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REVIEW

PHOTOSENSORY SIGNAL TRANSDUCTION IN *STENTOR COERULEUS*

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INTRODUCTION

Light plays fundamentally important roles for life on earth, primarily by being the energy source for photosynthesis in plants and certain bacteria. However, this is not the only way that light plays crucial roles in plant kingdom. Light is also used for its information in terms of intensity, direction, polarization and color/wavelength. Many organisms use light to gain information about their environment just as they react to other environmental stimuli, such as chemicals, gravity, and temperature, and then respond based on that information. Most organisms, ranging from bacteria to mammals, directly or indirectly react to light and exhibit a wide variety of responses at molecular, cellular and organism levels. These light induced responses are initiated by the absorption of light of specific wavelength by photosensor molecules. Only a few biological photosensor molecules have been structurally identified and functionally characterized. The best known photoreceptors are those containing retinal as a chromophore. Although retinal-containing photoreceptors are used in nature, most notably rhodopsin in animal vision, other rhodopsin-like photoreceptor molecules have also been found widely distributed outside the animal kingdom[24,48,64]. Another class of well-studied photoreceptors belongs to the tetrapyrrole family, which includes phytochrome, phycobilins and chlorophylls. Phytochrome regulates many processes and activities in plants[25] and has been found in green algae, as well as in spermatophytes, ferns and mosses. The phycobilins are accessory pigments which absorb visible light and transfer excitation energy to chlorophylls for photosynthesis[54]. Flavins have been implicated as a major group of blue light receptors.[1-3,12,28,32,57]

Recently, a new photoreceptor molecule, stentorin[67], has been identified in the ciliated protozoan *Stentor coeruleus*(class Ciliata, subclass Spirotricha order Heterotricha). Interestingly, the photosensor molecule blepharismin of *Blepharisma japonicum*, a heterotrichous protozoan very closely related to *Stentor coeruleus*, was also found to be a hypericin analog[6,43]. This class of hypericin-like photoreceptor molecules is unique among the photobiological light sensors.

Investigations of the role of hypericin-like molecules in photosensory transduction should broaden our understanding of the basic nature of stimulus-response systems at the single cell level. *Stentor coeruleus* is particularly interesting in this regard since it is unicellular and the problems associated with complex interactions that occur between mutually interdependent cells in higher organisms can be circumvented. In addition, *Stentor coeruleus* is one of the largest ciliates, with a typical size of up to 700 micrometers in length. It is deeply pigmented, and is thus readily visible to naked eyes. It also exhibits well-defined, light induced responses. *Blepharisma japonicum* is also capable of both light intensity- and color/wavelength-sensory "vision".

In this review, we will discuss the way the ciliate cells respond to light at both cellular and molecular levels. However, we must emphasize that much more research is warranted before the photosensory transduction in *Stentor coeruleus* and related ciliate cells can be fully described. For the purpose of discussing what is currently known about the photosensory transduction pathways in *Stentor coeruleus* and *Blepharisma japonicum*, the photosensory transduction processes involved in the photoresponses of these ciliates can be described schematically as follows:

Light signal → Absorption by photoreceptor → Signal generation

and amplification →Response (reversal of motor and rotation)

PHOTOMOVEMENT RESPONSES

(A) Photophobic responses

Light-avoiding behaviors of Stentor coeruleus has been known for many decades. However, it was relatively recent that the light-avoiding behavior was characterized as being due to a step-up photophobic response and a negative phototactic respons[57,58]. A sudden increase in light intensity causes Stentor to stop swimming when it moves from the dark side to the illuminated spot, tumble and reverse the direction of its ciliary stroke. The stroke reversal results in a change in the direction of swimming, i.e. away from the light (Fig. 1). *Ble-*

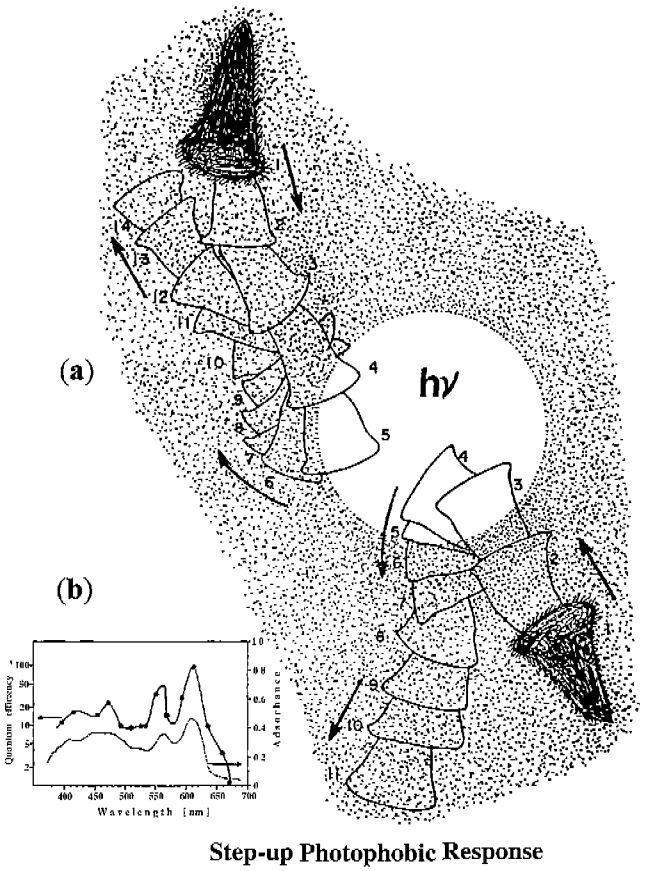

Figure 1. (a)The cells exhibit stop-turn response upon encountering the illuminated area (center)[60,63]. (b) The action spectrum for the step-up photophobic response and the absorption spectrum (dotted line) of *Stentor* cell suspension[49].

pharisma japonicum displays similar photophobic response.

(B) Phototaxis

In addition to the light-avoiding response, Stentor is also able to detect the direction of the incident light and swims along the axis of the incident light and away from the light source. Since negative phototaxis of a free swimming cell, moving away from the stimulus light source, can result from a series of step-up photophobic responses, whether or not *Stentor coeruleus* displays a true negative phototactic response had to be answered by a specific experimental design[61].

(C) Photokinesis

Whether or not there is a dependence of swimming speed on fluence in *Stentor coeruleus* is not well established[58]. Recently, Iwatsuki[34] reported that, in fact, the ciliate cell exhibited a positive photokinesis. If this is indeed true, question arises as to the driving force (for example, ATP) for the positive photokinesis, as *Stentor coeruleus* is non-photosynthetic. It would be interesting to explore the suggestion that cellular free ATP level of the ciliate is modulated by light[13].

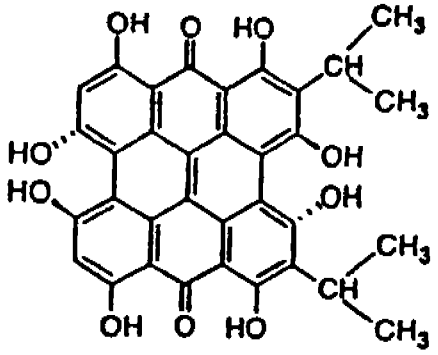

Figure 2. The chromophore structure of stentorin[67]. The structure was synthetically confirmed[4].

PHOTOSENSORY TRANSDUCTION IN STENTOR COERULEUS

(A) Photosensors: Pigment chromophores

Although rhodopsin-like pigments are generally believed to serve as universal visual photoreceptors, there is no evidence for their existence in *Stentor coeruleus*, or for their participation in the photophobic and phototactic responses. Among a number of methods available to identify the photoreceptors involved in various light induced responses, action spectrum measurement is one of the most widely used methods[41]. An action spectrum can be determined by plotting the reciprocal of the fluence rate at which 50% of the cells exhibit photoresponses against wavelength. On the basis of the resemblance of the action spectra for the photophobic and phototactic responses in *Stentor coeruleus* to the absorption spectrum of the whole cells and that of the extracted pigment, the photoreceptor pigment was identified as stentorin [38,60,61,69]. It was also found that a colorless mutant, as well as caffeine-bleached *Stentor coeruleus* lost its photosensitivity, further supporting that stentorin is the photoreceptor[58]. The action spectrum for the step-up photophobic response of *Stentor coeruleus* is similar to the absorption spectrum of stentorin. The action spectrum for the negative phototactic response is similar to that for the photophobic response [60,61]. However, the action spectra of the photomovement responses of *Stentor coeruleus* differ greatly from the absorption spectra of the typical photoreceptor molecules, such as the retinal-based visual pigments in higher animals. The action spectra for the light-induced receptor potential and ciliary stroke reversal are also consistent with the absorption spectra of the pigment stentorin[16,60,61,74]. Both *Stentor coeruleus* and *Blepharisma japonicum* display the action spectra for the membrane receptor potentials that spectrally correlated with the absorption spectra of stentorin and blepharismin, respectively[16,17].

The blepharismin structure is particularly unique among the hypericin compounds because of its conjugation-disrupting bridge carbon (Fig. 3). This accounts for the distinct absorbance spectrum of the blepharismin chromophore. It

remains to be seen if ciliates other than Stentor and Blepharisma and even some non-ciliate cells make use of the hypericin-based pigment molecules for photoreception.

(B) Photoreceptor proteins

The "photoreceptor apparatus" of both Stentor and Blepharisma is the pigment granule[39,68]. The pigment granules in *Stentor coeruleus* are located in sub-pellicular ectoplasm and are distinct in nature from mitochondria[33,72]. Two distinct forms of stentorin chromoproeins can be chromatographically isolated. These are stentorin I and II. Their absorbance spectra are very similar with respect to peak shape. However, the absorption peaks of stentorin II in the visible region are red-shifted for about 10 nm. The chromophores in stentorin I and II are similar in structure, while the difference in peak position implies that chromophore environment may be responsible for the spectral shift. Only the stentorin I is strongly fluorescent while stentorin II is only weakly or non-fluorescent. Both stentorin I and II displayed anomalous behaviors on SDS-PAGE gels. For example, the apparent molecular mass of stentorin I was strongly dependent on the gel concentration. Stentorin II did not enter the gel. These anomalies presented the possibility that stentorins were probably glycosylated since the dependence of the apparent molecular weight on gel concentration is characteristic of glycoproteins [56]. However, neither stentorin I nor stentorin II were found to be glycosylated[66,68]. Stentorin I is not larger than cytochrome c (12.5 kDa). Detergent-solubilized stentorin I, previously thought to be a 100 kDa chromoprotein[37], is very likely a chromophore-detergent complex. Stentorin II differs from stentorin I in that it can be precipitated by TCA. Detergent-solubilized stentorin II behaves like a large complex of mol mass greater than 500,000 dalton. The stentorin II can be further resolved into stentorin II-A and stentorin II-B by hydrophobic interaction chromatography[11]. It contains the chromophore covalently bound to an approximately 50 kDa protein as determined by SDS-urea-PAGE. The amino acid composition of the approximately 50 kDa blue-green band from SDS-urea-PAGE of stentorin II-B is known[11]. Because the N-terminus is blocked, direct sequencing from the N-terminus is not feasible. However, two partial sequences, (1) NPFTAELVETA and (2) SILAADESTGTIG, were obtained for stentorin II-B tryptic fragments. Even though these sequences are too short to search for homologous proteins, a computer search of the Swiss-Prot and Genembl database using WORDSEARCH turned up several proteins exhibiting high homology to these sequences. Two highly homologous gene products from photoreceptor cells were among those identified. The first is the gene product from Drosophila ninaC, which encodes a protein containing 1,501 amino acid residues with domains homologous to protein kinases and myosin[44]. Results from the BESTFIT program revealed that the similarity of sequence 1 to -(279)HPFLTELIENE (289)-of *ninaC* is 73%, while its identity is 55%. The similarity of sequence 2 is 50% and the identity 42% to -(596) KVLAAILNIGNI(607)- of ninaC. The homologous fragment for sequence 1 is located in the kinase domain (17-282). There is a total of 10.2 % of aspartate and glutamate residues in this kinase domain. The homologous fragment for sequence 2 is located in the myosin domain (329-1,053). This myosin domain contains 13% aspartate and glutamate residues. Another protein homologous to the limited N-terminal sequences of stentorin II-B digest is a sodium-calcium exchanger from bovine rod photoreceptors[51]. Sequence 1 exhibits 60% similarity and 40% identity to -(995)QPLSLEWPET(1004)- of this protein, while sequence 2 displays 75% similarity and 42% identity to -(1056)VWWAHQVGETIG(1067)-. This 1,199 amino acid protein contains 17% aspartate and glutamate residues. Hydrophobicity analysis provides 12 hydrophobic segments in this protein so that 12 transmembrane segments in topology are defined. It also contains 6 N-linked glycosylation sites near the N-terminus. An unusual feature is the presence of 26 acidic residues which cluster in the segment -(967) EDEEEEDEEEEDEEEEEEEEE EEEEEEENE(994)-.

The homologous segment (995-1004) for sequence 1 is located following this acidic segment. That of sequence 2 is located near the C-terminus.

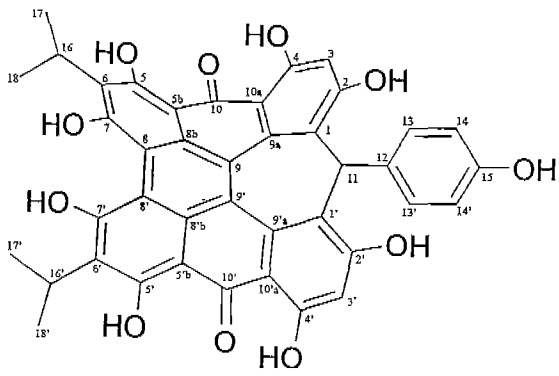

Figure 3. The chemical structure of blepharismin.

(C) Signal trigger: Primary photoprocesses

Photomovement responses of microorganisms such as ciliates involve a chain of chemical and/or physical events triggered by the photoexcited photoreceptor molecule/apparatus. The nature and efficiency of the photomovement initiation trigger is provided by the primary photoprocess of the photoreceptor molecules. It was suggested that stentorin II, rather than stentorin I, serve as the photosensor as implicated by the fact that the former displays very low fluorescence quantum yield with ultrafast decay in picosecond time scale [62,53].The picosecond pump-probe spectroscopy study indicated that a unique initial photoprocess occurred within 3 ps in excited stentorin II but not in the free chromophore species, hypericin or stentorin I[53]. Blepharismin in its protein bound form also exhibits a picosecond process[77,78]. Unlike rhodopsin, photoactive yellow protein (PYP) and phytochromes, both stentorin and blepharismin do not exhibit a photochemical transformation cycle that can be readily detected by spectrophotometry under freeze-thaw cycling conditions. The chemically parent compound hypericin for stentorin and blephar-

cally parent compound hypericin for stentorin and blepharismin is an efficient photosensitizer, mediating various photodynamic actions in vivo and *in vitro* via singlet oxygen [13,79]. It has been suggested that singlet oxygen functions as a signal messenger for the photomovements in some microorganisms. However, at least in *Stentor coeruleus*, singlet oxygen does not seem to play a significant role in the photomovement transduction pathway, since stenorin II is a poor singlet oxygen generator, unlike its free chromophore and hypericin[10]. A singlet oxygen quencher did not specifically inhibit the photophobic response of *Blepharisma japonicum*, also suggesting that the active oxygen is not a photosensory transducer component for this organism[5]. What then is the primary photochemistry involved in triggering the photosensory transduction pathway in Stentor and related photoresponsive ciliates? Stentorin can mediate electron transfer processes in their excited state[73]. For example, the stentorin model chromophore, hypericin, can serve as an electron acceptor from an electron donor with sufficient reducing potential. Hypericin undergoes two reversible one-electron reductions for hypericin at -0.87 V and -1.18 V vs. NHE [50]. Additionally, an oxidation is observed at ca. +0.90 V in DMSO. These formal potentials are consistent with the visible absorbance maximum of hypericin in DMSO at 599 nm (=2.1 eV). Given this information, we estimate that the excited state potentials for hypericin (Hyp) are approximately -0.90 V for the formation of hypericin cation radical Hyp+ [Due to uncertainty of these numbers, the oxidation potential of the excited state hypericin can be as high as +1.2V;[73,78]

$$Hyp + h\nu \rightarrow Hyp^* \rightarrow Hyp^{+} + e^-$$

and +0.90 V for the formation of hypericin anion radical Hyp$^-$,

$$Hyp + h\nu \rightarrow Hyp^* + e^- \rightarrow Hyp^-$$

where electron e^- is supplied by an electron donor including the ground state Hyp itself. Thus, in the excited state, hypericin can be a good electron donor, as well as being a good electron acceptor, depending on the redox potentials of the donor/acceptor pairs present in solution. Yamazaki *et al*[78]. demonstrated that *p*-benzoquinone, which functions as a classical electron acceptor, quenched the fluorescence of hypericin. The quenching is due to electron transfer from hypericin to *p*-benzoquinone at a diffusion-controlled rate ($1.43 \times 10^{10} M^{-1}s^{-1}$). The electron donor property of hypericin in its excited state is consistent with the picosecond absorption difference measurements of hypericin that a new species was formed upon excitation in ~5 ps having a transient absorption in the red/far-red region. This species was not present in ground state absorption[26,27]. The long wavelength absorption is quenched by the electron scavenger solvent, acetone, which may have resulted from a solvated electron[26,27]. Weiner and Mazur[71] reported that the formation of cation and anion radicals upon photolysis of hypericin. Photolysis of stentorin II yields radical species, apparently producing stentorin cation radicals, in a manner similar to the scheme shown for the excited state hypericin[73]. Electron transfer probably occurs from the excited stentorin chromophore to a suitable acceptor/amino-acid residue. An efficient electron transfer process may account for the quenching of the fluorescence from the excited state stentorin. The rate for this process can be estimated from the short fluorescence decay lifetime, ~$1 \times 10^{11} M^{-1}s^{-1}$. This value is close to the diffusion limited rate constant for electron transfer from the excited state hypericin to *p*-benzoquinone. This reaction is likely a reversible process in native stentorin.

Hypericin can serve as an electron acceptor in the photoreduction with NADH. As a hydroxyquinone, the electron acceptor function of hypericin can also be demonstrated by photolytically producing a hypericin anion species (λ_{max} 734 nm) in piperidine as solvent[42]. Hypericin can also be photooxidized in the presence of dithiodiethanol with a quantum yield of ca. 0.001. Photooxidation of hypericin with dithiodiethanol ($HOCH_2CH_2$-S-S-CH_2CH_2OH) generates mercaptoethanol (HS-CH_2CH_2OH) and oxyhypericin[9]. Similarly, photooxidation of stentorin (HO-ST-OH) would likely produce a new quinone form of oxy-stentorin (O=ST=O) according to the following scheme:

$$\begin{aligned} &HO\text{-}ST\text{-}OH + h\nu \rightarrow HO\text{-}ST\text{-}OH^* \\ &HO\text{-}ST\text{-}OH^* + R\text{-}S\text{-}S\text{-}R \rightarrow HO\text{-}ST\text{-}OH^{\bullet+} + [R\text{-}S\text{-}S\text{-}R]^{\bullet-} \\ &\qquad\qquad \rightarrow O{=}ST{=}O + 2H^+ + 2R\text{-}S^- \end{aligned}$$

In native stentorin, two electrons and two protons resulting from the similar reactions reduce the suitable amino acid residue(s) such as cystine. The potential for the oxidation of mercaptoethanol is +0.02 V at pH 8[80], while a potential of +0.6 V is necessary for the reduction of oxy-hypericin in DMSO[50]. Thus, a spontaneous reduction of oxy-hypericin in the ground state is feasible. Similarly, an oxy-stentorin can be reduced to stentorin to complete the photo-cycle, with the possible vectorial release of protons from the stentorin-bound pigment granule to the cytoplasm. It remains to be seen if electron/proton transfer to and from the stentorin chromophore is accelerated by the apoprotein to initiate the functionally viable "photo-cycle" in *Stentor coeruleus*. The redox potential of the cysteine-cystine system is estimated to be -0.21 V at pH 7.0[8]. This redox potential would enhance the photooxidation of hypericin with respect to dithiodiethanol-mercaptoethanol system (-0.9 V at pH 10 for oxidation).

Electron transfer to and from the excited state of blepharismin is likely to play a signal initiating role in the photosensory transduction of *Blepharisma japonicum*. Because of the similarities in their molecular structures between blepharismin and hypericin (also stentorin), we expect blepharismin and its model compounds to exhibit similar electrochemical behaviors and efficient electron transfer in the excited state.

(D) Signal generation and amplification

The motility of *Stentor coeruleus* is propelled by ciliary

strokes and its ultimate reaction to light is ciliary reversal. Therefore, it is important to look at what governs the ciliary movement. Membrane potentials control the ciliary movement. Evidence has been gathered indicating that ciliary activity is controlled primarily through electrical properties of the cell membrane[15,45]. The membrane is believed to control ciliary beatings by regulating the internal ionic environment, especially the intracellular calcium ions[14]. The motile mechanoresponse of *Stentor coeruleus* is controlled by ion permeabilities of the cell membrane that determine resting, action, and mechanoreceptor potentials in the ciliate cell[74,75]. Similarly, the light-responses in *Stentor coeruleus* have been investigated using electrophysiological approaches. The photic receptor potential appeared after the onset of light stimulation of *Stentor coeruleus* with a delay as long as 0.5 s at temperatures below 10 °C. This contrasts markedly with the receptor potentials produced by mechanical and electrical stimulation[76]. For example, mechanical stimulus initiates a membrane depolarization within 10 ms of the stimulus onset, which within another 20 ms triggers an action potential[75]. These differences in time course indicate that there are at least kinetic and possibly biochemical differences in the generation of the photic and mechanical receptor potentials.

The photomovement responses in *Stentor coeruleus* are pH dependent, with lower responses at acidic pH and higher responses at neutral and basic pH's[19,20,70]. It was also demonstrated that heavy water (D_2O) enhanced the photophobic response and inhibited the phototactic response in *Stentor*[35]. Upon addition of the protonophores, the photoresponses of Stentor decreased significantly. It is hypothesized that the protonophores would dissipate the pH gradient across the membrane, which could be generated by electron/proton transfer from the excited state stentorin[60,70]. As a result of the primary photoprocess of stentorin, a transient intracellular pH change may be coupled directly or indirectly to open the voltage-sensitive calcium channels. Indeed, in a model system consisted of hypericin imbedded in phospholipid liposomes, a light-induced pH drop within the liposome has been reported[23]. An intracellular pH drop can also be triggered by the photoexcitation of hypericin or hypocrellin incorporated in 3T3 mouse fibroblast cells[7]. The model studies suggest that the primary photoprocess can lead to an intracellular pH change. Such a transient pH change could serve as the initial signal for the subsequent signal transduction pathway involved in the photomovement of the ciliate cells.

The membrane resting potential in ciliate cells is usually negative so that the cell interior is electrically negatively charged with respect to the extracellular medium. *Stentor coeruleus*, as in most organisms, maintains a negative resting potential of about -50~-55 mV across the cell membrane[16,75]. Upon excitation of the cell with a step-up light stimuls, a graded membrane receptor potential is generated, followed by an all-or-none action potentia[16,74].The pH-dependent action potential has also been observed by Kim[37]. In addition, Ca^{2+} ionophores such as calimycin, α-phosphatidic acid and Ca^{2+} blockers, including Ruthenium Red and methoxyverapamil, specifically inhibit both the step-up photophobic and negative phototactic responses in Stentor coeruleus[59]. Although it is obvious in *Paramecium* as well as in other species, no membrane hyperpolarization has been observed in *Stentor coeruleus*. As in most ciliated protozoa, it seems that locomotion in *Stentor coeruleus* is regulated by membrane-limited Ca^{2+} fluxes[35,49]. Also, as will be discussed later, actinic photon signals are amplified by the influx of Ca^{2+} ions across the cell membrane via depolarization voltage-dependent calcium channels.

Perhaps the most convincing evidence for the generation of a transient pH change as an early intracellular transduction signal comes from the photoresponses of the ciliate cells to protonophores and exogenous ammonium chloride[20]. Ammonium chloride serves as a membrane permeable weak acid that lowers the intracellular pH. We suggest the artificially lowered intracellular pH in the presence of NH4Cl counteracts any light-induced pH drop as the early signal for the subsequent transduction cascade. *Stentor coeruleus* shows similar responses to ammonium chloride[20].

As described earlier, electrophysiological studies suggested a temporal correlation between light-induced membrane potential and photomovement responses, indicating that the membrane electrical events are involved in the photosensory tansduction in *Stentor coeruleus*. *Blepharisma japonicum* exhibits similar time courses correlating the membrane potential changes with the photophobic response. These results are not unexpected since modification of the motile responses by ionophores and ion channel blockers ($TPMP^+$, CCCP, FCCP, calimycin, and verapamil, etc.) as well as by extracellular ions (Ca^{2+}, H^+) have been observed before[63]. These agents are known to disrupt or alter cell membrane potential and are therefore expected to influence the photomotile responses in *Stentor coeruleus*.

The final motile response in the photomovement of *Stentor coeruleus* consists of at least the following steps, namely, a stop reaction of its forward swimming, followed by brief backward swimming when light intensity is strong enough, and resumption of forward swimming. When higher light intensity than that required to elicit a receptor potential exceeding the threshold is applied, *Stentor coeruleus* cells reacted with a receptor potential which triggers rapidly the action potential, and after the decay of the action potential, a plateau of depolarization follows. With stimulation of light of similar intensity, *Stentor coeruleus* reacted by altering its swimming direction with a stop reaction and a period of backward swimming. The latency [delay time between the initial onset of light stimulus and the appearance of action potential] of the stop reaction is shorter and the duration of backward swimming is longer with increasing light intensity. There is a temporal, and possibly functional connection between the light-induced membrane potential changes and the phobic response[16,17].

Fig. 4 schematically shows the ionic model for the photomovement of *Stentor coeruleus* based on electrophysiological and physiological studies. On the basis of the electrophysiological measurements[16,18,22], a similar transduction model can be proposed for the photomovement response of

Blepharisma japonicum. Further investigation of the ionic basis underlying the light-induced membrane potential changes is warranted to shed light on our understanding of the photosensory tansduction in *Stentor coeruleus* and *Blepharisma japonicum*. One direction of future work could be the application of patch clamp techniques[30,47]. Patch clamp techniques[46] have allowed the measurement of the currents in single ionic channels. The patch clamp technique has been successfully applied to single channel current recording in plasma membrane blisters of Paramecium[52]. Only recently, this technique has been applied to identify cGMP-dependent ion channels in Stentor cells[40].

Signal transduction model

In the previous section, we discussed the possible role of an intracellular pH change (ΔpH) as an early transducing signal generated by the photoexcitation of photoreceptors stentorin and blepharismin in the pigment granules of *Stentor coeruleus* and *Blepharisma japonicum*, respectively. A signaling role of the ΔpH in the photomovement of the ciliates warrants direct experimental confirmation. In the proposed transduction scheme, the light signal is amplified in terms of Ca^{2+} ion influx from the extracellular medium, which elicits structural changes in the ciliary contractile axonemes for the reversal of ciliary stroke. Fig. 4 presents a schematic model for the photosensory transduction pathway for the photomovement responses such as the step-up photophobic response in *S. coeruleus*, on the basis of photophysiological and electrophysiological results highlighted above[63]. The *Blepharisma japonicum* cell appears to use similar ionic mechanism[21,22]. The biochemical mechanisms underscoring each of the ionic events depicted in this scheme are virtually unknown. Here, we can only speculate about the possible biochemical mechanisms based on indirect physiological observations.

Pertussis toxin blocks the inhibition of adenylate cyclase by catalyzing the covalent modification of the inhibitory G protein[65]. The G-protein activator fluoroaluminate sensitized the ciliate cells, *Stentor coeruleus* and *Blepharisma japonicum*, to the actinic light stimuli and enhanced their photophobic responses. Incubation of both ciliates in the presence of 8-bromo-guanosine 3′, 5′-cyclic monophosphate (8-Br-GMP) resulted in specific inhibition of their photophobic responses, while lengthening the latency for the generation of action potentials, thus desensitizing the cells to the actinic light stimul[18,19]. IBMX, an inhibitor of cGMP-dependent phosphodiesterase[29], and *l-cis*-diltiazem, a potent blocker of cGMP-dependent ion channels[31], also inhibited the photophobic response of *Stentor coeruleus* and *Blepharisma japonicum*[18,19,21]. On the other hand, 6-anilino-5,8-quinolinedione (LY83583) significantly stimulated their photophobic responses, presumably due to the lowering of cellular cGMP. These results suggest that G-protein and cGMP/GMP play a signaling role in the photosensory transduction in *Stentor coeruleu* and *Blepharisma japonicum*.[It is not clear how the "dark" activation of G-protein and the lowering of cellular cGMP by fluoroaluminate and LY83583, respectively, led

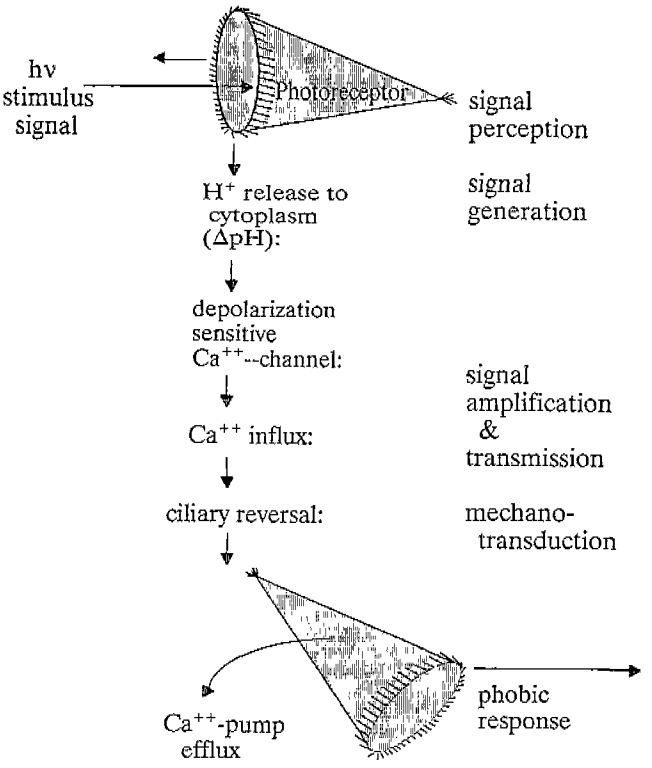

Figure 4. Schematic representation of the ionic model for the photomovement of *Stentor coeruleus*.

to the enhanced photosensitivity of the ciliates. Since the cells treated with these drugs did not exhibit ciliary stroke reversals in the dark, these drugs result in a net increase in the photosensitivity of the ciliate cells toward the photophobic responses].

In summary, the photoexcitation of stentorin in the pigment granule is proposed to generate a transient intracellular pH change, which can serve as a cellular signal associated with the depolarizing receptor potential. Subsequently, the initiating signal appears to be amplified by a sudden trigger of Ca^{2+} ion influx into the cell. The involvement of these photo-signal transduction events in the ciliate cells is consistent with the inhibitory effects of protonophores and Ca^{2+} channel blockers. It has also been suggested that a heterotrimeric G-protein plays an important role as a signal transducer in *Stentor coeruleus* and *Blepharisma japonicum*. Thus, these cells provide an analogy to "visual" excitation system reminiscent of the rhodopsin-coupled transducin of higher animals. In both cases, G-protein activates a phosphodiesterase as an effector molecule, which leads to the lowering of cellular cGMP level. In the vertebrate visual system, the light-induced, rhodopsin-mediated lowering of intracellular cGMP level transiently closes cGMP-gated cation channels. However, in Drosophila, the light-induced, rhodopsin-mediated activation of the phospholipase Cα results in the opening of the cation channels, transient receptor potential in the plasma membrane, and influx of Ca^{2+} ions[55]. No experiments have been performed to identify a phospholipase C isoform, activation of which may elicit influx of Ca^{2+}

The scheme proposed here combines vertebrate effector cGMP-dependent phosphodiesterase and invertebrate effector phospholipase C. The hypothetical model proposed here is also based on limited physiological evidence for the photophobic responses in *Stentor coeruleus* and *Blepharisma japonicum*, but the fact that the light-induced action potentials in the ciliates are depolarizing, consistent with the influx of Ca^{2+} ions.

The working hypothesis includes the photoactivation sequence stentorin → G-protein → phosphodiesterase → lowering of cGMP →opening of Ca^{2+}-channel →ciliary stroke reversal. This hypothesis is illustrated in a schematic model in Fig. 5. A similar model is likely to be operative in *Blepharisma japonicum* mediated by its photoreceptor blepharismin.

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