Journal of Photoscience, Vol. 2, No. 1, pp. 31–35, 1995
Printed in the Republic of Korea.

1225-8555/95

INVITED REVIEW

THE PHOTO-MECHANICAL RESPONSES IN THE UNICELLULAR CILIATES

PILL-SOON SONG*
Department of Chemistry University of Nebraska Lincoln,
NE 68588-0304, USA

(Received 9 December 1994, *accepted* 20 *February* 1995*)*

Abstract — Light signals in the form of intensity gradient, propagation direction, and wavelength elicit diverse mechanical responses ("photomechanical responese")in most organisms. The single cell ciliates, *Stentor coeruleus and Blepharisma japonicum*, are particularly sensitive to the light of visible wavelengths. In this paper, the way in which the seemingly sophisticated light signal transduction is triggered by the photosensory apparatus will be described in terms of the photoreceptor structure and photochemical function.

INTRODUCTION

Most organisms exhibit varied mechanical responses to light signals; such as intensity, propagation direction, polarization, wavelength and light-dark rhythm. The "photo-mechanical responses" include changes in motility, such as sudden stop and turn (photophobic response), rotation, movement parallel/anti-parallel to the direction of light propagation (phototaxis), bending (phototropism) and velocity of movement (photokinesis), *etc.* How do the colored unicellular ciliates *Stentor coeruleus* and *Blepharisma japonicum* stop and swim backwards momentarily when suddenly exposed to higher intensity light? Figure 1 depicts the so-called "step-up" photophobic response of the former.[1]

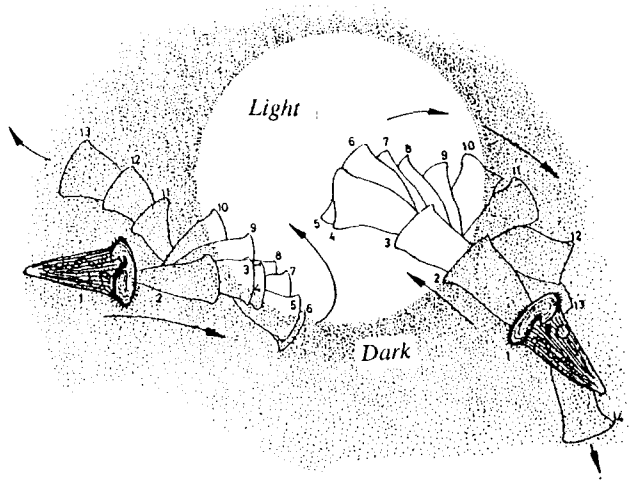


Figure 1. This figure shows 0.2-second sequential tracings from the microscopic video tape recordings for the ciliate cells which responded to a sudden change in light intensity as they swim from the dark to the illuminated area of the medium. Each cell is seen to stop at the light-dark boundary, resulting from the reversal of the ciliary stroke when exposed to light.[1]

To discuss the mechanism of the photo-mechanical response in the unicellular ciliates, it is convenient to define a sequence of light signal transduction steps as follows(Scheme 1):

Light Signal
↓ Signal Perception
Photochemical Process
↓ Signal Generation
Biochemical Process
↓ Signal Amplification
Electrophysiological Event
↓ Mechano-Transduction
Mechanical Response(Ciliary Stroke Reversal)

Scheme 1. Light Signal Transduction in Photoresponsive Ciliates

Unfortunately, our understanding of the molecular assemblies and membrane architecture, which underscore the light signal transduction chain, is still

at its preliminary level. Some steps are better understood than others, as described below.

THE CHEMICAL STRUCTURE AND SENSORY TRANSDUCTIONAL ROLE OF THE PHTOSENSOR MOLECULES

Signal Perception. Light signals are perceived by the photosensor, or photoreceptor molecules. The photoreceptor molecule in *Stentor coeruleus* is stentorin, which is covalently linked to the 50-kdalton apoprotein.[2] In our negative ion fast atom bombardment (FAB) mass spectrometry, stentorin showed an $(M-1)^-$ ion at 591.1304, consistent with the $(M-1)^-$ of $C_{34}H_{24}O_{10}$ [$(M-1)^-$ calculated to be 591.1291]. Results from acetylation of stentorin, in combination with FAB-MS, indicated that there are eight hydroxyl groups in stentorin. The NMR spectrum of stentorin revealed the presence of isopropyl groups in stentorin. According to the NMR data, the structure of stentorin involves one of two possible symmetrical arrangements of those protons and isopropyl groups, namely, 2,2', 4,4', 5,5', 7,7'-octahydroxyl-3,3'-diisopropylnaphthodianthrone(**1**) , or 2,2', 4,4', 5,5' ,7,7'-octahydroxyl-3,6'-diisopropylnaphthodianthrone (**2**).[3]

Structure **1** has now been identified as the chromophore of free stentorin on the basis of a comparison of the spectroscopic properties (NMR, CD, UV-Vis, and fluorescence) of synthetic compounds **1** and **2**[4] with native stentorin[5]. Compound **1** has also been synthesized by Hideo Iio and his group at Osaka City University (private communication, 1995). Similar studies are in progress to elucidate the chromophore structures of blepharismins, the photosensor molecules in *Blepharisma japonicum*. The structures of blepharismins are distinctly different from either (**1**) or (**2**) and contains an extra carbonyl group and a benzenoid ring. A tentative structure (**3**) has been assigned for one of the blepharismin pigments isolated from the dark-adapted pink fluorescent cells.

The ciliate photosensor molecules represent a structurally unique class of biological photoreceptor chromophores. Figure 2 shows a rather exclusive

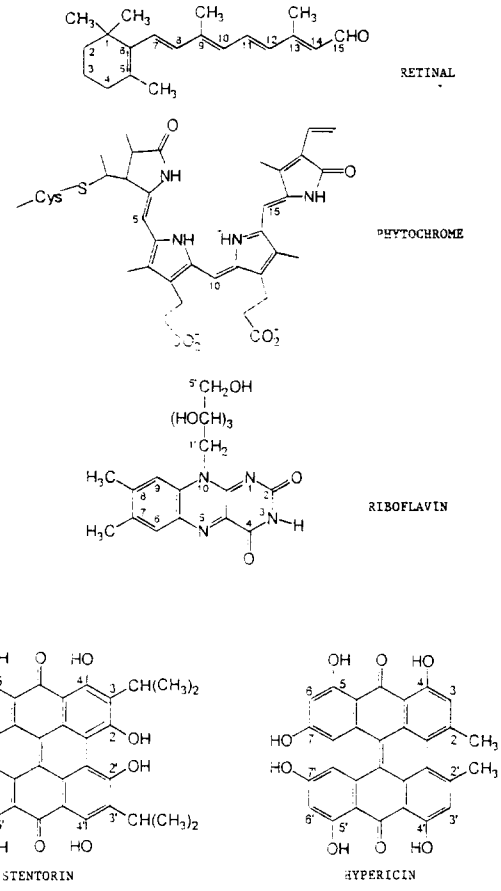
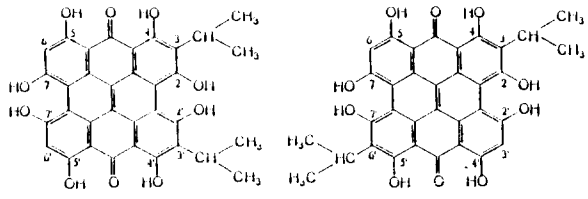
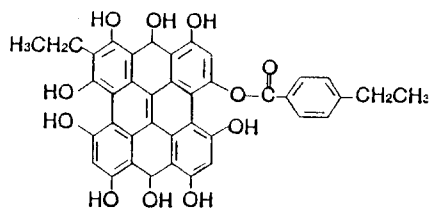


Figure 2. The established chromophores of the photobiological light sensors including stentorin and its structural analog hypericin. Although flavins are likely candidates as the chromophore of blue-light receptors, their definitive identifications remain to be inverstigated. Riboflavin shown is a typical flavin.



1 2



3

listing of the established photoreceptor chromophores for both prokaryotic and eukaryotic photo-responsive organisms. It remains to be seen if other photo-responsive ciliates and organisms utilize chromophores similar to stentorin and blepharismins.

Photoreceptor Assembly. Stentorin is bound to the 50-kdalton protein (stentorin-2B). When isolated under native conditions, stentorin forms a large molecular assembly (stentorin-2, up to 800-kDa in

mass) comprised of the 50-kdalton protein and additional non-chromophore-bearing protein subunit(s) (stentorin-2A). The chromophore structure of stentorin-2B appears to be identical with the structure **1** for free stentorin, as we recently probed the structures of stentorin chromoproteins and hypericin by surface-enhanced resonance Raman scattering spectroscopy (SERRS).[6] The supramolecular architecture of the photoreceptor assembly is not understood well. The pigment proteins are localized within the pigment granules, 0.3-0.7 μm in diameter, which are distributed longitudinally along the cell body between ciliary rows. They are localized near the ciliary basal bodies fused into the pellicle, or plasma membranes. A recent scanning electron micrograph study showed that pigment granules were closely packed in a random array within each of their rows. A transmission electron micrograph study revealed that the pigment granules were striated with alternating layers having high and low electron density. The striation pattern shown in Figure 3 is somewhat reminiscent of the membrane stacks of another photoactive organelle, chloroplast.

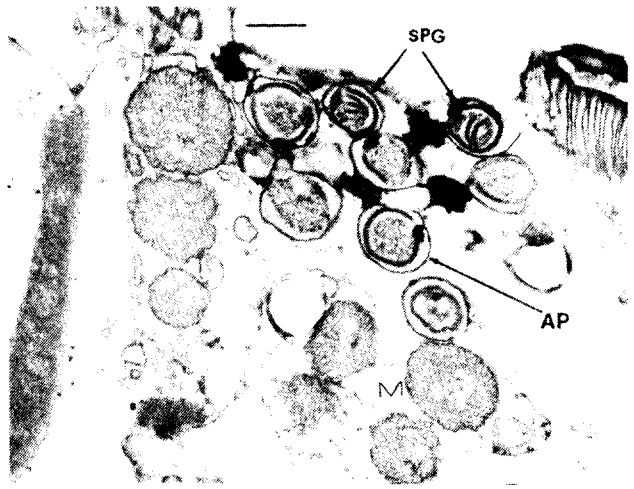


Figure 3. Transmission electron micrographs showing both striated (sPG) and amorphous (AP) pigment granules. Mitochondria (M) are also seen on the lower part and upper left-hand side of the micrograph. Upper horizontal bar = 500 nm.

Similar to stentorin, blepharismins are located within the pigment granule, 0.5 μm in diameter, of *Blepharisma japonicum*. A recent study by Matsuoka and his coworkers at Kochi University showed that the granules surrounded by membrane were connected to a plasma membrane. The pigment granules contain a honeycomb-like structure and a stacked lamella structure.

Signal Generation. Light signals must be transduced to eventually elicit mechanoresponses, such as the reversal of ciliary stroke in *Stentor coeruleus* and *blepharisma japonicum*. The initial step of the signal transduction chain involves generation of cellular signal(s) *via* the photoprocesses of the photosensor molecules. The photochemical mechanisms of stentorin and blepharismin are unknown at present. Preliminary steady state photolysis of stentorin and ble-pharismin failed to yield spectrophotometrically observable photochemical cycles. However, the photoprocess leads to a lowering of intracellular pH in *Stentor coeruleus*.

Deprotonation of stentorin may serve as the initial photosensory transduction step. Also, the photoresponses of *Stentor coeruleus* were shown to be dependent on pH. Additionally, intracellular pH-modulating reagents [including ammonium chloride, protonophores carbonylcyanide *m*-chlorophenyl-hydrazone (CCCP) and carbonylcyanide *p*-(trifluoromethoxy)-phenyl-hydrazone (FCCP)] were found to reduce the photo-motile responses of the ciliate cell.[7]

A pump-probe experiment showed that stentorin-2 undergoes an absorbance decrease(bleaching) in less than 3 ps; whereas free stentorin chromophore did not exhibit the ultrafast photoprocess.[8] This process is faster than the fluorescence decay occurring with a lifetime of about 10 ps. The ultrafast bleaching process, monitored at 565-630 nm, may be an intermolecular proton transfer to an appropriately situated amino acid residue in the photoreceptor molecule. Alternatively, the ultrafast process may involve electron transfer. Recent studies in this laboratory have shown that stentorin and blepharismins are a strong electron donor. The 3-ps absorption transient observed may result from electron transfer. A series of time-resolved fluorescence spectra of stentorin, in collaboration with Yamazaki's laboratory at Hokkaido University, are consistent with electron transfer as a primary photoprocess in this molecule.[9]

Since blepharismin is structurally similar to stentorin, we expect that similar photochemistry occurs in blepharismin. An added proof is that the photophobic response in *Blepharisma japonicum* can be modulated by ammonium chloride, CCCP, and FCCP, as in *Stentor coeruleus*. Also, an intracellular pH decrease, upon light irradiation, was observed in *Blepharisma japonicum*.[10]

Signal Amplification. A cellular signal generated by the photoprocess of the photosensor molecules must be amplified. Figure 4 shows a schematic illustration of the light signal transduction chain in ciliate cell *Stentor*. A similar chain appears to operate in *Blepharisma*. We have shown a kinetic correlation between the light-induced membrane potentials and

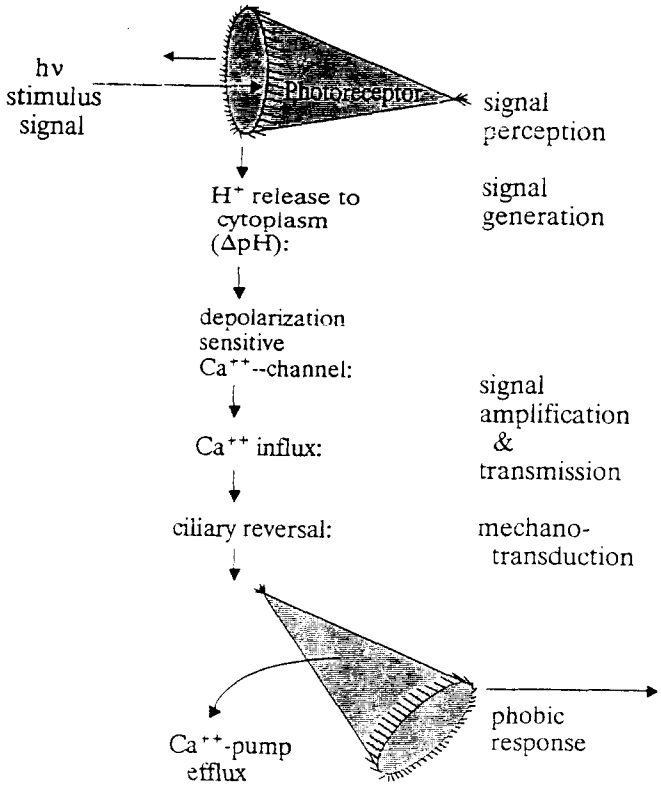


Figure 4. Tentative scheme for a step-up photophobic response in Stentor coeruleus. The light signal cascade is represented by four steps.

the photomotile responsed in both ciliates, resulting in light-triggered calcium ion influx (and the reversal of ciliary beat stroke). Every photon absorbed by stentorin in *Stentor coeruleus* results in influx of approximately 130 Ca^{+} ions. Thus, the signal amplification factor here is 3 to 4 orders magnitude smaller than that in rhodopsin-triggered signal amplifications in terms of number of Na^{+} ions(Na^{+}-channel closure)per photon absorbed. Low extracellular Ca^{2+} ion concentrations and Ca^{2+} ion channel blockers, such as diltiazem and pimozide, inhibit the photo-mechanoresponses of *Stentor* and *Blepharisma*.

The biochemical reactions underscoring the signal generation and amplification are poorly understood at the present time.

We are exploring two possible hypotheses: (1) The photoexcitation of stentorin resulting in a transient drop in intracellular pH may then be coupled to the activation of a transducin-like G-protein, or (2) a meta-stable stentorin activates the G-protein, followed by a transient drop in intracellular pH for the generation of the photoreceptor potential (Fig. 4). How either one of these, or an entirely unexplored mechanism, is coupled to the generation of an action potential resulting from the influx of Ca^{2+} ions is also unknown.

A cGMP-dependent phosphodiesterase is a possible signal amplifier in the photosensory signal cascade in *Stentor coeruleus* and *Blepharisam japonicum*. We observed that a G-protein-coupled phosphodiesterase is likely to be a signal amplifier in these ciliate cells. Experiments with phosphodiesterase inhibitors and activators also suggest that the cytosolic cGMP level and its modulation play an important role in the photosensory transduction chain involved in both the photophobic and phototactic responses.[11]

CONCLUSION AND FUTURE RESEARCH

A fundamental problem in photosensory biology is the unavailability of *in vitro* assay of the functional activities of photoreceptors, unlike enzymes and proteins with activities that can be readily assayed. Once the photoreceptor is separated from the photoreceptor assembly and decoupled from the signal cascade chain, its functional activity is lost, making it difficult to assay its activity. The photoreactivity *per se* of a photoreceptor, such as the well established photocycle for rhodopsin, is not necessarily a measure of its functional activity. Thus, the discovery of transducin and the modulation of its activation by rhodopsin represented a major step in establishing a functional assay of rhodopsin *in vitro*.[12] It appears that the light signal cascade in the photophobic responeses of *Stentor coeruleus* and *Blepharisma japonicum* also utilizes a G-protein. To explore the involvement of a G-protein in the photosensory transduction in the nonneural ciliate cells, we are carrying out the following studies.

(a) *Cloning of 36-and 39-kDa G_{α}-proteins*. The presence of G-proteins (36- and 39-kDa) from a membrane fraction of *Stentor* cell was confirmed by using and antiserum raised against bovine transducin's carboxyl-terminal decapeptide (KENLKDCGLF). The degree of amino acid sequence homology of G-protein α-subunits is 50% with respect to bovine transducin, and they have several highly conserved motifs, particularly those regions involved with GTP-binding and hydrolysis sites. The AS7 antibody raised against the transducin decapeptide reacts with not only transducin-α, but also with G_i-α from rat, which contains the sequence KNNLKDCGLF. Thus, there are two G-proteins from *Stentor* membrane fractions. The second G-protein sequence is more homologous to the rat G_i (67%) than to the transducin (56%). Full sequences of these G-proteins are being determined.

(b) *Primary structure and cloning of stentorin gene.*

To facilitate deduction of the primary structure of stentorin, it is necessary to clone the stentorin-2B gene for structure-function studies of stentorin. So far, we have been able to microsequence three peptide fragments of the N-blocked stentorin-2B.[2]

(c) *Stentorin:G-protein interactions*. Using a GTP photoaffinity label such as azidoanilido GTP, it should be possible to demonstrate a functional linkage for light/stentorin-induced GTP binding to the 36- and 39-kDa G-proteins, If confirmed, the stentorin:G-protein assembly represents a minimal molecular transducer in the early stage of the light signal transduction. Similar studies are warranted for the possible blepharismin:G-protein transducer assembly.

Acknowledgement — The work described in this lecture was in part supported by the U.S. Army Research Office (28748-LS-SM) and by U.S. P.H.S. NIH (NS14526). I wish to acknowledge the contributions of Drs. S. and H. Fabczak, N. Tao, and R. Dai, Ms. Susanne Metha-Keuthen as well as my collaborators Professors T. Cotton, F. Lenci, W. Struve and I. Yamazaki to the studies described.

REFERENCES

1. Song, P.-S. (1983) Protozoan and related photoreceptors: Molecular aspects. *Annu. Rev. Biophys. Bioengin.* **12**, 35-68.
2. Dai, R., T. Yamazaki, I. Yamazaki and P.-S. Song (1995) Initial spectroscopic characterization of the ciliate photoreceptor stentorin. *Biochim. Biophys. Acta*, submitted.
3. Tao, N. M. Orlando, J.-S. Hyon, M. Gross and P.-S. Song (1993) A new photoreceptor molecule from *Stentor coeruleus*. *J. Am. Chem. Soc.* **115**, 2526-2528.
4. Cameron, D. W. and A. G. Riches (1995) Synthesis of stentorin. *Tetrahedron Lett.*, in press.
5. Sommer, D., T. Wells, N. Tao and P.-S. Song (1995) *J. Phys. Chem.* To be submitted.
6. Wynn, J. L., J.-H. Kim, N. Tao, R. Dai, P-S. Song and T. M. Cotton (1995) Characterization of the photoreceptor of *Stentor coeruleus* by surface-enhanced resonance Ranman scattering spectroscopy. *J. Phys. Chem.* **99**, 2208-2213.
7. (a) Fabczak, H., S. Fabczak, P.-S. Song, G. Checcucci, F. Ghetti and F. Lenci (1993a) Photosensory transduction in ciliates. Role of intracellular pH and comparison between *Stentor coeruleus* and *Blepharisma japonicum*. *J. Photochem. Photobiol. Part B.* **21**, 47-52.
   (b) Fabczak, S., H. Fabczak, N. Tao and P.-S. Song (1993b) Photosensory transduction in ciliates. I. An analysis of light-induced electrical and motile responses in *Stentor coeruleus*. *Photochem. Photobiol.* **57**, 696-701.
   (c) Fabczak, S., H. Fabczak and P.-S. Song (1993c) Photosensory transduction in ciliates. III. The temporal relation between membrane potentials and photomotile responses in *Blepharisma japonicum*. *Photochem. Photobiol.* **57**, 872-876.
8. Savikhin, S., N. Tao, P.-S. Song and W. S. Struve (1993) Ultrafast pump-probe spectroscopy of the photoreceptor stentorins from the ciliate *Stentor coeruleus*. *J. Phys. Chem.* **97**, 12379-12386.
9. Song, P.-S., I.-H. Kim, S. Florell, N. Tamai, T. Yamazaki and I. Yamazaki (1990) Structure and function of the photoreceptor stentorins in *Stentor coeruleus*. II. Primary photoprocess and picosecond time-resolved fluorescence. *Biochim. Biophys.* Acta **1040**, 58-65.
10. Matsuoka, T., Y. Murakami, T. Furukohri, M. Ishida and K. Taneda (1992) Photoreceptor pigment in *Blepharisma*: H+ release from red pigment. *Photochem. Photobiol.* **56**, 399-402.
11. (a) Fabczak, H., P. B. Park, S. Fabczak and P.-S. Song (1993d) Photosensory transduction in ciliates. II. Possible role of G-protein and cGMP in *Stentor coeruleus*. *Photochem. Photobiol.* **57**-702-706.
    (b) Facbczak, H., N. Tao, S. Fabczak and P.-S. Song (1993) Photosensory transduction in ciliates. IV. Modulation of the photomovement response of *Blepharisma japonicum* by cGMP. *Photochem. Photobiol.* **57**, 889-892.
12. (a) Stryer, L. (1986) Cyclic GMP cascade of vision. *Annu. Rev. Neurosci.* **6**, 87-119.
    (b) Stryer, L. (1986) Molecular mechanism of visual transduction, In: *Membrane Proteins: Targeting and Transduction*: R. A. Welch Foundation Conf. on Chem. Research, Vol. 33, Houston, TX, pp. 291-300.