SF 298 MASTER COPY KEEP THIS COPY FOR REPRODUCTION PURPOSES

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB NO. 0704-0188*



| 1. AGENCY USE ONLY *(Leave blank)* | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | Feb 1, 1996 | Final Tech Report, 9/30/92-12/31/95 |

**4. TITLE AND SUBTITLE**
Photo-Signal Transduction in Motile Cilia Blepharisma

**5. FUNDING NUMBERS**
DAAL03-92-G0356

**6. AUTHOR(S)**
Pill-Soon Song

**7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)**
The Board of Regents of the Univ. of NE-Lincoln
303 Canfield Administration Building
Lincoln, NE 68588-0430

**8. PERFORMING ORGANIZATION REPORT NUMBER**
LWF/05-139-10402

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**
ARO 29597.4-LS-EPS

**11. SUPPLEMENTARY NOTES**
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release; distribution unlimited.

**12 b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200 words)*

*Blepharisma japonicum* and *Stentor coeruleus*, single cell protozoan ciliates, possess both light intensity- and wavelength-sensitivity. Their light sensor molecules, blepharismin and stentorin, respectively, contain hypericin-derived structures as unique photoreceptor chromophores. Under this grant, we recently elucidated the chemical structures of both blepharismin and stentorin. How these light sensor molecules mediate the intensity- and wavelength-sensitive light-sensory responses in these single cell organisms is still under investigation in this laboratory, results so far strongly suggest that both blepharismin and stentorin initiate their primary photoprocesses via electron transfer.



**14. SUBJECT TERMS**
Light-signal transduction, photosensor molecules, protozoan ciliates, Blepharisma japonicum, Stentor coeruleus

**15. NUMBER IF PAGES**
4

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OR REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| UNCLASSIFIED | UNCLASSIFIED | UNCLASSIFIED | UL |

# Photo-Signal Transduction in Motile Cilia Blepharisma

FINAL PROGRESS REPORT

Pill-Soon Song

U.S. ARMY RESEARCH OFFICE

Funding Number: DAAL03-92-G-0356

University of Nebraska-Lincoln
Department of Chemistry

REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

Specific aims of the study were to elucidate: (a) the chemical structures of light-sensor molecules blepharismin and stentorin, and (b) their mechanisms of action in light-sensory signal processing events, namely, detection of sudden changes in light intensity and light-avoiding behaviors of the single cell ciliate *Blempharisma japonicum* and its closely related organism *Stentor coeruleus*.

During the period of this grant, two significant results have been achieved. For the first time, we have been able to isolate and purify the photosensor-bound protein, stentorin, with molecular weight of 55,000. This light-sensor protein retains the functional characteristics of native stentorin protein complex of more than half a million molecular weight located within the pigment granules of *Stentor coeruleus*. In addition, we have been able to determine a tentative chemical structure for the light sensor chromophore of *Blepharisma japonicum*. It appears that the chemical structure of blepharismin is distinctly different from that of stentorin. Both stentorin and blepharismin add to the exclusive list of only a limited number (four to five) of light sensor molecules in nature.

Light signals perceived by the single cell ciliates are transduced by transducin-like G-proteins, as suggested by our study of the effects of various G-protein activators and inhibitors on the photo-sensory responses of both organisms. To characterize the G-proteins in the ciliate cells, Phun Bum Park and Elisabetta Bini supported by this grant partially cloned and sequenced Blepharisma and Stentor G-proteins. Photo-activation of G-proteins appears to be coupled to a cGMP-dependent phosphodiesterase. If this result is further confirmed by directly isolating and/or cloning the latter and establishing its light activation via G-proteins, the single cell photo-signal transduction system will provide an interesting comparison to the visual excitation system in mammals.

---

(1) LIST OF MANUSCRIPTS published under ARO sponsorship during this grant period (includes publications arising from both DAAL03-92-G-0356 and 28748-LS-SM with identical project title):


1. N. Tao, M. Orlando, J.-S. Hyon, M. Gross and **Pill-Soon Song**, A New Photoreceptor Molecule from *Stentor coeruleus*. J. Am. Chem. Soc. **115**, 2526-2528 (1993).

2. **Pill-Soon 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.

3. I. Yamazaki, T. Yamazaki, Y. Nishimura, R. Dai and **Pill-Soon Song**, Time-Resolved Fluorescence Spectroscopy and Photolysis of the Photoreceptor Blepharismin. Biochim. Biophys. Acta **1143**, 319-326 (1993).

4. T. Yamazaki, N. Ohta, Y. Nishimura, I. Yamazaki and **Pill-Soon Song**, Excited State Properties of Hypericin: Electronic Spectra and Fluorescence Decay Kinetics. J. Phys. Chem. **97**, 7870-7875 (1993).

5. S. Fabczak, H. Fabczak, N. Tao and **Pill-Soon Song**, Photosensory Transduction in Ciliates. I. An Electrophysiological Analysis of the Photophobic Response in *Stentor coeruleus*. Photochem. Photobiol., **57**, 696-701 (1993).

6. H. Fabczak, P.B. Park, S. Fabczak and **Pill-Soon Song**, Photosensory Transduction in Ciliates. II. Role of G-Protein and cGMP in *Stentor coeruleus*. Photochem. Photobiol., **57**, 702-706 (1993).

7. S. Fabczak, H. Fabczak and **Pill-Soon Song**, Photosensory Transduction in Ciliates. III. The temporal Relation Between Membrane Potentials and Photomotile Response in *Blepharisma japonicum*. Photochem. Photobiol., **57**, 872-876 (1993).

8. H. Fabczak, N. Tao, S. Fabczak and **Pill-Soon Song**, Photosensory Transduction in Ciliates. IV. Modulation of the Photomovement Response of *Blepharisma japonicum* by cGMP. Photochem. Photobiol., **57**, 889-892 (1993).

9. H. Fabczak, S. Fabczak, **Pill-Soon Song**, G. Checcucci, F. Ghetti and F. Lenci, Photosensory Transduction in Ciliates. Role of Intracellular ΔpH and Comparison Between *Stentor coeruleus* and *Blepharisma japonicum*. J. Photochem. Photobiol.,Part B., **21**, 47-52 (1993).

10. S. Savikhin, N. Tao, **Pill-Soon Song** and W.S. Struve, Ultrafast Pump-Probe Spectroscopy of the Photoreceptor Stentorins from the Ciliate *Stentor coeruleus*. J. Phys. Chem., **97**, 12379-12386 (1993).

11. **Pill-Soon Song**, Light Signal Transduction in *Stentor coeruleus*. In: Recent Advances in Photosciences (Edited by M. Yoon and P.-S. Song), Chungnam National Univ., Taejon, Korea pp. 143-157.

12. N. Tao and **Pill-Soon Song**, New Light Sensor Molecules of Single-Cell Ciliates. Smart Structure and Materials: SPIE Proceedings, Vol. **2189**, 238-248 (1994)

13. S. Fabczak, H. Fabczak and **Pill-Soon Song**, $Ca^{2+}$ Ions Mediate the Photophobic Response in *Blepharisma* and *Stentor*, Acta Protozoologica, **33**, 93-100 (1994).

14. N. Tao, L. Deforce, M. Romanowski, S. Meza-Keuthen, **Pill-Soon Song,** and M. Furuya, *Stentor* and *Belpharisma* Photoreceptors: Structure and Function, Acta Protozool. **33**, 199-211 (1994).

15. R. Dai, T. Yamazaki, I. Yamazaki and **Pill-Soon Song**. Initial spectroscopic characterization of the ciliate photoreceptor stentorin. Biochim. Biophys. Acta **1231**: 58-68 (1995).

16. J. L. Wynn, J.-H. Kim, N. Tao, R. Dai, **Pill-Soon Song**, and T. M. Cotton. Characterization of the photoreceptor of *Stentor coeruleus* by surface-enhanced resonance Raman scattering spectroscopy. J. Phys. Chem. **99:** 2208-2213 (1995).