OFFICE OF DIRECTOR

Director-General: *Hideo Mohri*
Associate Professors: *Shigeru Itoh*
Ryuji Kodama
Kohji Ueno
Research Associate: *Kaoru Ohno*
Hiroki Kokubo (on leave)

Phylogeny of outer arm dynein in flagella and cilia

Hideo Mohri

Dynein is a motor protein essential for microtubule-dependent cell motility. In axonemes of flagella and cilia, outer arm and inner arm dyneins attach to outer doublet microtubules. The isolated outer arm dynein molecule is a three-headed bouquet in protists (e.g. *Chlamydomonas* and *Tetrahymena),* whereas the molecule is two-headed in multicellular animals (e.g. Deuterostomia and Protostomia). The number of heads corresponds to the number of heavy chain subunits. Alpha, β and γ heavy chains compose the outer arm in protists and α and β heavy chains are present in the outer arm of multicellular animals. We examined the outer arm dynein isolated from sperm flagella of a sea anemone, a species located just prior to the divergence of the two main branches in the animal kingdom phylogenic tree. The outer arm was the two-headed molecule typical of multicellular animals. Fig.1 shows schematic drawings of both animal (metazoan) and protist (and some lower plant) flagella and cilia with special reference to the outer arms. Since molecular phylogenetic analyses of dynein heavy chains indicate that animal α and β heavy chains are related to protist γ and β heavy chains, respectively, topographic relationship among these heavy chains in the cross-sections are also indicated in the figure. Based on the phylogenetic tree of dynein genes, *Chlamydomonas* α and β appear to be produced by gene duplication. Combined with other phylogenetic analyses, it is plausible that there was (or is) the organism more ancient than *Chlamydomonas* and *Tetrahymena*, which has two-headed outer arm dynein and consequently, the third heavy chain acquired in protists may have been lost during the evolution of animals from their ancestral unicellular organisms.

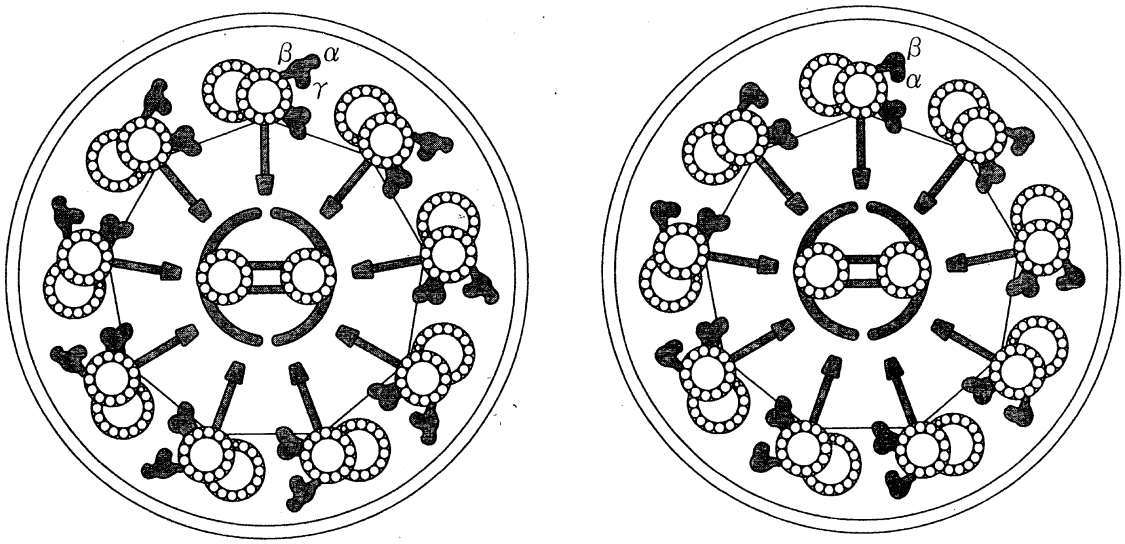

Fig.1. Schematic cross-sections of flagella or cilia in protists and lower plants (left) and in animals (right). Positions of each heavy chain subunit (head) are also shown.

Molecular evolution of photosynthesis.

Shigeru Itoh

We study the molecular evolution of photosynthesis through physicochemical research of various types of photosynthetic organisms. Oxygen-evolving photosynthesis of cyanobacteria seems to be evolved from the anoxygenic bacterial photosynthesis at 2.7-3.5 billion years ago just after the evolution of life. Symbiosis of cyanobateria inside the eukariotic cells, then, produced the first plant 2 billion years ago. The process of evolution from anoxygenic to oxygenic photosynthesis has been our main target of research.

(1)Discovery of anomalous photosynthesis.

Acaryochloris marina, a newly discovered oxygen-evolving cyanobacteria-like unicellular organism, was found to use a far-red absorbing new pigment chlorophyll *d* in its photosystem I reaction center pigment-protein complex in collaboration with Marine Biotechnology Institute Kamaishi (Fig.2). Although chlorophyll *a* that absorbs visible red light has been known to be indispensable for the oxygenic photosynthesis of all the plants and cyanobacteria, *A. marina* efficiently undergoes oxygenic photosynthesis even with far-red light of lower quantum energy compared to the red light absorbed by chlorophyll *a*. We named the special pair chlorophyll *d* of the newly identified photosystem I reaction center *P740*. The organism can be a missing link between the anoxygenic photosynthesis that uses 800-860 nm far-red light and the oxygenic photosynthesis that uses 650-700 nm red light.

Photosynthesis in a newly discovered bacterium *Acidiphilium rubrum* isolated from acidic mine drainage, was also shown to be quite different from the photosynthesis in all the ever-known oxygenic and anoxygenic photosynthesis. *A. rubrum* uses Zn-containing bacteriochlorophyll in its anoxygenic photosynthesis. This was the first case of photosynthesis based on pigments other than chlorophylls that are Mg-containing pigments. These new organisms show the wide variability of photosynthesis.

(2) Evolution of optimization mechanism of photosynthesis.

Structure-function relationships of the photosynthetic light reaction in the newly found organisms as well as plants and in anaerobic green sulfur bacteria *Chlorobium* and *Heliobacteria*, are studied by modern physicochemical techniques. We modified the reaction center complexes of these organisms by replacing quinone (vitamin K1) or chlorophyll cofactors by the artificial compounds or by site-directed mutagenesis, and studied the ultra-fast reaction kinetics by picosecond laser fluorometry, spectroscopy and spin-echo pulse ESR spectroscopy at 4 -280 K. Molecular architectures of plant and bacterial photosynthetic reaction center proteins are shown to be highly optimized in utilization of solar energy, however, in somewhat different direction in each case. The design of ancestral photosynthetic apparatus that is now lost, is to be traced based on new findings.

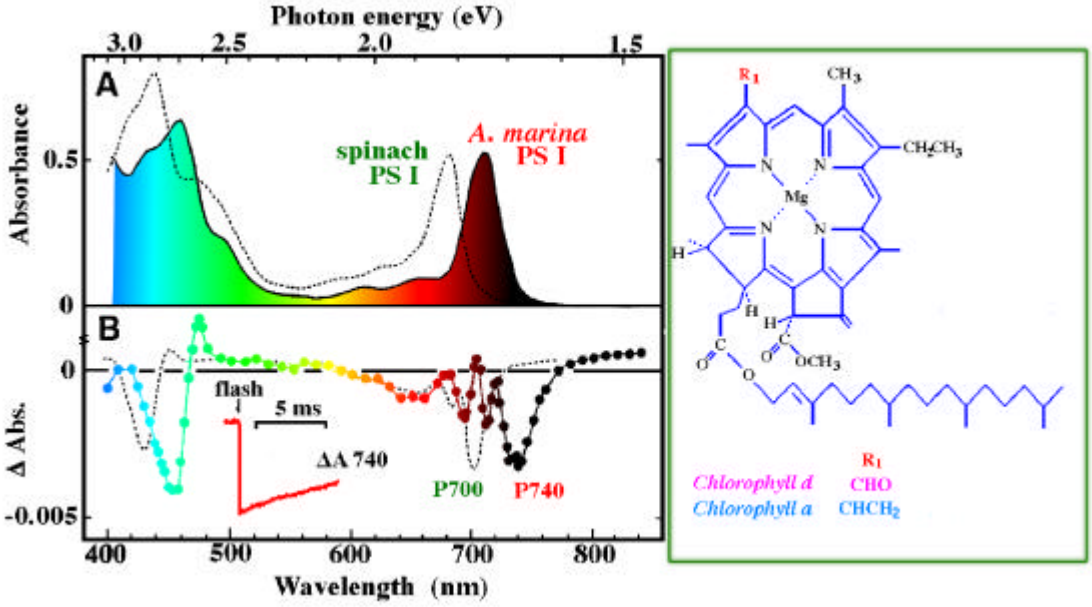

Fig.2. Absorption spectrum (A) and light-induced difference absorption spectrum of reaction center chlorophyll P740(B) of newly identified photosystem I (PS I) reaction center complex of *Acaryochloris marina* that has far-red absorbing chlorophyll *d*. Broken lines in (A) and (B) represent absorption and difference spectra (P700) in plant (spinach) PS I reaction center containing chlorophyll *a*, respectively. Molecular structures of chlorophylls *a* and *d* are also shown.

Mechanisms determining the outline shape of the adult lepidopteran wings

Ryuji Kodama

Wings of the lepidopteran insects (butterflies and moths) develop from the wing imaginal disc, which is a hollow sac made of simple epithelium. When the pupariation is completed, the wing, which was hidden inside the body wall of the larvae, is exposed on the surface of the pupa, which gradually turns into the adult wing. The outline shape of the adult wing is often different from that of the pupal wing. This difference is brought about by the programmed cell death of the marginal area of the pupal wing, while the internal area develops as adult wing blade. The marginal dying area is called the degeneration region and the internal area is called the differentiation region, hereafter.

The cell deaths in the degeneration region proceeds very rapidly and completes in a half to one day period in *Pieris rapae* or several other species examined. It was shown that the dying cells in the regeneration region have two characteristics common with the apoptotic cell death in mammalian cells. These are i) the presence of apoptotic bodies, which are heavily condensed cells or their fragments engulfed by other cells or macrophages, shown by transmission electron microscopy and ii) the presence of conspicuous accumulation of fragmented DNA evidenced by the TUNEL histological staining (Kodama, R. et al., Roux's Arch. Dev. Biol. 204, 418-426, 1995).

The cells in the degeneration region are actively engulfed by the macrophages in the cavity beneath the wing epithelium. Moreover, the macrophages seem to be concentrated beneath the degeneration region by the strong adhesion between basal surfaces of the dorsal and ventral epithelium in the differentiation region. By injecting the india ink or ferritin solution to the body cavity of the pupa, we have confirmed that this adhesion is tight enough to exclude the macrophages from the differentiation region, because the injected probes was found mostly concentrated in the degeneration region when observed several minutes later (Yoshida, A. (Biohistory Research Hall) and Kodama, R., unpublished).

Studies using another lepidopteren species, *Orgyia recens approximans*, provided by Drs. Y. Arita and K. Yamada (Meijo University) is underway . In this species, the wing is normally formed until the beginning of the pupal period, but becomes conspicuously degenerated only in the female adult. In our preliminary study, it was shown that the pupal wing is normally formed both in male and female pupa, but after about two days, female pupal wing starts degeneration on its margin, as if the degeneration region is continuously formed deep into the center of the wing (Kodama, R. et al., unpublished). It is thus suggested that the control mechanism which demarcates the region to be degenerated is defective in the female in this species. Further investigation using this species might give important insight into such mechanisms.

Another collaborative work with the laboratory of Dr. K. Watanabe (Hiroshima University) concerns mostly on the development of trachea and tracheole pattern in the swallow tail butterflies. Trachea and trcheoles are both important in delivering air into the wing and their pattern coincide with that of the boundary of degeneration and differentiation zones at the distal end of the wing. According to the observations, the pattern formation of wing epithelium is often dependent on tracheal and tracheole patterns. Basic research on the development of tracheal pattern formation is being done through the scanning electron microscopy and the bright field light microscopy of the fixed or fresh specimens to describe the exact pathway and the time course of the formation of elaborate pattern of trachea and tracheoles and to establish the cytological and developmental relationship between the formation of tracheal pattern and epithelial cell pattern, such as scale cell pattern. The Fig.3 depicts how the tracheoles protrude from the primary trachea at the pre-pupa stage. These fine threads are arranged with even spaces and may closely related with the scale cell pattern formation.

Fig.3. The tracheoles (fine threads) and the primary trachea (thick tube in the center) at the late stage of the pre-pupa.

Protein palmitoylation and developmental mechanism at embryogenesis in invertebrate and vertebrate

Kohji Ueno

We have studied the molecular mechanisms of the development of cells and organs in the silkworm *Bombyx mori* and have elucidated that the abdominal leg development was regulated by a homeotic gene which specifies the identities of abdominal segments. We have found that a high molecular weight protein (p260/270) was expressed in abdominal leg cells during early embryonic stages. p260/270 was identified to be a protein palmitoylase which transfers palmitate to cysteine residues of proteins. Almost of small GTP-binding, heterotrimeric G, and G-protein-linked receptor proteins are known to be modified with palmitate through thioester linkages. These dynamic modifications thought to be important in regulation of signal transduction. Therefore we speculated that p260/270 may be involved in regulation of signal transduction and may function in abdominal leg development.

To better understand the molecular mechanism how the modification of protein palmitoylation regulates the development of cells and organs, a search for a homolog of p260/270 in vertebrate was undertaken. Homology search of an ESTdb (Expressed Sequence Tags data base) with the amino acid sequences of p260 and p270 identified mouse embryonic cDNA clones which were highly homologous to the amino acid sequences of p260 and p270. This result suggested that a homolog of p260/270 was expressed in mouse embryos. *In situ* hybridization of mouse embryos revealed that the transcripts are detected mainly in the central and peripheral nervous system in mouse embryos from embryonic day 11.5. The transcripts were detected in the regions of forebrain, midbrain, hindbrain and spinal cord in the central nervous system and also detected in the cranial ganglia and dorsal root ganglia in the peripheral nervous systems. Immunocytochemical analyses of cultured mouse primary embryonic brain cells were performed to identify which cells express mouse p260/270 homolog. This analysis revealed that mouse homolog was expressed specifically in neural cells, but not in neuroepithelial and glial cells. Fig.4 showed a result of immunocytochemical analysis of primary cultured cells. From these results, we speculate that a protein palmitoylase may function in the development of neural cells in the central and peripheral nervous system during mouse embryogenesis.

We found that protein palmitoylase were expressed specifically in a few types of cells during early embryogenesis in invertebrate and vertebrate. Further study is necessary to elucidate the common mechanism how the modification of protein palmitoylation regulates the development of the cells in invertebrate and vertebrate.

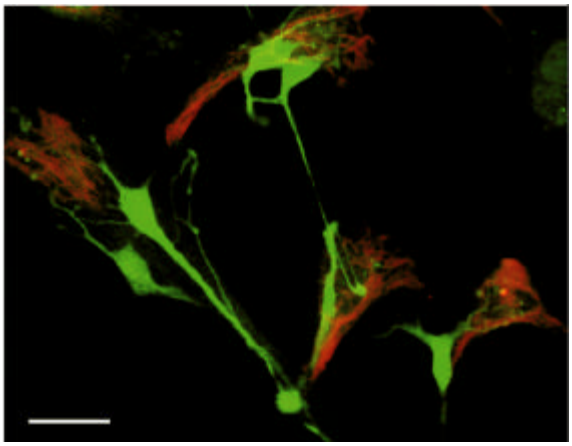

Fig.4. Immunocytochemical analysis of cultured primary cells. Cells were double labeled with an antibody against mouse p260/270 homolog and anti-nestin antibody. Mouse homolog (green) was detected in neurons whereas nestin (red) was detected in neuroepithelial cells. Bar represents 25μm.

Publication Lists:

Akiyama, M., Kobayashi, M., Kise, H., Takaichi, S., Watanabe, T., Shimada, K., Iwaki, M., Itoh, S., Ishida, N., Koizumi, M., Kano, H., Wakao, N. and Hiraishi, A. (1999) *Acidiphilium rubrum* and Zinc-bacteriochlorophyll part I: Molecular structure of the zinc-containing bacteriochlorophyll. In *"Photosynthesis:Mechanism and Effects"* (ed. Garab, G.), pp. 731-734. Kluwer.

Hu.Q., Ishikawa, T., Inoue, Y., Iwasaki, I., Miyashita, H., Kurano, N., Miyachi, S., Iwaki, M. and Itoh, S. (1999) Heterogeneity of chlorophyll *d*-binding photosystem I reaction centers from the photosynthetic prokaryote *Acaryochloris marina.* In *"Photosynthesis: Mechanism and Effects"* (ed. Garab,G.), pp. 437-440. Kluwer.

Itoh, S., Iwaki, M., Hu, Q., Miyashita, H. and Miyachi, S. (1999) A novel photosystem type reaction center from oxygenic prokaryote *Acaryochloris marina* that contains chlorophyll *d*. In "*The Photosynthetic prokaryotes"* (eds. Peschek et al.), pp. 183-186. Kluwer.

Itoh, S., Iwaki, M., Hu, Q., Miyashita, H., Iwasaki, I., Kurano, N., Miyachi, S., Kobayashi, M., and Shimada, K. (1999) New types of reaction centers using chlorophyll-*d* and Zn-bacteriochlorophyll *a*. In *"Photosynthesis: Mechanism and Effects"* (ed. Garab, G.), pp. 521-526. Kluwer

Iwaki, M., Itoh, S., Kamei, S., Matsubara, H. and Oh-oka, H. (1999) Time-resolved spectroscopy of chlorophyll-a like electron acceptor in the reaction center complex of the green sulfur bacterium *Chlorobium tepidum*. *Plant and Cell Physiol.*, **40**, 1021-1028.

Kubo-Irie, M., Ohta, K., Meguro, T., Nakazawa, T. and Mohri, H. (1999) Morphological evidence that spermatogenic cells undergo apoptosis within testis of diapausing swallowtail pupa (*Atropheneura alcinous*). *J. Exp. Zool*.**283**, 194-201.

Matsunami, K., Kokubo, H., Ohno, K., Xu, P.-x., Ueno K. and Suzuki, Y. (1999) Embryonic silk gland development in *Bombyx*: molecular cloning and expression of the *Bombyx trachealess* gene. Dev. Genes Evol. **209**, 507 – 514.

Mohri, H. (1999) Tubulin 30 Years Later. *Cell Struct. Funct.* **24**, 265-267.

Mohri, H., Inaba, K., Kubo-Irie, M., Takai, H. and Yano-Toyoshima, Y. (1999) Characterization of outer arm dynein in sea anemone, *Anthopleura midori*. *Cell Motil. Cytoskel*. **44**, 202-208.

Noguchi, T., Kamimura, Y., Inoue, Y. and Itoh, S. (1999) Photoconversion of a water-soluble chlorophyll protein from *Chenopodium Album*: Resonance Raman and FTIR study of protein and pigment structures. *Plant and Cell Physiol.*, **40**, 305-310.

Oh-oka, H. Iwaki, M., and Itoh, S. (1999) The tight coupling of quinol oxidoreductase to the P840 reaction center complex in *Chlorobium*. In "*Photosynthesis:Mechanism and Effects*" (ed. Garab, G.), pp. 1525-1528. Kluwer.

Shimada, K., Itoh, S., Iwaki, M., Nagashima, K. V. P., Matsuura, K. and Kobayashi, M., Wakao, N. (1999) Reaction center complex based on Zn-bacteriochlorophyll from *Acidiphilium rubrum.* In *"Photosynthesis: Mechanism and Effects"* (ed. Garab G.), pp. 909-912. Kluwer.

Wakao, N., Hiraishi, A., Shimada, K., Kobayashi, M., Takaichi, S., Iwaki, M. & Itoh, S. (1999) Discovery, characteristics, and distribution of Zn-BChl in aerobic acidophilic bacteria including *Acidiphilium* species and other related acidophilic bacteria. In " *The Photosynthetic prokaryotes*" (eds. Peschek et al.), pp. 745-750. Kluwer.

Yoshii, T., Hara, H., Kawamori, A., Akabori K., Iwaki, M. & Itoh, S. (1999) ESEEM study of the location of spin-polarized chlorophyll-quinone radical pair in membrane-oriented spinach photosystems I and II complexes. *Appl. Magn. Reson.* **16**, 565-580.