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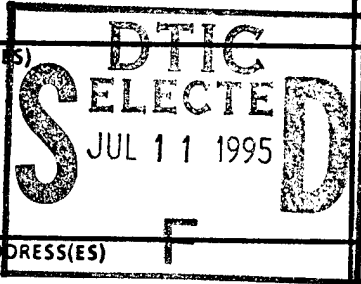
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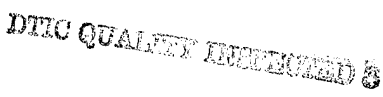
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13. ABSTRACT (Maximum 200 words)

The photosensing processes in *Stentor coeruleus* and *Blepharisma japonicum* can be described within the photo-signal transduction cascade shown above, with stentorin and blepharismismin serving as the photosensor molecules, respectively. In the photophobic responses of these ciliates, light signal (red wavelength light and intensity gradient) is perceived by the photosensor molecules localized in the pigment granule. A signal transduction then leads to the reversal of the direction of ciliary beating that is entailed in the stop-turning motion of the cell away from the source of light. In this paper we briefly describe the photosensory transduction which utilizes stentorin and blepharismismin as the photosensor molecules.



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## Statement of the Problem Studied:

Title: Photo-Signal Transduction in Motile Ciliate *Blepharisma*

The single cell ciliate, *Blepharisma japonicum*, is responsive to a sudden increase in light intensity and is capable of discriminating colors of different wavelengths ("color vision"). This remarkable single cell vision is mediated by the photosensor molecule called blepharismine. The objectives of the research conducted included:

- (1) Elucidation of the chemical structure of the photosensor molecule
- (2) Study of its photochemical mechanism of action
- (3) Exploration of the light signal transduction cascade triggered by the light excitation of the photosensor molecule

## Summary of the Most Important Results:

During the three year period of research supported by the Army Research Office grant, the following important results have been obtained to solve the problem outlined above:

- (1) The chemical structures of the two forms of the *Blepharisma* photosensor molecule, red and blue blepharismine, have been elucidated by means of UV-visible spectroscopy, NMR, FTIR and mass spectrometry. We have also determined the structure of stentorin, the photosensor molecule in the closely related ciliate *Stentor coeruleus*. Both blepharimins and stentorin represent a structurally unique class (hypericin derivatives) of the biological light receptor molecules that have been added to the select few photosensors that are known up to this point (i.e., rhodopsin, phytochrome, and blue light cryptochromes, based on retinal, tetrapyrrole and flavin as the chromophore molecules, respectively).

- (2) The primary photochemical mechanism of the ciliate photosensor molecules appears to be electron transfer coupled with proton release. This reaction occurs in a few picosecond time scale in both blepharismin and stentorin. The resulting intracellular pH change serves as an initial physiological signal.
  
- (3) The light signals in terms of intensity gradient and different wavelength colors are transduced and amplified in the cells via a transducin-like G-protein coupled to a cGMP-dependent phosphodiesterase. Activation of the phosphodiesterase enzyme depolarizes the cell membrane by opening the voltage-dependent calcium channels. The calcium influx results in a transient reversal in ciliary stroke, thus eliciting the phototile response in *Blepharisma japonicum* and *Stentor coeruleus*.

### List of all publications and technical reports published:

- (1) Renke Dai, Tomoko Yamazaki, Iwao Yamazaki and Pill-Soon Song: "Initial Spectroscopic Characterization of the Ciliate Photoreceptor Stentorin" *Biochimica et Biophysica Acta*, in press (1995).
- (2) S. Fabczak, H. Fabczak and P.-S. Song,  $Ca^{2+}$  Ions Mediate the Photophobic Response in *Blepharisma* and *Stentor*, *Acta Protozoologica*, **33**, 93-100 (1994).
- (3) N. Tao, L. Deforce, M. Romanowski, S. Meza-Keuthen, P.-S. Song and M. Furuya, *Stentor* and *Blepharisma* Photoreceptors: Structure and Function, *Acta Protozool.* **33**, 199-211 (1994).
- (4) N. Tao and P.-S. Song, New Light Sensor Molecules of Single-Cell Ciliates. *Smart Structure and Materials: SPIE Proceedings*, Vol. **2189**, 238-248 (1994)
- (5) H. Fabczak, P.B. Park, S. Fabczak and P.-S. Song, Photosensory Transduction in Ciliates. II. Role of G-Protein and cGMP in *Stentor coeruleus*. *Photochem. Photobiol.*, **57**, 702-706 (1993).
- (6) D. Gioffre, F. Ghetti, F. Lenci, C. Paradiso, R. Dai and P.-S. Song. Isolation and characterization of the presumed photoreceptor protein of *Blepharisma japonicum*. *Photochemistry and Photobiology*, **58**: 275-279 (1993).
- (7) T. Yamazaki, I. Yamazaki, Y. Nishimura, R. Dai and P.-S. Song. Time-resolved fluorescence spectroscopy and photolysis of the photo-receptor blepharimin. *Biochimica et Biophysica Acta*, **1143**: 319-326 (1993).
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- (10) S. Fabczak, H. Fabczak, N. Tao and P.-S. Song. Photosensory transduction in ciliates. I. An electrophysiological analysis of the photophobic response in *Stentor coeruleus*. *Photochemistry and Photobiology*, **57**: 696-701 (1993).
- (11) H. Fabczak, P.B. Park, S. Fabczak and P.-S. Song. Photosensory transduction in ciliates. II. Role of G-protein and cGMP in *Stentor coeruleus*. *Photochemistry and Photobiology*, **57**: 702-706 (1993).
- (12) S. Fabczak, H. Fabczak and P.-S. Song, Photosensory Transduction in Ciliates. III. The temporal Relation Between Membrane Potentials and Photomotile Response in *Blepharisma japonicum*. *Photochemistry and Photobiology*, **57**: 872-876 (1993).
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- (14) P.-S. Song, Structure and Function of the Ciliate Photoreceptors. In: *Frontiers of Photobiology* (Edited by A. Shima, M. Ichihashi, Y. Fujiwara and H. Takebe), International Congress Series 1021. Excerpta Medica, Amsterdam and New York, 1993. pp. 153-157.

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- (19) G. Checcucci, F. Lenci, F. Ghetti and P.-S. Song, A Videomicroscopic Study of Singlet Oxygen Quencher on *Blepharisma japonicum* Photobehavior. *J. Photochem. Photobiol., B.* **11**: 49-55 (1991).
- (20) D.L. Farrens and P.-S. Song, Subnanosecond Single Photon Time Measurements Using a Pulsed Diode-Laser. *Photochem. Photobiol.* **54**: 313-317 (1991).

### Participating Scientific personnel:

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Hanna Fabczak, Postdoctoral Fellow (1990-1992)  
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Elisabetta Bini, Predoctoral Trainee (1994-1995)  
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Phun Bum Park (Ph.D. degree expected 1995)  
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### Inventions:

No patents planned or pending