterations that are then relayed to the first post-receptor transducing component. With the development of molecular genetic methods for introducing structural alterations into membrane proteins and tools for time resolution of structural changes, we are poised at the onset of what promises to be an exciting adventure into sensory receptor activation and signal relay.

Sensory rhodopsin I can help fulfill this promise as a model system. Its relatively simple and elegant color-sensing capability provides a facile means for the experimenter to drive the molecule to the conformational extremes of attractant and repellent signaling (Spudich and Bogomolni, 1984). Its similarities and intriguing differences from the proton pump bacteriorhodopsin in protein structure (Blanck *et al.*, 1989; Henderson *et al.*, 1990), photochemical reaction cycle (Bogomolni and Spudich, 1987), and chromophore/protein interactions in the photoactive site (Fodor *et al.*, 1989; Yan *et al.*, 1990a, b; Yan *et al.*, 1991) provide clues and focused questions: What are

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0145-479X/92/0400-0193$06.50/0 

the key differences in the common design of these two proteins that make one a proton pump and the other a photosensory receptor? Which photochemical reactions do they share which are specific to proton translocation in the one case and receptor activation in the other?

The goal of this review is to cover emerging information and approaches for study of sensory rhodopsin I. Other recent reviews of the sensory rhodopsins of halobacteria are by Spudich and Bogomolni (1988) and Oesterhelt and Marwan (1990).

## THE FAMILY OF RETINYLIDENE PROTEINS IN *Halobacterium halobium*

In *H. halobium* both photoenergy transduction and photosensory signaling are carried out by a family of intrinsic membrane chromoproteins which use retinal for light absorption. Two of these rhodopsin-like proteins, bacteriorhodopsin (BR, $\lambda_{max}$ 568 nm) and halorhodopsin (HR, $\lambda_{max}$ 578 nm), are light-driven electrogenic pumps for protons and chloride, respectively (Oesterhelt and Stoeckenius, 1973; Schobert and Lanyi, 1982; see articles in this issue by Lanyi, Oesterhelt, and Rothschild). BR consists of a single polypeptide (26 kDa) which folds in the membrane into seven membrane-spanning α-helical segments forming an internal pocket where the chromophore *all-trans* retinal is bound (reviewed by Khorana, 1988; Henderson and Schertler, 1990). From its primary sequence and other similarities to BR, HR (27 kDa) presumably has a similar structure. The other two, sensory rhodopsins I and II (SR-I and SR-II) are phototaxis receptors which modulate cell swimming behavior as discussed below.

## SENSORY RHODOPSINS

*H. halobium* are polarly flagellated and their motility is modulated by light, which can attract or repel the cells depending on its wavelength (Hildebrand and Dencher, 1975). This color-sensitive phototaxis enables the cells to migrate toward an environment optimal for light-energy absorption by BR and HR while avoiding potentially damaging shorter wavelengths. *H. halobium* mutants in light-activated ion transport (Flx mutants, $BR^-HR^-$) are still fully phototactic (Spudich and Spudich, 1982), indicating that unlike other phototactic bacteria, which use light-induced energy changes through photosynthesis to modulate swimming behavior (Armitage and Evans, 1981; Taylor, 1983), *H. halobium* has evolved dedicated sensory receptors for phototaxis. Absorption spectroscopy and flash photolysis of Flx mutant membranes revealed two phototaxis receptors: SR-I, $\lambda_{max}$ 587 nm (Bogomolni and Spudich, 1982) and SR-II, also called phoborhodopsin, $\lambda_{max}$ 487 nm (Takahashi *et al.*, 1985).

BR has been suggested to affect phototaxis of *H. halobium* in a number of studies. Early action spectra of wild-type phototaxis exhibit an attractant peak at 565 nm which coincides with the absorption maximum of BR (Hildebrand and Dencher, 1975). Based on studies of phototactic responses of deenergized cells, BR was suggested to be a receptor for attractant responses of *H. halobium* to green-orange light (Baryshev *et al.*, 1981). Later, attempts were made (Bibikov and Skulachev, 1989) to separate effects of BR on phototaxis from those by SR-I by comparing energized with deenergized cells in which BR effects would presumably be selectively accentuated. These measurements were made in the presence of SR-I, HR, and SR-II. Because of their spectral overlap, photoactivation of BR results in concurrent photoactivation of SR-I and HR.

To examine the effects of BR activation in the absence of the other photoactivated pigments, we have constructed a $BR^+HR^-SR\text{-}I^-SR\text{-}II^-$ strain by genetic transformation (B. Yan *et al.*, 1992). Pho81 is a $BR^-HR^-SR\text{-}I^-SR\text{-}II^-$ mutant isolated after a series of selection steps from wild-type strain S9 (Spudich and Spudich, 1982; Sundberg *et al.*, 1985; Spudich *et al.*, 1986). It does not exhibit phototaxis or light-induced proton fluxes. We have expressed bacterioopsin in Pho81 and studied the effects of BR activation on cell swimming behavior and photoenergetics of the transformed cells *in vivo*. Under usual culture conditions, photoexcitation of BR results in only a nonadaptive upward shift in the spontaneous reversal rate. Although BR does not mediate phototaxis responses in energized transformed Pho81 cells under our culture conditions. Proton pumping by BR in transformed Pho81 cells partially deenergized by inhibitors of energy metabolism results in a small attractant response. Our data confirm the observations by Skulachev and coworkers (Baryshev *et al.*, 1981; Bibikov and Skulachev, 1989) that $\Delta\mu_{H^+}$ generated by BR photoactivation induces a taxis response by the cell when $H^+$ translocation by the $H^+$-ATPase and by respiration are inhibited. Based on our

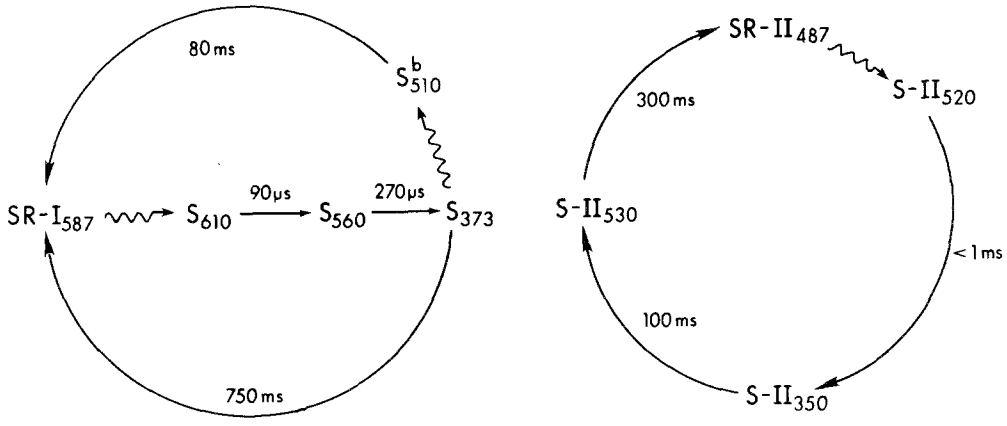


**Fig. 1.** Photochemical reaction cycles of SR-I and SR-II. Wavy arrows indicate light reactions, other arrows indicate thermal reactions for which half-lives are shown, and subscripts indicate absorption maxima of photointermediates. Photoexcitation of $SR\text{-}I_{587}$ generates an attractant signal, while photoexcitation of $S_{373}$ or $SR\text{-}II_{487}$ generates a repellent signal to the flagellar motor. SR-I photocycle from Spudich and Bogomolni (1984) and Bogomolni and Spudich (1987). SR-II photocycle from Tomioka *et al.* (1986) and Imamoto *et al.* (1991).

measurements, we attribute the effects of BR photoactivation on swimming behavior to secondary consequences of electrogenic proton pumping affecting metabolic or signal-transduction pathways, rather than to primary sensory signaling analogous to that mediated by SR-I.

## COLOR DISCRIMINATION BY SENSORY RHODOPSIN I

SR-I is unusual among known photoreceptors in that it exists in two spectrally distinct forms, each of which can be photoactivated to generate sensory signals (Spudich and Bogomolni, 1984). The thermally stable species $SR\text{-}I_{587}$ (Fig. 1) is an attractant receptor; its photoexcitation suppresses directional reorientation (swimming reversals), thereby favoring cell migration into higher-intensity regions of orange-red light. A long-lived intermediate species ($S_{373}$, $\lambda_{max}$ 373 nm) in the SR-I photocycle accumulates in the photostationary state maintained by photoexcitation of $SR\text{-}I_{587}$ (Fig. 1). $S_{373}$ is photochemically reactive and functions as a repellent receptor; its photoexcitation induces swimming reversals, avoidance responses to high UV-blue light intensity. The activation of as few as one to two $S_{373}$ molecules is sufficient to elicit a response (Spudich and Bogomolni, 1984). Sensory rhodopsin II is a distinct chromoprotein (E. N. Spudich *et al.*, 1986) which mediates repellent responses in the blue-green spectral region (Takahashi *et al.*, 1985; Wolff *et al.*, 1986).

## STRUCTURE OF SENSORY RHODOPSIN I

Incorporation of radiolabeled retinal into the chromophoric pockets of SR-I and SR-II allowed identification of their apoproteins in denaturing gels as distinct polypeptides at 25 and 23 kDa $M_r$, respectively (E. N. Spudich *et al.*, 1986), similar in size to BR. The retinal binding pockets of BR and SR-I have been shown to be closely similar in their electrostatic and hydrophobic interactions with the chromophore by studies of retinal analog effects on absorption (J. L. Spudich *et al.*, 1986; Baselt *et al.*, 1989; Yan *et al.*, 1990a, b, 1991) and comparison of their resonance Raman spectra (Fodor *et al.*, 1989). The SR-I apoprotein gene (*sopI*) has been cloned by Blanck *et al.* (1989). Hydropathy analysis indicates seven transmembrane helices as in BR and the deduced amino acid sequence (Fig. 2) bears substantial homology to the BR retinal-binding regions. A structural model for SR-I has been developed based on the structure of BR, and > 80% of the residues placed in the BR retinal-binding pocket appear to be conserved in SR-I.

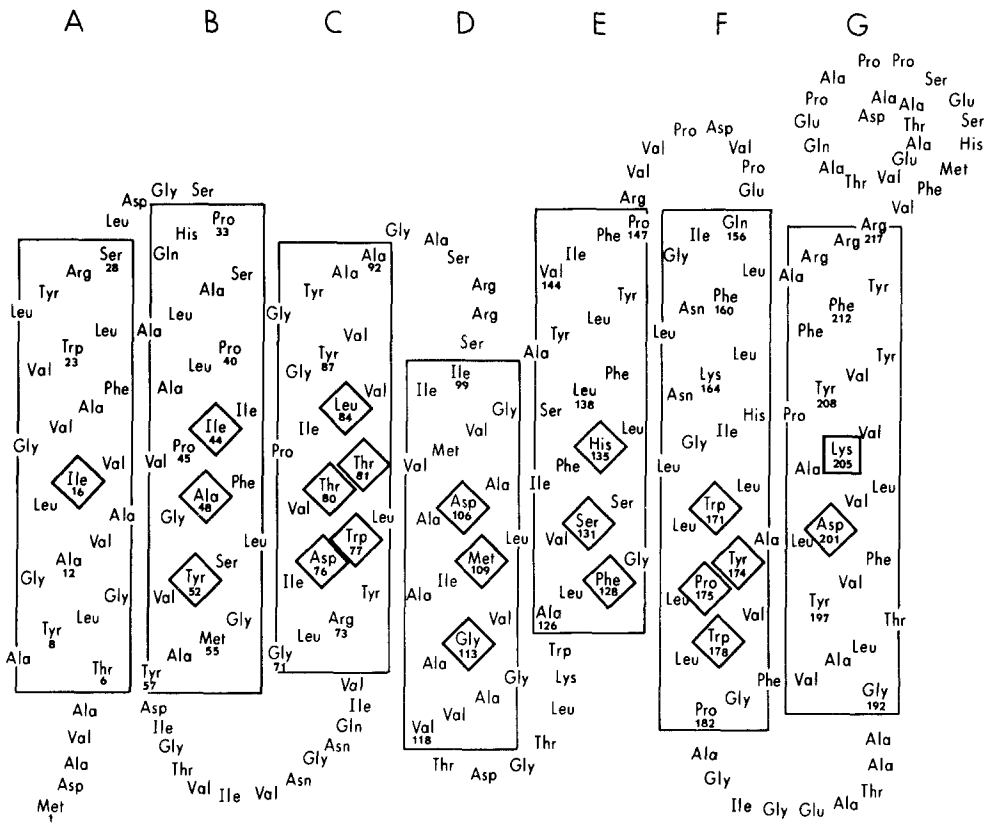


**Fig. 2.** Two-dimensional folding model of SR-I. The large boxes represent the seven α-helical transmembrane segments (A–G). The retinal-binding lysine 205 (square) and other residues suggested to be in the retinal binding pocket (diamonds) are based on homology with BR. Redrawn from Henderson *et al.* (1990); sequence from Blanck *et al.*, 1989.

## SENSORY RHODOPSIN I ACTIVATION

### Signaling States

In SR-I, *all-trans* retinal is bound in a protonated Schiff base linkage to the ε-amino group of a lysine (Fodor *et al.*, 1989), inferred to be Lys205 by homology to BR and HR. Light absorption causes *all-trans* to 13-*cis* isomerization of the chromophore (Tsuda *et al.*, 1985) and blocking this reaction prevents formation of photocycle intermediates and sensory signaling *in vivo* (Yan *et al.*, 1990a). From the absorption spectra of intermediates in the SR-I photocycle, one expects deprotonation of the Schiff base to occur in $S_{373}$ formation and reprotonation in its decay (Bogomolni and Spudich, 1987). In BR Schiff base deprotonation and reprotonation occur in the formation and decay of the analogous intermediate $M_{412}$ (Lewis *et al.*, 1974). These intramolecular proton transfers are crucial reactions in the transmembrane proton translocation process (see articles by Lanyi, Oesterhelt, and Rothschild, in this issue; Mathies *et al.*, 1991). However, SR-I photoexcitation does not cause proton fluxes (Spudich and Spudich, 1982; Bogomolni and Spudich, 1982) or sustained hyperpolarization of the membranes of intact cells or vesicles (Ehrlich *et al.*, 1984). Therefore SR-I is not an electrogenic ion pump. Our working hypothesis is that photoactivation of the chromoprotein generates active conformations of the receptor (signaling states) which interact with post-receptor components to control the flagellar motor switch.

Based on retinal analog reconstitution of the SR-I apoprotein, the $S_{373}$ intermediate, the repellent receptor form of SR-I, appears to function as the attractant signaling state (McCain *et al.*, 1987; Yan and Spudich, 1991). The lifetime of $S_{373}$ was modulated by incor-

porating retinal analogs into SR-I apoprotein *in vitro* and *in vivo*. Photocycles of the SR-I analog pigments exhibit the same reaction scheme as native SR-I. The analog $S_{373}$-like species exhibit similar formation rates, but different decay rates as monitored by flash spectroscopy in membrane vesicle suspensions. The attractant receptor signaling efficiencies determined by physiological measurements are proportional to the lifetimes of the $S_{373}$-like intermediates, indicating $S_{373}$ is active in producing the attractant signal. Analogously, an $S_{373}$ photoproduct $S^b_{510}$ is possibly active in producing a repellent signal.

## Evidence for a Steric Trigger

Retinal/apoprotein interactions have been probed in SR-I and BR with analogs missing $\beta$-ionone ring or polyene chain methyl groups, acyclic and dihydro analogs, and analogs with C—C bridged ("locked") and shortened polyene chains (Spudich *et al.*, 1986; Baselt *et al.*, 1989; Yan *et al.*, 1990a, b, 1991). Overall close similarity is observed in the absorption spectra of the corresponding analog SR-I and BR pigments; however, differences are observed in the apoprotein environments near the retinal 13-methyl group and near the $\beta$-ionone ring. Further investigation has led Bing Yan to discover a key difference in the role of the 13-methyl group in SR-I compared to BR (Yan *et al.*, 1991). As discussed below, the different roles of the 13-methyl group provide a clue to how SR-I and BR have been modified by nature to perform their distinct functions.

The SR-I and BR proteins share many common features, such as comparable molecular weights, similar secondary structures, and highly conserved retinal binding pockets, the requirement for a specific *all-trans*/13-*cis* isomerization of retinal, and homologous photocycles. But differences are also evident. The $M_{412}$-like intermediate of SR-I, $S_{373}$, is 40 nm shifted to the blue and its decay rate is greatly reduced compared to $M_{412}$ ($t_{1/2}$, 750 ms vs. 5 ms). Moreover, the expected O-like intermediate in the SR-I photocycle must be a fast-decaying intermediate since no accumulation of this intermediate is seen on the $S_{373}$ decay pathway in contrast to BR. The two dramatic differences are:

1. Unlike the retinal binding pocket of BR in which both *all-trans* and 13-*cis* retinal form retinylidene linkages, SR-I, in native membranes, has an exclusive *all-trans* chromophore binding pocket. 13-*cis* retinal does not form a retinylidene pigment with the SR-I apoprotein (Yan *et al.*, 1991), although this isomer binds to the BR apoprotein even more rapidly than *all-trans* retinal, the functional isomer of both pigments (Dencher *et al.*, 1976; Schreckenbach *et al.*, 1977).

2. The 13-desmethyl BR analog, when photostimulated in its *all-trans* form, produces an $M_{412}$-like intermediate at a normal rate and is functional in proton pumping (Fendler *et al.*, 1987; Gaertner *et al.*, 1983, 1988), but *all-trans* 13-desmethyl SR-I analog exhibits no photochemical production of an $S_{373}$-like intermediate nor physiological activity (Yan *et al.*, 1991).

The results provide compelling evidence for the requirement of a specific steric interaction between the retinal 13-methyl group and protein residues for SR-I activation. We suggest this interaction functions as a trigger for generating protein conformational changes to produce the attractant signaling state of the receptor, $S_{373}$ (Yan *et al.*, 1991). In BR, the activation driving force appears to be the isomerization-induced $pK_a$ changes in the retinal Schiff base and protein residues (Stoeckenius, 1979; Kalisky *et al.*, 1981; Henderson *et al.*, 1990). These $pK_a$ changes and the formation of $M_{412}$ (the $S_{373}$-like intermediate of BR) do not require 13-methyl/protein interaction. On the other hand, activation of bovine rhodopsin requires a steric interaction between the retinal 9-methyl and the protein residues (Ganter *et al.*, 1989). Lack of this interaction in 9-desmethyl-rhodopsin leads to an abnormal photochemical reaction which does not produce its signaling conformation (Metarhodopsin-$II_{380}$), and inhibits biological function by $> 90\%$ (Ganter *et al.*, 1989). The steric trigger in rhodopsin uses the 9-methyl group, which is close to the isomerizing 11,12-double bond. Analogously, the results indicate that the 13-methyl group, which is close to the isomerizing 13,14-bond in SR-I, serves as a trigger for SR-I activation, i.e., converts photon absorption by the chromophore into protein conformational changes. Relevant to this observation, photo-induced conformational changes in SR-I as monitored by FTIR differ from those in BR. In particular, signals indicating lipid disordering accompany the $SR\text{-}I_{587} \rightarrow S_{373}$ transition (Bousché *et al.*, 1991), as observed also in the rhodopsin $\rightarrow$ Metarhodopsin-$II_{380}$ transition.

## SIGNAL RELAY

### MPP-I

A methylation system with similarities to that of eubacterial chemotaxis was demonstrated in *H. halobium* (Schimz, 1981), and proteins chemically similar to the intrinsic membrane methyl-accepting chemotaxis proteins (MCPs) which generate the signals modulating the flagellar motor in *Escherichia coli* are present in *H. halobium* membrane preparations (Spudich *et al.*, 1988; Alam *et al.*, 1989). The expression of one of these proteins (a 97-kD $M_r$ protein) is quantitatively correlated with that of SR-I chromoprotein in several mutants (Spudich *et al.*, 1988; Spudich *et al.*, 1989). There is evidence for a distinct methyl-accepting protein associated with SR-II (Spudich *et al.*, 1989). Methylation/demethylation as assessed by changes in carboxylmethyl turnover rate is regulated by chemostimuli and photostimuli (Alam *et al.*, 1989) and specifically by the attractant and repellent receptor forms of SR-I and by SR-II (Spudich *et al.*, 1989).

The type of methylation (carboxylmethylesterification) of the 97-kDa protein [called MPP-I (*methyl-accepting phototaxis protein I*)] is characteristic of the MCPs in eubacteria (reviewed in Simon *et al.*, 1985; Stewart *et al.*, 1988). Like MCPs, MPP-I appears also to be an integral membrane protein since it requires harsh detergents to be solubilized from membrane preparations. Partial sequencing of the MPP-I protein and its gene recently cloned reveals homology with the cytoplasmic signaling domain of eubacterial MCPs (Virginia Yao and John L. Spudich, unpublished). The MCPs function in both chemoreception and signal transduction through cytoplasmic components to the flagellar motor. An attractive hypothesis is that the signaling states of the SR-I chromoprotein interact with MPP-I, which relays the signal to the flagellar motor analogously to the relay of chemoeffector binding by the *E. coli* MCPs. Our working hypothesis is that the MPP-I protein interacts physically with the SR-I chromoprotein (Fig. 3).

Results have been presented suggesting that cyclic nucleotides and $Ca^{++}$ (Schimz and Hildebrand, 1987), and a G-protein-like component (Schimz *et al.*, 1989), are possibly involved in the photosensory transduction chain in *H. halobium*. Also fumarate has been implicated in the signaling pathway (Marwan *et al.*, 1990).

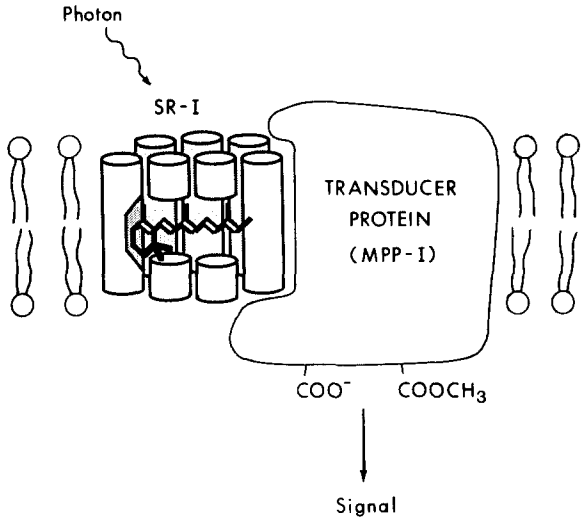


**Fig. 3.** Working model for the SR-I signaling system. In the model, photoactivation of SR-I results in an altered interaction with MPP-I, which relays the signal to a sensory pathway controlling the switching probability of the flagellar motors.

## SYNTHETIC sopI GENE

To facilitate structure/function studies of SR-I, we are developing methods for site-specific mutagenesis and expression of the protein in a functional form. An efficient method for mutagenesis is replacement of a restriction fragment by a synthetic counterpart containing the desired codon alterations (Lo *et al.*, 1984). However, a frequent limitation in the application of this method to native genes is that they do not contain suitable restriction sites at the desired regions or the restriction sites that are present may be in multiple locations in the gene. Therefore a synthetic SR-I apoprotein gene (*sopI*) was constructed and optimized for mutagenesis in all parts of the gene by the method of cassette replacement, without changing the encoded amino sequence [synthesized by M. P. Krebs, E. N. Spudich, and J. L. Spudich in the laboratory of H. G. Khorana (Massachusetts Institute of Technology)]. The synthetic *sopI* and flanking sequences contain 32 unique restriction sites for readily available, stable, and efficient restriction enzymes, 30 of which produce staggered ends (to ensure efficient ligation during duplex replacement reactions). Within the coding region, unique sites are spaced an average of 24 nucleotides apart.

The synthetic *sopI* has been expressed (E. N. Spudich, M. P. Krebs, H. G. Khorana, and J. L. Spudich (1992), *Biophys. J.*, **61**: A531) in

*H. halobium* strains transformed with a derivative of the multicopy plasmid pMPK52 developed by Krebs *et al.* (1991) and previously used to express BR. Analysis of the SR-I produced in the mutants with and without MPP-I and site-specific mutagenesis of SR-I are currently in progress.

## ACKNOWLEDGMENTS

We are grateful to Alexander Kiselev, Karl Olson, Elena Spudich, Istvan Szundi, Virginia Yao, and Bing Yan for helpful comments on the manuscript. Work from the author's laboratories cited here was supported by grants from NIH, NSF, and ONR.

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