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Review

Proton transport by sensory rhodopsins and its modulation by transducer-binding

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Abstract

The study of light-induced proton transfers in the archaeal sensory rhodopsins (SR), phototaxis receptors in *Halobacterium salinarum*, has contributed important insights into their mechanism of signaling to their cognate transducer subunits in the signaling complex. Essential features of the bacteriorhodopsin (BR) pumping mechanism have been conserved in the evolution of the sensors, which carry out light-driven electrogenic proton transport when their transducers are removed. The interaction of SRI with its transducer blocks proton-conducting channels in the receptor thereby inhibiting its proton pumping, indicating that the pump machinery, rather than the transport activity itself, is functionally important for signaling. Analysis of SRII mutants has shown that the salt bridge between the protonated Schiff base and its counterion Asp73 constrains the receptor in its inactive conformation. Similarly, in BR, the corresponding salt bridge between the protonated Schiff base and Asp85 contributes to constraining the protein in a conformation in which its cytoplasmic channel is closed. Transducer chimera studies further indicate that the receptor conformational changes are transmitted from the sensors to their cognate transducers through transmembrane helix–helix interaction. These and other results reviewed here support a signaling mechanism in which tilting of helices on the cytoplasmic side (primarily outward tilting of helix F), similar to that which occurs in BR in its open cytoplasmic channel conformation, causes structural alterations in the transducer transmembrane helices.

Keywords: Phototaxis; Signal transduction; Protein–protein interaction; Transmembrane helix–helix interaction; Proton transport

1. The receptors

Light provides organisms a source of energy as well as information to recognize spatial patterns in the environment. The halophilic archaeon, *Halobacterium salinarum* (formerly called *Halobacterium halobium*), which inhabits high salt environs, such as ponds formed as a result of evaporation of sea water by exposure to intense solar radiation, makes use of light for both energy and sensory transduction by exploiting a family of light-sensitive proteins: the archaeal rhodopsins.

Four different rhodopsins have been identified in the plasma membrane of *H. salinarum*, two of which are light-driven ion pumps and two of which are receptors for phototaxis. The pumps, bacteriorhodopsin (BR) and halorhodopsin (HR), use green-orange light to electrogenically transport protons and

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chloride, respectively. The resultant electrochemical potential drives ATP synthesis by a H^+-ATPase, motility by proton-driven flagellar motors, Na^+-driven transport of metabolites and other energy-requiring membrane processes. The two phototaxis receptors are sensory rhodopsin I and II (SRI and SRII), which control the cell's swimming behavior. *H. salinarum* migrates up gradients of orange light absorbed by SRI, an attractant receptor [1]. A photocycle intermediate (S373) of the same protein is a repellent receptor form of SRI that mediates avoidance of UV-light in the presence of an orange light background [2]. The attractant effect brings the cells to light used by BR and HR for ion pumping, and the repellent effect ensures the cells will avoid photooxidative damage by a shorter wavelength length. On the other hand, SRII appears to function exclusively as a photoreceptor for avoidance of blue–green light [3–6]. SRII is preferentially made under highly aerobic conditions when the danger of photooxidation is greatest and when the transport rhodopsins are not made and, hence, the cells seek darkness.

Several other halophilic archaeons have been shown to contain members of the archaeal rhodopsin family with functions corresponding to those of BR, HR, SRI or SRII, which are believed to have evolved through gene duplications in view of their amino acid sequence identities, primarily in the retinal-binding pocket [7]. So far, 25 archaeal retinal proteins from different species have been reported and all of them can be classified into the four subgroups, H^+-pump, Cl^--pump, sensor-I and sensor-II, according to phylogenetic analysis [8,9]. Recently a photoactive homolog of this family has also been demonstrated in the eukaryotic microbe *Neurospora crassa* [10,11]. Heterologously expressed *Neurospora* rhodopsin exhibits a photochemical reaction cycle very similar to that of SRII, suggesting a photosensory role of this new protein [11].

The protein sequences of this family predict structures composed of seven transmembrane (TM) helices, as do those of higher eukaryotic receptor proteins such as visual pigments, and cholinergic and adrenergic receptors. Among the archaeal proteins and *Neurospora* rhodopsin, the 22 amino acid residues composing the binding pockets for the chromophore retinal are highly conserved, providing a readily recognizable motif. The binding pockets encase all-*trans* retinal which is covalently bound to a lysine residue on the seventh helix (designated helix G) through a protonated Schiff base.

The binding of retinal to the apoprotein gives rise to a significant red-shift, called the opsin shift, of the absorption maximum of the chromophore. Several factors are responsible [12] and one major role in the tuning mechanism is played by the 'complex counterion' for the positively charged protonated Schiff base, consisting of an aspartate and an arginine (at positions 85 and 82 in BR) on helix C and an aspartate (212 in BR) on helix G. These residues are also conserved in all archaeal rhodopsins with the exception of HRs in which the residue corresponding to Asp85 is Thr with chloride bound nearby. This substitution is crucial for the specification of the ion to be transported as revealed by analysis of the BR mutant D85T, in which the proton pump was converted to a chloride ion pump [13,14]. Interestingly the salt bridge between the corresponding helix C aspartate (Asp73 in SRII) and the protonated Schiff base on helix G is also crucial in sensory signaling, since SRII mutants D73N and D73Q are constitutively active [15; Spudich et al., unpublished results]. As discussed below, this effect has been interpreted as evidence that the electrostatic interaction between helices C and G constrains the receptor conformation in the inactive state.

All of the archaeal rhodopsins exhibit cyclic photoreactions that go through several quasi-stable intermediates until returning to the initial state (photocycle). The initial light-dependent event in the photocycles is isomerization of the retinylidene chromophore from all-*trans* to 13-*cis*. In the case of the proton pump BR, the first proton transfer, from the Schiff base to Asp85, accompanies formation of the M intermediate. As a consequence of the protonation of Asp85, a hydrogen bonding network in the extracellular half channel of the protein is perturbed, resulting in proton release toward the extracellular side from somewhere near Glu204 and Glu194 [16]. The disruption of the salt-bridge between the Schiff base and Asp85 is also important for the subsequent cytoplasmic opening of the molecule by tilting B, F and G helices as occurs in N intermediates, allowing for the deprotonation of Asp96 coupled with reprotonation of the Schiff base [17–19]. Subsequently with the protonation of Asp96 from the cytoplasmic aque-

ous phase, the conformation switches back with reisomerization of the chromophore to the all-*trans* form during formation of the O intermediate, in which the absorption maximum is red-shifted relative to the initial state, in large part due to the lack of a negative charge at the 85 position because Asp85 remains protonated. The vectorial proton transport is completed with the final proton transfer from the protonated Asp85 to the proton release group in the extracellular side [16]. Extensive mutant analyses of BR have shown that the residues identified as participating in proton release and uptake (Glu194, Glu204 and Asp96) are important for facilitating pumping activity but the elimination of their carboxyl groups does not abolish the activity.

The only essential residues found for BR pumping activity are the retinal-binding lysine and Asp85, both of which are present in SRI and SRII and which, therefore, might be expected to pump protons. In fact, SRI and SRII photocycles involve Schiff base deprotonation as is evident in the formation of near-UV-absorbing species similar to M of BR. Therefore, it has been an important question whether SRs are capable of transporting protons and whether proton movements play a critical role in signal transduction by SRI and SRII.

The first discovered of the SRs, SRI, however, was shown in the earliest measurements to have non-electrogenic photoreactions in its functionally active state in halobacterial membranes [1,20]. In fact, SRI was first identified in mutants specifically selected for the loss of light-induced hyperpolarization of the membrane (Flx mutants). These mutants exhibited retinal-dependent phototaxis behavior comparable to that of wild-type [20], indicating the existence of light-sensing proteins distinct from electrogenic pumps. The photosensor was initially named slow-cycling rhodopsin (SR), because of its $\sim$100-times slower turnover of the photocycle compared to BR and HR. The rate limiting step of the photocycle is the decay of the M-like intermediate, S373 (also called SRI-M), which was identified as an active state of SRI for attraction to light, because its lifetime when altered by incorporation of analogue retinals was proportional to the efficiency of the attractant response of the cells to orange light [21]. Photoexcitation of S373 causes a repellent response and, thus, SRI is color-sensitive, mediating attractant responses to orange light and repellent responses to near-UV light.

Later the second photoreceptor (SRII) for the repellent taxis for blue–green light was identified in Flx mutant derivatives which either lack SRI synthesis [3] or produce higher concentrations of SRII [4–7]. Measurements of the SRII photocycle revealed the formation of two consecutive slow-decaying intermediates with absorption maxima near 350 and 540 nm, attributable to M and O intermediates as in BR [22]. Both the M and O intermediates appear to be active signaling states of SRII because the elongation of the lifetimes of these states by use of analogue retinals proportionally increases the efficiency of the response of the cells to blue–green light [23].

2. The transducers

The first of the transducer proteins for phototaxis signaling by the SRs was identified by analysis of phototaxis mutant membranes which revealed a membrane protein, HtrI, whose concentration correlated with attractant response sensitivity and which was methylated in a similar manner as eubacterial chemotaxis receptor/transducers [24]. It was also demonstrated that SRI and SRII activation with light stimuli modulated methyl group turnover in vivo [25,26]. The *htrI* gene was cloned [27] and found to be immediately upstream of the *sopI* gene (gene encoding SRI opsin) and under the control of the same promoter [27,28]. The revealed primary sequence of HtrI indeed verified the homology to the eubacterial methyl-accepting chemotaxis receptors. Transducer genes for *H. salinarum* SRII and for the repellent receptor from another halobacterial species (*Halobacteria pharaonis* SRII; pSRII) were subsequently cloned and named HtrII and pHtrII, respectively [29,30]. Several more SR-encoding genes have now been identified [8,9,31] and a cognate Htr-encoding gene precedes each of them. HtrII but not HtrI or pHtrII contains a large periplasmic loop corresponding to the ligand-binding site of chemotaxis receptor/transducers and it has been reported that HtrII indeed functions as a chemotaxis receptor for serine [32]. Genes encoding a two-component phospho-relay pair homologous to those of chemotactic eubacteria have been cloned and shown

to be essential for photo- as well as chemotaxis [33,34]. Thus, SR–Htr interactions translate light stimuli into the same signals as chemotaxis effectors and exploit the machinery for locomotion and chemotactic migration by the organism.

3. SRI photochemistry and pumping

With the genes in hand, the study of SR molecules in membranes devoid of Htrs became available by molecular genetic manipulation. SRI expressed without HtrI exhibited marked differences from the receptor in the presence of HtrI. First noted was the strong pH-dependence of SRI-M decay in the HtrI-free receptor, indicating that the Schiff base becomes reprotonated by taking up protons from the outer milieu, contrasting with the pH-independent decay in the presence of HtrI [35,36]. Furthermore HtrI-free SRI was found to function as a light-driven electrogenic proton pump in neutral to alkaline pH conditions, unlike the SRI associated with HtrI [37]. Also the pK_a of the blue-to-purple transition (blue shift of the absorption maximum from 587 to 552 nm) was shifted from 8.5 in wild-type membranes to 7.4 in HtrI-deficient membranes [37]. The blue shift was found to originate from deprotonation of Asp76 (the residue corresponding to Asp85 in BR) which then functions as a primary counterion for the protonated Schiff base and also as a proton acceptor for the Schiff base when SRI-M is formed by photoreaction [38], in analogy to BR-M formation. Indeed, it is this blue-shifted form (SRI552) that exhibits light-driven proton pumping activity across the membrane in the same direction as BR [37,39] and in a similar kinetic manner in which proton release to the extracellular side is followed by uptake from the cytoplasmic side concurrent with SRI-M formation and decay, respectively [40].

The red-shifted form at acidic pH (SRI587) has the primary counterion Asp76 protonated, preventing the proton transfer from the Schiff base to this residue [41]. However, SRI587, and also SRI-D76N, still exhibit substantial SRI-M formation, unlike the case of the acid form of BR and BR-D85N. One candidate for the alternative proton acceptor is His166 located in the cytoplasmic side of the Schiff base and conserved among SRIs identified so far [9], since its absence in mutants inhibited the formation of SRI-M [42]. In wild-type HtrI-free SRI587, the decay rate of SRI-M is still pH-dependent, indicating that the Schiff base becomes reprotonated from the medium, but the pumping activity driven by orange light is lost. The interpretation is that proton release to the cytoplasmic side occurs with SRI-M formation and that SRI-M returns spontaneously to the initial state taking up the proton from the cytoplasmic side without net proton transport (Fig. 1). However, with blue light, which induces SRI-M photoreaction, an inverted proton pumping activity was demonstrated [39]. This interesting behavior was rationalized by assuming that the second photoreaction isomerizes the chromophore back to all-*trans* and switches the accessibility of the Schiff base from the cytoplasmic to the extracellular side, whereupon the Schiff base reprotonates from the extracellular side resulting in a net proton transport from outside to in [39,43].

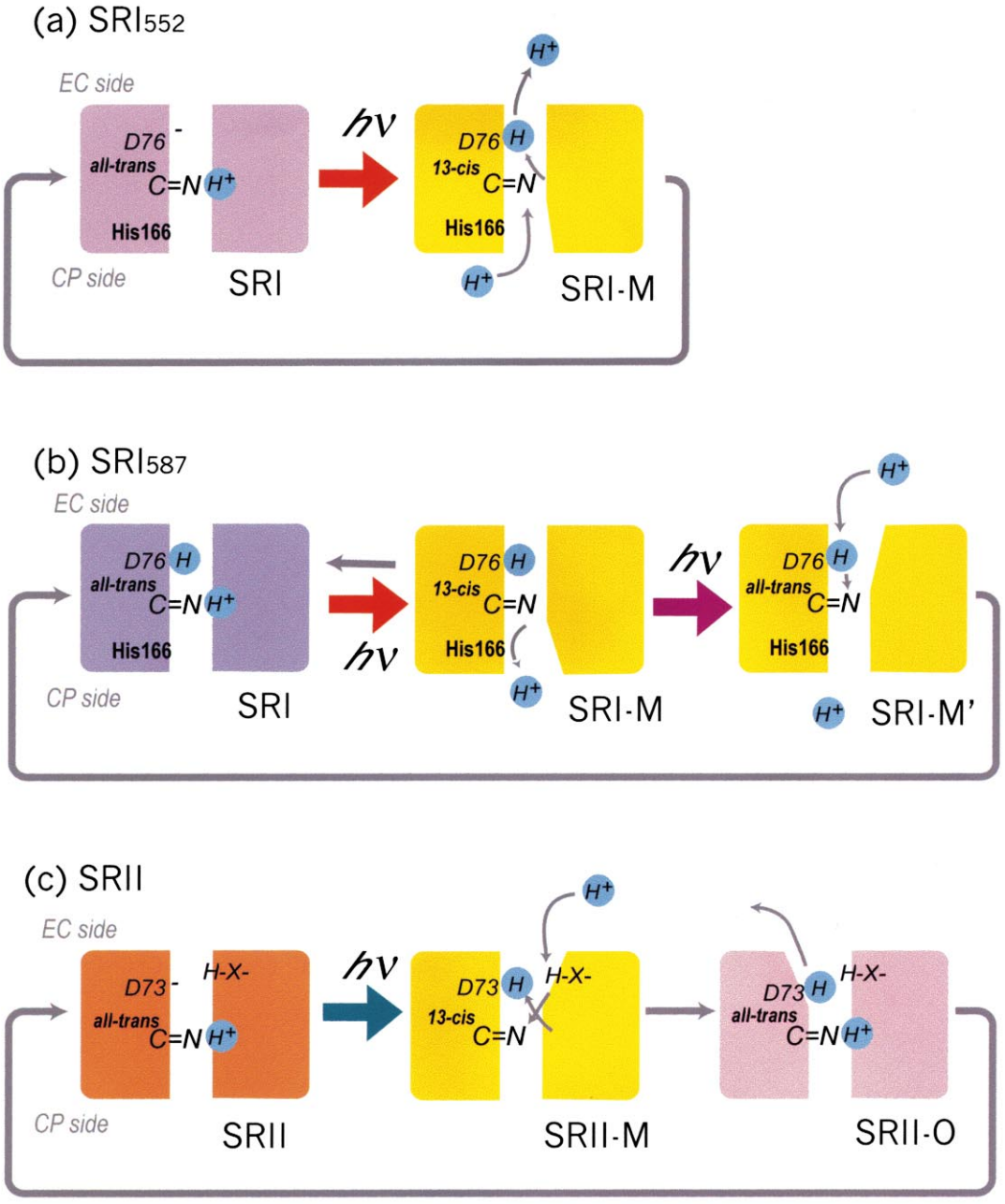
4. Effects of HtrI on SRI: clues to the signaling mechanism

The electrogenic transport function of transducer-free SRI shows that the essential features of the BR pumping mechanism have been conserved in the evolution of the sensor. This conservation of the pump mechanism and its inhibition by HtrI interaction led to the conclusion that the pump machinery, but not the transport activity itself, is functionally relevant for signaling, i.e. interaction with HtrI converts SRI from a proton pump to a sensory receptor [44].

When SRI and HtrI are coexpressed in the membrane, the pK_a of Asp76 is elevated to $\sim$8.5, producing therefore almost exclusively the red-shifted species, SRI587, at neutral pH. The photocycle is also modulated so that the SRI-M-decay rate is unaffected by external pH between 4 and 8, and proton movements on and off the receptor are blocked, indicating Schiff base reprotonation from an internal proton donor, in contrast to that in the absence of HtrI where the reprotonation occurs from the cytoplasm [36,40]. The pK_a of the Schiff base, which is 9.5 in the free SRI, is elevated above 12 with a steep slope titration curve in the presence of HtrI, indicating the titration of groups other than the Schiff base being responsible for disruption of the interactions

stabilizing the protonated Schiff base [12]. The susceptibility of the Schiff base to hydrolysis by hydroxylamine was also considerably lowered in the presence of HtrI. These lines of evidence indicated that SRI and HtrI both in the dark and in the light are in a molecular complex, in which the cytoplasmic entry of SRI is blocked, inhibiting the pumping activity [44,45]. This may well be an important clue to the signaling mechanism, strongly suggesting that the process that opens the cytoplasmic channel in BR creates the signaling conformation in SRI.

The modulation of the photocycle kinetics by HtrI is such that both deprotonation and reprotonation of the Schiff base are facilitated [46]. Co-purification of HtrI with his-tagged SRI by Ni^{2+}-affinity chromatography provides biochemical confirmation of the complexation (Spudich and Spudich, unpublished).



Similar effects of transducer-binding on SRI-M decay to those reported by Olson and Spudich [40] were independently obtained by Krah et al. [47], who reached the same conclusion that SRI and HtrI are physically complexed. However, black lipid membrane measurements indicated that SRI in complex with HtrI exhibited measurable light-driven proton transport [43], in conflict with the earlier finding that HtrI blocked proton release [40]. The apparent discrepancy may arise either from the different conditions used, or from the different sensitivity of the techniques applied for the pumping measurements, or a contribution from a small portion of transducer-free SRI in the later measurements. Recent pH-electrode measurements of proton transport comparing HtrI-free and HtrI-complexed SRI confirmed that HtrI blocks SRI pumping in *H. salinarum* envelope vesicles [45].

The portion of HtrI necessary for interaction with SRI was assessed by deletion constructs to identify the shortest transducer that ensures the pH-independent photocycle characteristic of the complexed SRI. The N-terminal 147 residues of HtrI which contains its two TM segments (TM1 and TM2) and a hydrophilic cytoplasmic region of ~90 residues, fused to the C-terminal 36 residues, was shown to fulfill the requirements [48]. Site-specific mutagenesis of HtrI in the cytoplasmic region has identified seven protonatable residues ranging from positions 56 to 108 whose neutralization affects SRI photochemistry or function [49]. These data indicated that the physical contact of HtrI with SRI responsible for the modulation of the photocycle is contained within the TM segments and cytoplasmic 90 residues, which may have electrostatic interactions with SRI. Further, analysis of HtrI/HtrII chimeras revealed that the two TM helices are sufficient to define the specificity of functional interaction of Htr proteins with their cognate receptors [50]. Therefore, the crucial interactions between SR and Htr pairs are likely to be restricted to their membrane domains.

5. SRII photochemistry

SRII is typical of sunlight avoidance receptors in that its absorption maximum near 500 nm is matched to the peak in the spectrum of solar radiation at the Earth's surface.

The smaller opsin shift of SRII as compared to other archaeal rhodopsins and the vibronic structure of its absorption spectrum have been attributed to lack of electronic perturbation which is responsible for the greater red shifts and vibrational broadening of the absorption spectra of BR, HR and SRI [51]. The amino acid residues for providing such a perturbation are likely to be residues near the retinal and conserved among BR, HR and SRI pigments but missing in SRII proteins (namely, Met, Ser and Ala at positions 118, 141 and 215 in BR). The reintroduction of any one or the combination of these residues in pSRII did show appreciable red shifts up to 517 nm, indicating that there must be other factors to be uncovered to account fully for the red shifts of

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Fig. 1. Proton transfer processes during the photocycles of SRI and SRII. (a) Purple form of SRI (SRI552) which has Asp76 unprotonated in the unphotolyzed state: Asp76 becomes protonated concomitantly with deprotonation of the Schiff base as SRI-M is formed as the result of photoisomerization of the chromophore with orange light. Proton release from Asp76 (possibly involving other proton transfer groups in the extracellular (EC) domain) to the EC side, followed by reprotonation of the Schiff base by proton uptake from the cytoplasmic (CP) side with the decay of M, accomplishes the photocycle with net outward transport of one proton. (b) Blue form of SRI (SRI587) which has Asp76 protonated in the unphotolyzed state: orange light-induced isomerization of the chromophore switches accessibility of the Schiff base proton from the EC to the CP side in SRI-M formation, driving (in transducer-free SRI587) proton release from the Schiff base to the CP side which is facilitated by His166. Stoichiometric proton release is observed in free SRI587, but is blocked by transducer (HtrI)-binding. M returns to the initial state through thermal processes, during which the Schiff base is reprotonated by taking up a proton from the CP side without net proton translocation. Additional near-UV light drives a two-photon pathway in which the 13-*cis* chromophore with unprotonated Schiff base is photoisomerized to all-*trans* with subsequent proton uptake from the EC side, resulting in net inward proton transport across the membrane. (c) SRII: the first proton transfer occurs from the Schiff base to Asp73 with the formation of SRII-M, which is followed by the reprotonation of the Schiff base by uptake of one proton from the EC side in the M-to-O conversion. This process appears to be facilitated below neutral pH by an unknown protonatable residue (X) with p*K* near 7.5. Protonated Asp73 finally releases the proton to the EC side as O decays to the initial state, without net proton transport.

the other pigments (Shimono et al., personal communication).

Apart from its color regulation, SRII and pSRII are in some sense more similar to BR than is SRI in that Asp73 in SRII (Asp75 in pSRII) is the counterion of the Schiff base and is unprotonated in the dark state at neutral pH (the pK_a was determined to be 3.0 for SRII [52] and 5.5 for pSRII [53]), thus functioning as a proton acceptor for the Schiff base when M is formed [54,15]. As in the BR photocycle, M decay in the SRII and pSRII photocycles is accompanied by the formation of visible-absorbing intermediates N and O, attributable to the reprotonation of the Schiff base and the subsequent reisomerization of the chromophore, respectively, as occur in BR [53,55].

6. Pumping activity of SRII

The most crucial residues for the electrogenic machinery for proton pumping in BR, the Schiff base and the Asp counterion, in the SRII photocycle successively change their protonation states as occurs in BR, i.e. proton transfer from the Schiff base to Asp73, and reprotonation of the Schiff base followed by Asp73 deprotonation. Hence, one might have expected that SRII would pump protons. Moreover, SRII-M decay was pH-dependent in a manner consistent with proton uptake during M decay from the outer milieu, although a protonatable residue with pK_a near 7.5 appears to assist the proton uptake, rendering this process pH-independent below neutral pH [45].

However, SRII in the native membrane was found not to be pumping ions, but circulating protons only in the extracellular side, i.e. taking up a proton from the extracellular side as the Schiff base reprotonates in M-to-O conversion and releasing it to the same side as Asp73 deprotonates when O decays to the initial state [45] (Fig. 1). In measurements with the highly sensitive black lipid membrane technique wild-type pSRII showed noticeable but small photocurrent signals which were enhanced either by azide or by a mutation introducing Asp in the position corresponding to Asp96 of BR [56]. Hence, the negligible or very low pumping activities of SRII or pSRII appear to be attributable to a low proton conductance in the cytoplasmic channel, which could be restored by introducing a protonatable residue into the channel. Consistent with this view, M decay was accelerated by mutations in which hydrophilic residues in the cytoplasmic channel corresponding to Asp96 and Thr46 of BR were introduced into pSRII [57].

Since the measurements of proton movements of SRII in complex with HtrII have been reported only with wild-type SRII and under conditions in which the SRII cytoplasmic channel exhibits low proton conductivity, it remains to be tested whether HtrII-binding will close the cytoplasmic channel, as does HtrI-binding to SRI. Such an effect would be predicted by the model of the signaling mechanism in which transducers are coupled to outward movements of helices (primarily helix F) on the cytoplasmic side of their receptors [58].

7. Effects of HtrII and D73 mutants of SRII: further clues to the signaling mechanism

Unlike HtrI, HtrII does not raise its cognate SR's Schiff base aspartate counterion pK_a to an alkaline value. Hence, at neutral pH Asp73 is unprotonated in the unphotolyzed state both in the transducer-complexed and in the free SRII, whereas Asp76 in SRI is unprotonated only in the absence of its transducer. Therefore, SRII complexed with HtrII displays an identical absorption spectrum as that of free SRII near neutral pH, unlike SRI which shows a 35 nm red shift due to the protonation of Asp76 caused by HtrI-binding.

Effects of HtrII on SRII were observed in the receptor photocycle kinetics [55]. HtrII modulates the photocycle so that M forms more rapidly, M converts to O without appreciable formation of N, and O decay is significantly accelerated. These effects of HtrII complexation suggest transducer modulation of the conformational changes of SRII that occur late in the photocycle and facilitation of Asp73 deprotonation by altering the hydrogen bonding network in the extracellular channel of SRII.

Conversely, in the signaling process, SRII must modulate the structure of HtrII. This modulation presumably occurs via the conformational changes of SRII that occur in the late photocycle states, since

they appear to be the active signaling states of SRII [23]. In the second half of the photocycle, the salt bridge between the Schiff base and Asp73 is disrupted because Asp73 is protonated. In BR, electron and X-ray projection maps have revealed protein structural changes resulting from the disruption of the corresponding salt bridge (i.e. between the Schiff base and the Asp85 counterion). In the BR mutants, D85N at alkaline pH and D85N/D96N at neutral pH helices B, F and G are tilted, as occurs also in the N intermediate in the wild-type photocycle [17,18,59]. This observation emphasizes the small energy difference between the two conformational states of BR which can be switched simply by disruption of the salt bridge even without photoisomerization and deprotonation of the Schiff base, which are however indispensable for electrogenic proton transport.

Similar mutations that shift the conformation of BR are found to activate SRII. The SRII mutant D73N, in which the salt bridge is eliminated, exhibits constitutive activity as revealed by elevated reversal frequencies of cells in the dark, attenuated responses of the cells to the light and adaptive demethylation of HtrII in the dark [15]. SRII mutant D73Q is activated to an even greater extent (E.N. Spudich et al., unpublished). Other mutants of the same residue substituted with other amino acids without carbonyl groups, such as Ala, Ser, Thr or Leu, also showed constitutive activity in the dark, but they regain near-normal activity after repetitive stimulation (Sasaki and Spudich, unpublished). Our interpretation is that these mutations activate the receptor to a lesser extent than D73Q or N, and that adaptational processes that follow the stimuli suppress the constitutive activity. An interesting possibility to explain the apparently less constitutive activation by these mutations is that they permit a weak counterion interaction between the protonated Schiff base and a bound chloride ion, partially shifting the conformations back to the inactive state. This possibility is supported by the relatively strong anion-dependent spectral shifts in the case of the weakly activating D73A and D73S compared to anion-independence of the D73N spectrum (Sasaki and Spudich, unpublished).

SRI, on the other hand, has an already-disrupted salt bridge between the Schiff base and Asp76 in the dark because Asp76 is protonated when SRI is complexed with HtrI. In accordance with the view expressed above from SRII studies, SRI would be partially constitutively activated in the dark. A possible role of dark activation is to poise the SRI receptor in an intermediate state (i.e. in an equilibrium between its two conformations), capable of being shifted by photostimuli with different wavelengths toward one or the other extreme. In fact, one photon-stimulation of SRI with orange light causes an attractant response whereas 2-photon-stimulation with orange followed by near-UV light causes a repellent response [2]. Moreover, genetic suppressor analysis strongly supports the notion that attractant and repellent signals from SRI result from shifting of the same equilibrium in opposite directions [60]. Therefore, a key feature in the design of SRI as a color-discriminating receptor may be its metastability caused by disruption of the salt bridge.

8. Summary

The study of light-induced proton transfers in SRI and SRII has contributed important insights into the mechanism of phototaxis signaling to their transducer proteins (HtrI and HtrII): (i) electrogenic proton transport by transducer-free SRs shows that the essential features of the BR pumping mechanism have been conserved in the evolution of the sensors. (ii) Proton pumping by SRI, readily demonstrable in its transducer-free form, is blocked by HtrI-binding, showing that the pump machinery, rather than the transport activity itself, is functionally important for signaling. (iii) SRII mutant analysis has demonstrated that the salt bridge between the protonated Schiff base and its counterion Asp73 constrains the receptor in its inactive conformation. A corollary is that light-induced transfer of the proton, which disrupts the salt bridge, is a major contributor to inducing the conformationally changed signaling state. (iv) Some SRII mutants in which the salt bridge is disrupted and light-induced deprotonation of the Schiff base is blocked still produce appreciable phototaxis signals showing that the proton transfer is not the only determinant of the signaling state and that deprotonation of the Schiff base is not essential for signaling.

Transducer chimera studies further indicate that the receptor conformational changes are transmitted

from the sensors to their cognate transducers through TM helix–helix interaction. With this in mind, the proton transfer results support a signaling mechanism in which tilting of helices on the cytoplasmic side (primarily outward tilting of helix F), similar to that which occurs in BR, causes structural alterations in the transducer TM helices.

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