NATIONAL INSTITUTE FOR BASIC BIOLOGY

岡崎国立共同研究機構

基　礎　生　物　学　研　究　所

ANNUAL REPORT

1998

The cover photograph shows the flower variegation of the common morning glory caused by excision of the transposable element *Tip100* from the *CHS-D* gene for flower pigmentation. (See Division of Gene Expression and Regulation I)

Post Doctral Fellow

1 NIBB Research Fellow
2 JSPS Postdoctral Fellow
3 JSPS Reserch Associate
4 JST Fellow

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INTRODUCTION

We present here the 1998 Annual Report of the National Institute for Basic Biology (NIBB). NIBB, a government-supported, basic research institute, was established in 1977. It aims to stimulate and promote the biological sciences by conducting first-rate research on site as well as by cooperating with other universities and research organizations. The Institute concentrates on the study of cellular functions, reproduction and development, neuronal and environmental control, gene expression and regulation, and molecular evolution of eukaryotic organisms.

In 1998, the Center for Transgenic Animals and Plants was established. Dr. E. Watanabe was appointed as an Associate Professor of the Center in September. At the end of March, Professor Masayuki Yamamoto (University of Tokyo) of the Adjunct Division of Cell Proliferation and Professor Masatoshi Takeichi (Kyoto University) of the Adjunct Division of Behavior and Neurobiology successfully completed their terms at NIBB. In turn, Professor Motoya Katsuki of the University of Tokyo, Professor Mitsumasa Wada of the Tokyo Metropolitan University and Professor Fujio Murakami of the Osaka University were respectively appointed as Adjunct Professors in the Division of Cell Proliferation, the Division of Cellular Regulation and the Division of Behavior and Neurobiology in April. In September, Professor Ken-ichirou Morohashi of the Division of Speciation Mechanisms II moved to the Division of Cell Differentiation. Drs. Y. Ozeki and M. Nakafuku were appointed as Associate Professors of the Adjunct Division of Cell Proliferation and the Adjunct Division of Behavior and Neurobiology, respectively. The positions were vacated when Adjunct Associate Professors M. Maejima and H. Kobayashi departed the NIBB. We have also recently appointed 6 research associates, 8 institute research fellows and 1 technician to replace 7 research associates, 9 institute research fellows and 2 technicians who moved to other positions (e.g. associate professors, senior scientists, research associates, etc.) either within or outside of NIBB. In addition numerous postdoctoral fellows are actively engaging in various research at NIBB. The activities and future goals of NIBB, especially in the field of plant science, were subjected to an external peer review.

NIBB plays an important role as a national and international center for biological research and is responsible for conducting research projects in cooperation with various research groups. As a part of such cooperative activities, NIBB carried out a Special Program which is currently focused on "Adaptation and Resistance to the Environment." In support of this program, NIBB held the 40th NIBB Conference entitled "Stress Responses. Sensing, Signal Transduction and Gene Expression" (Professor Norio Murata, organizer). The 41st NIBB Conference was an International Symposium sponsored by the Ministry of Education, Science, Sports and Culture and entitled "Frontier of the Genome Biology of *Escherichia coli*" (Professor Takashi Horiuchi, organizer). In August, the Director-Genelas of NIBB and the Biological Research Center of the Hungarian Academy of Sciences signed a "Memorandum of Understanding between the NIBB and the Biological Research Center of the Hungarian Academy of Sciences" to promote international cooperation between the two institutions. A similar agreement between NIBB and the Australian National University allowed the Australia-Japan Binational Seminar entitled "Molecular Physiology of Photosynthesis in Stress Environments" (Professors C. Barry Osmond and N. Murata, organizers) to be held in 1998. In addition, NIBB continues to sponsor interdisciplinary symposia and study meetings on current topics by inviting leading scientists from around the world to the institute. NIBB also provided a training course in biological sciences for young investigators. To continue our improvement welcome any suggestions concerning the research activities of NIBB.

H. Mohri

Finally, I would like to congratulate Drs. S. Itoh, H. Shibuya and M. Hayashi for being awarded the Plant and Cell Physiology Award, the Young Investigator Award of the Japanese Biochemical Society and the Botanical Awards for Young Scientists, respectively. Mr. Y. Tomoyasu received the Nagakura Award. Following Drs. T. Kinoshita and J. Yuasa, he is the third graduate student from NIBB to win the Award.

Hideo Mohri, D.Sc.
Director-General

ORGANIZATION OF THE INSTITUTE

The National Institute for Basic Biology, NIBB, is a part of the Okazaki National Research Institutes (ONRI) located on a hill overlooking the old town of Okazaki. The NIBB was established in 1977 and its activities are supported by Monbu-sho (the Ministry of Education, Science, Sports and Culture) of Japan. The ONRI are composed of three independent organizations, National Institute for Basic Biology (NIBB), National Institute for Physiological Sciences (NIPS) and Institute for Molecular Science (IMS).

Policy and Decision Making

The Director-General oversees the operation of the Institute assisted by two advisory bodies, the Advisory Council and Steering Council. The Advisory Council is made up of distinguished scholars representing various fields of science and culture, and advises the Director-General on the basic policy of the Institute. The Steering Council is made up of professors of the Institute and an equal number of leading biologists in Japan outside NIBB, and advises the Director-General on the scientific activities of the Institute. The Council advises on faculty appointments and on the Institute's annual budget as well as its future prospect.

Administration

Administration of the Institute is undertaken by the Administration Bureau of the Okazaki National Research Institutes under the direct auspices of the Ministry of Education, Science, Sports and Culture.

Research

The Institute conducts its research programs through three departments and one laboratory organized into 17 divisions.

Each division has its own research project and is staffed by a professor, an associate professor and two research associates. A division forms, in principle, an independent project team. Six of the divisions are for adjunct professorship and are under professors who hold joint appointments with other universities. The adjunct division has resident research associates. The arrangement aims to facilitate exchange in research activities in Japan. The Technical Department manages the activities of research techniques and helps to promote research activities of each division and also to maintain the research resources of the Institute. The Department also undertakes the technical education of its staff.

Research Support Facility

The research support facility of the NIBB consists of the Large Spectrograph Laboratory, Tissue and Cell Culture Laboratory, Computer Laboratory, Plant Culture Laboratory, Plant Cell Culture Laboratory, Experimental Farm, Laboratory of Stress-Resistant Plants and Center for Transgenic Animals and Plants. In addition, seven facilities are operated jointly with the NIPS; they consist of the Radioisotope Facility, Electron Microscope Center, Center for Analytical Instruments, Machine Shop, Laboratory Glassware Facility, Animal Care Facility, and Low-Temperature Facility.

Campus

The Okazaki National Research Institutes covers an area of 150,000 m^2 with four principal buildings. The NIBB's main research building has a floor space of 10,930 m^2. Two-thirds of the space was completed in 1982 and the remaining third in June, 1983. The buildings which house the research support facility were also completed in June, 1983. A building for Laboratory of Gene Expression and Regulation (2,577 m^2) was newly built in December, 1996.

Department/Laboratory	Divisions
Department of Cell Biology	Cell Mechanisms Bioenergetics Cell Proliferation (adjunct) Cell Fusion (adjunct) Cellular Communication (adjunct)
Department of Developmental Biology	Reproductive Biology Cell Differentiation Morphogenesis Developmental Biology (adjunct)
Department of Regulation Biology	Molecular Neurobiology Cellular Regulation Biological Regulation (adjunct) Behavior and Neurobiology (adjunct)
Laboratory of Gene Expression and Regulation	Gene Expression and Regulation I Gene Expression and Regulation II Speciation Mechanisms I Speciation Mechanisms II

GRADUATE PROGRAMS

The NIBB carries out two graduate programs.

1. Graduate University for Advanced Studies

The NIBB constitutes the Department of Molecular Biomechanics in the School of Life Science of the Graduate University for Advanced Studies. The University provides a three year Ph.D. course. Those who have completed a master's course in a university or are qualified at an equivalent or higher level are eligible to apply.

The Department consists of the following Divisions and Fields:

Divisions	Fields
Molecular Cell Biology	Biomolecular Systems Cell Dynamics
Developmental Gene Expression and Regulation	Gene Expression Morphogenesis Transgenic Biology
Regulation Biology	Biological Regulation Biological Information

2. Graduate Student Training Program

Graduate students enrolled in other universities and institutions are trained to conduct research for fixed periods of time under the supervision of NIBB professors.

OFFICE OF DIRECTOR

Director-General: *Hideo Mohri*
Associate Professors: *Shigeru Itoh*
Ryuji Kodama
Kohji Ueno
Research Associate: *Akinao Nose (- May 31, 1998)*
Masuo Goto (on leave)

Evolution of photosynthesis

Shigeru Itoh

We study the evolution of photosynthesis in molecular level. 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 cyanobacteria inside the eukariotic cells, then, produced the first plant 2 billion years ago. We recently focus our target of research on the evolutionary process from anoxygenic to oxygenic photosynthesis.

(1) Hunting for missing links of evolution "New photosynthesis".

A newly discovered bacterium *Acidiphilium rubrum* isolated from acidic mine drainage, was shown to use Zn-bacteriochlorophyll in its anoxygenic photosynthesis by us. This was the first case of photosynthesis based on pigments other than Mg-chlorophyll's that are ubiquitously used in the ever-known oxygenic and anoxygenic photosynthesis.

Acaryochloris marina, a newly discovered oxygen-evolving cyanobacteria-like organism, was shown to use a far-red absorbing new pigment chlorophyll-*d* in its photosystem I reaction center pigment protein complex in collaboration with a Marine Biotechnology Institute Kamaishi. Although chlorophyll *a* that absorbs visible red light has been known to be indispensable for the oxygenic photosynthesis of plants and cyanobacteria, this organism efficiently undergoes oxygenic photosynthesis even with far-red light that has lower quantum energy. We named the special pair chlorophyll-*d* of the newly identified photosystem I reaction center *P740* (Fig. 1). The organism can be a missing link between the anoxygenic photosynthesis that can use far-red light and the oxygenic photosynthesis that uses only visible-light. Molecular mechanisms and structures of the new photosynthetic systems are now being studied.

(2) Survey through optimization mechanism of photosynthesis.

We compared the structure-function relationships of the photosynthetic reaction center complexes of plants, green sulfur bacteria, *Heliobacteria* and newly found organisms. We replaced quinone cofactor molecules by artificial compounds inside the proteins and studied their localization by the ps-ns absorption, fluorescence and spin-echo ESR spectroscopy at 4-280 K. Comparison of structure-function correlation in various photosynthetic systems also revealed unique cytochrome reactions in Green-sulfur bacteria. Molecular architecture of plant and bacterial photosynthetic proteins are shown to be highly optimized but in different directions. This gave us a hint for the design of ancestral photosynthetic apparatus.

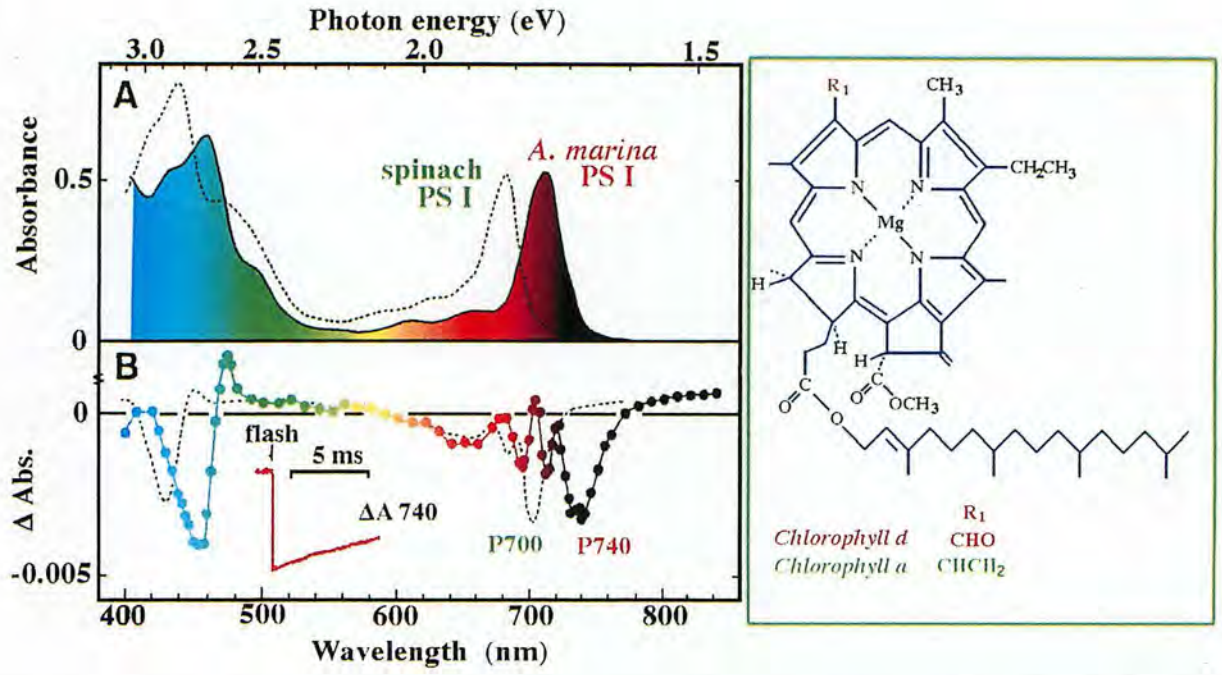

Fig.1. Absorption spectrum (A) and light-induced difference spectrum of reaction center chlorophyll-*d* P740(B) of newly identified photosystem I (PS I) complex of *Acaryochloris marina*. Broken lines in (A) and (B) represent absorption and difference spectra (P700) in spinach PS I reaction center containing chlorophyll-*a*, respectively. Molecular structures of chlorophylls 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 lepidopteran 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 butterfly. According to their 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 including SEM observation (Fig.2) and histological observations to provide the basis of the morphogenetic analysis of wing epithelium as a whole.

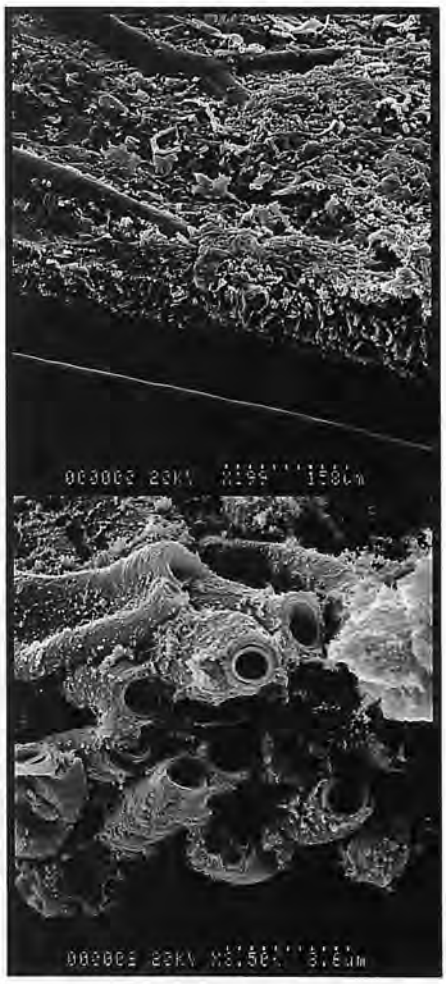

Fig.2. The inside of the larval wing disc of a swallow tail butterfly. The dorsal and the ventral epithelium of the disc were peeled apart with adhesive tape after critical-point-drying. The upper panel shows a low magnification view, whose lower right corner is shown in high magnification in the lower panel. Thick and smooth-surfaced tubes in the upper panel are trachea. A bundle of much thinner tubes are tracheoles, whose details are shown in the lower panel.

Protein palmitoylation and cell development at embryogenesis

Kohji Ueno

Thus far I have studied the molecular mechanisms of abdominal leg development in the silkworm *Bombyx mori*. From these studies, I have elucidated that abdominal leg development is regulated by a homeotic gene which specifies the identities of the abdominal segments. I have found that a high molecular weight protein, p260/270, is expressed in abdominal leg cells during early embryonic stages. The purified p260/270 was found to transfer palmitate to the cysteine residues of synthetic peptides *in vitro*. Most small, GTP-binding, heterotrimeric G, and G-protein-linked receptor proteins are known to be modified with palmitate through thioester linkages. Thus, these dynamic modifications may be important in the regulation of signal transduction. Therefore, I speculated that p260/270 may be involved in protein palmitoylation and may function in abdominal leg development (Ueno, K. and Suzuki, Y. J. Biol. Chem. 272: 13519-13526, 1997).

To understand the molecular mechanisms of how protein modification by palmitoylation regulates the development of cells and organs, I have attempted to find a vertebrate homologue of *Bombyx* p260/270. In order to accomplish this, I have searched an EST (Expressed Sequence Tag) data base for cDNAs encoding amino acid sequence residues corresponding to p260 and p270. From these searches, I found that several mouse embryonic cDNA clones were highly homologous to the amino acid sequences of p260 and p270. This suggested that a homologue of p260/270 was expressed in mouse embryos. One of these mouse p260/270 cDNAs was therefore used for *in situ* hybridization analyses of mouse embryos. Transcripts homologous to p260/270 were detected mainly in the central and peripheral nervous systems of mouse embryos from embryonic day 10 (E10). These transcripts were detected in the regions of the forebrain, midbrain, hindbrain and spinal cord of the central nervous system as well as in the cranial ganglia and dorsal root ganglia of the peripheral nervous system. The result of *in situ* hybridization on one mouse embryo section is shown in Fig.3. From these results, I speculate that a homologue of *Bombyx* p260/270 regulates development in the central and peripheral nervous system of mouse embryos. Further study is necessary to identify the specific cells which express these transcripts and understand the mechanisms of how this homologue regulates cell development.

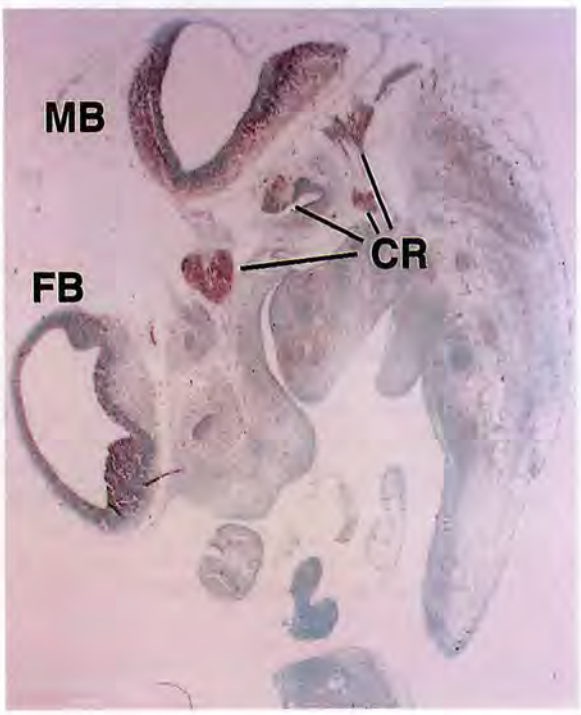

Fig.3. Localization of transcripts of a mouse homologue of p260/270 by *in situ* hybridization of a sagittal section of mouse E11.5 embryo. The transcripts are stained purple and nuclei are stained with methyl green. FB, forebrain; MB, midbrain; CG cranial ganglia.

Akinao Nose

How individual nerve cells find and recognize their targets during development is one of the central issues in modern biology. Our aim is to elucidate the molecular mechanism of axon guidance and target recognition by using the simple and highly accessible neuromuscular system of *Drosophila*.

The musculature of *Drosophila* embryos consists of 30 identifiable muscle fibers per hemisegment. Each muscle fiber is innervated by a few motoneurons in a highly stereotypic manner. The high degree of precision and previous cellular manipulations of neuromuscular connectivity suggest the presence of recognition molecules on the surface of specific muscle fibers which guide the growth cones of motoneurons. We have previously isolated several enhancer trap lines that express the reporter gene β-galactosidase (β-gal) in small subsets of muscle fibers prior to innervation. By molecularly characterizing these lines, we identified two genes, *connectin* and *capricious* that play roles neuromuscular connectivity.

I. connectin

Connectin is expressed on a subset of muscle fibers (primarily lateral muscles) and on the axons, growth cones of the motoneurons which innervate these muscles (primarily SNa motoneurons) and on several associated glial cells. When coupled with its ability to mediate homophilic cell adhesion in vitro, these results led to the suggestion that Connectin functions as an attractive signal for SNa pathfinding and targeting.

To study the role of *connectin* in vivo, we ectopically expressed Connectin on all muscles by using MHC (myosin heavy chain) promoter (*MHC-connectin*) in the P-element mediated transformants. In *MHC-connectin*, SNa nerves were observed to send

extra axon branches that form ectopic nerve endings on muscles 12, muscles they would never innervate in wild type. Furthermore, the ectopic innervation on muscle 12 was dependent on the *connectin* expression on SNa. These results showed that *connectin* functions as an attractive and homophilic guidance molecule for SNa in vivo.

II. *capricious*

capricious (*caps*) is expressed in subsets of neurons and muscles, including the RP5 motoneuron and its target, muscle 12. The cDNA cloning and sequencing revealed that caps encodes a novel transmembrane protein that like *connectin* belongs to LRR family. Within the LRR family, Caps protein was found to be most related to the product of the *Drosophila tartan* gene that have been implicated in neural and muscular development. We found that in the loss-of-function mutants of *caps*, the synaptic arborization pattern on muscle 12, a *caps*-positive muscle, was abnormal. The nerve terminal failed to stabilize on muscle 12, and instead extended to and arborized on the adjacent muscle, muscle 13. Ectopic expression of *caps* in all muscles by GAL4-UAS system also resulted in aberrant synapse formation on muscle 12. Like in the loss-of-function alleles, the muscle 12 terminal axon branch often formed collaterals that turned back and innervated muscle 13. These results suggest that Caps is involved in neuromuscular target recognition and/or stabilizaion of the synapses.

Analysis of meiosis

Masuo Goto

The major goal of our research is to elucidate regulatory mechanisms of meiosis. Meiosis is a crucial step in gamete formation and is essential for sexual reproduction. Meiotic steps are highly conserved among eukaryotic species. We have isolated and analyzed a number of cloned mouse genes which may be relevant to spermatogenesis. In addition, efforts have been also paid to elucidate the regulatory mechanisms of meiosis in fission yeast in more detail. Some examples of the analysis are presented below.

I. Regulators of meiosis II in fission yeast.

We analyzed the mechanisms of fission yeast meiosis using a meiosis II deficient mutant *mes1*. Even though the *mes1*$^+$ gene is essential to progress to meiosis II, its function has not yet been defined. To better understand the downstream pathway of *mes1* we decided to isolate a suppressor. We isolated our suppressor, *mes1* suppressor, from a *S. pombe* genomic library. The *mes1* mutation was suppressed by overexpression of the *slp1* gene. The *slp1* gene is relevant to spindle checkpoint in mitosis and radiation sensitivity. This gene is homologous to *S. cerevisiae CDC20*, but lacks the corresponding 35 amino acids C-terminal. The authentic *slp1*$^+$ gene encodes 488 amino acids, and its carboxyl region has significant homology to WD-repeat protein family. The C-terminal 35 amino acid portion includes a part of the seventh WD-repeat. In our experiments, full-length Slp1p could not suppress the mes1 defect. In addition, under nitrogen starvation, the *slp1* mutant cells produce mature asci containing one or two diploid spores. This indicates that the *slp1*$^+$ gene is essential in both meiosis I and meiosis II.

We determined that the physical interaction between Mes1p and Slp1p depends on the Slp1p C-terminal 35 amino acids. This physical interaction was prevented when an amino acid substitution occurred at aspartate-457 or tryptophan-463 in the seventh WD-repeat. The mutant Slp1p could suppress the *mes1* defect similarly to the truncated 35 C-terminal amino acids form. Therefore, the seventh WD-repeat of Slp1p is a key region for *mes1* suppression and binding to Mes1p. New insight into the crystal structure of G-protein heterotrimer and the G $\beta\gamma$ dimer revealed that G β is shaped like a seven-bladed propeller (seven WD-repeats) with a central shaft tunnel connecting the two faces. The seven WD-repeats of Slp1p could form a similar structure. Mutations in the seventh WD-repeat might destruct the propeller form since both the aspartate and the tryptophan residues are well conserved between WD-repeat protein families. This result indicates that the Slp1p may not be active in the seven propeller structure at the onset of meiosis II. In wild-type cells, the physical interaction between Mes1p and Slp1p is a critical step in activating Slp1p and overcoming the meiosis II checkpoint. This study implies that there are different cell cycle checkpoint mechanisms between mitosis and meiosis even though the same cell cycle regulators act at the appropriate steps.

II. Molecular cloning and characterization of mouse testis poly(A) binding protein II.

A cDNA clone from mouse testis cDNA library was isolated by a transcomplementation method using a *S. pombe* meiotic mutant (*sme2*) that arrests in the first meiotic division. The cDNA clone isolated has an open reading frame encoding 302 amino acids and has a strong similarity to mouse poly(A) binding protein II (mPABII) and bovine poly(A) binding protein II (PABII). PABII is known to bind the growing poly(A) tail. Northern blot analysis of the cDNA clone identified by mPABII revealed a single transcript of 1.2kb, detectable exclusively in adult testis. Immunohistochemical analysis using the polyclonal antibody demonstrated that mPABII protein was expressed in the nucleus at the specific stages from late pachytene spermatocytes to round spermatids. Genetic mapping showed the *Pabp3* gene encoding mPABII located near at position 19.5 on mouse chromosome 14. Although mPABII function is not clearly linked to spermatogenesis, these results suggest that mPABII might be involved in specific spermatogenetic cell differentiation.

Publication List:

Hara, H., Tang, J., Kawamori, A., Iwaki, M. and Itoh, S. (1998) Anomalous pulse-angle and phase dependence of Hahn's electron spin echo and multiple-quantum echoes of the spin correlated radical pair P700+A1- in Photosystem I. *Applied Magnetic Resonance* **14**, 367-379.

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Shishido, E., Takeichi, M. and Nose, A. (1998). Drosophila synapse formation: regulation by transmembrane protein with Leu-rich repeats, Capricious. *Science* **280**, 2118-2121.

Yoshida, A., Arita, Y., Sakamaki, Y., Watanabe, K. and Kodama, R. (1998) Transformation from the pupal to adult wing in *Oidaematophorus hirosakianus* (Lepidoptera: Pterophoridae). *Ann. Entomol. Soc. Am.* **91**, 892-857.

DEPARTMENT OF CELL BIOLOGY

Chairman: Mikio Nishimura

The department consists of two regular divisions and three adjunct divisions. One of the adjunct divisions started in 1998. The department conducts studies on molecular dynamics of the cell in higher plants and animals such as organelle differentiation, autophagy, cell motility, cytokinesis and neural development.

DIVISION OF CELL MECHANISMS

Professor:	*Mikio Nishimura*
Associate Professor:	*Ikuko Hara-Nishimura*
Research Associates:	*Makoto Hayashi*
	Tomoo Shimada
Post doctoral fellows:	*Tetsu Kinoshita*
	Akira Kato (~ Aug. 30)
	Nagako Hiraiwa (~ March 30)
	Yasuko Ishimaru
	Kanae Shirahama
	Shoji Mano
Graduate Students;	*Hiroshi Hayashi*
	Kenji Yamada
	Naoto Mitsuhashi
	Kazumasa Nito
	Yuki Tachibe
	(from Hiroshima University)
Technical Staffs:	*Maki Kondo*
	Katsushi Yamaguchi
JSPS Technical Staffs:	*Miwa Kuroyanagi*
	Kanako Toriyama (~ Nov. 30)
	Miki Kinoshita (April .1 ~)
	Chizuru Ueda (April .1 ~)
	Chihiro Nakamori (April .1 ~)
Visiting Scientists:	*Roland R. Theimer*
	(Bergische Univ. Germany)
	Yasuko Koumoto

Higher plant cells contain several distinct organelles that play vital roles in cellular physiology. During proliferation and differentiation of the cells, the organelles often undergo dynamic changes. The biogenesis of new organelles may occur, existing organelles may undergo a transformation of function, while other organelles may degenerate. Because the dynamic transformation of organellar function (differentiation of organelles) is responsible for flexibility of differentiation events in higher plant cells, the elucidation of regulatory mechanisms underlying organelle transformation are currently studied in this division.

I. Regulation at the level of protein transport to microbodies during the microbody transition.

Dramatic metabolic changes which underlie the shift from heterotrophic to autotrophic growth occur in greening of seed germination. Accompanying these metabolic changes, many constitutive organelles are functionally transformed. For example, etioplasts differentiate into chloroplasts and mitochondria acquire the ability to oxidize glycine. Glyoxysomes which are microbodies engaged in the degradation of reserve oil via β-oxidation and the glyoxylate cycle, are transformed into leaf peroxisomes that function in several crucial steps of photorespiration. After the functional transition of glyoxysomes to leaf peroxisomes during the greening of pumpkin cotyledons, the reverse microbody transition of leaf peroxisomes to glyoxysomes occurs during senescence. Microbody enzymes function after their transport to microbodies. Since the enzyme compositions and functions of glyoxysomes and leaf peroxisomes differ from each other, it is likely that the two types of microbodies possess different machineries for protein import.

Microbody proteins are synthesized in the cytosol on free polysomes and are transported post-translationally into microbodies. Two types of targeting signals to microbodies have been reported. One type of targeting signal is a part of the mature protein. One such signal, the tripeptide Ser-Lys-Leu, occurs at the C-terminal. has been identified as a targeting signal. Ser-Lys-Leu and related amino acid sequences commonly function in mammals, insects, fungi, and plants.

To characterize the microbody targeting signal in plants, we have examined the ability of 24 carboxy-terminal amino acid sequences to facilitate the transport of a bacterial protein, β-glucuronidase (GUS) into microbodies in green cotyledonary cells of transgenic Arabidopsis. Immunocytochemical analysis of the transgenic plants revealed that carboxy-terminal tripeptide sequences of the form [C/A/S/P]-[K/R]-[I/L/M] function as a microbody-targeting signal, while tripeptides with proline at the first amino acid position and isoleucine at the carboxyl terminus show weak targeting efficiencies. In contrast, another small group of microbody proteins are synthesized as precursors with N-terminal cleavable presequences; these include 3-ketoacyl-CoA thiolase, glyoxysomal citrate synthase (gCS), glyoxysomal malate dehydrogenase (gMDH) and long-chain acyl-CoA oxidase. These N-terminal sequences also function as a targeting signal to microbodies and are designated as PTS2. We generated a transgenic *Arabidopsis* plant that accumulated GUS-chimeric proteins with the N-terminal presequences of the pumpkin gCS or pumpkin gMDH. Immunocytochemical studies of the transgenic plant showed that the N-terminal sequences of gCS and gMDH function as targeting signals to plant microbodies. Site-directed mutagenesis studies of the chimeric proteins indicated that several amino acids in the consensus regions of the presequences were essential for targeting to microbodies or were necessary for the processing of the presequence. The fusion protein was transported into functionally different microbodies, such as glyoxysomes, leaf peroxisomes and unspecialized microbodies, and was subsequently processed. These observations indicated that the transport of gCS and gMDH is mediated by their amino-terminal presequences and that the transport system is functional in all plant microbodies. Therefore, it is unlikely that glyoxysomes and leaf peroxisomes posses different targeting machineries.

II. Microbody defective mutant of Arabidopsis.

It has been suggested that the functional conversion between glyoxysomes and leaf peroxisomes is controlled by gene expression, protein translocation, and protein degradation. A genetic approach is an effective strategy toward understanding the regulatory mechanism(s) of peroxisomal function at the level of

gene expression, protein translocation, and protein degradation. We isolated and characterized 2,4-dichlorophenoxybutyric acid (2,4-DB)-resistant mutants. It has been demonstrated previously that 2,4-dichlorophenoxybutyric acid (2,4-DB) is metabolized to produce a herbicide, 2,4-D, by the action of peroxisomal fatty acid β-oxidation in higher plants. To isolate mutants that have defects in peroxisomal fatty acid b-oxidation, we screened mutant lines of Arabidopsis seedlings for growth in the presence of toxic levels of 2,4-DB. Twelve of the mutants survived; of these, four required sucrose for post-germinative growth (Figure 1, A B). This result suggests that these mutants have defects in peroxisomal fatty acid β-oxidation, because peroxisomal fatty acid β-oxidation plays an important role in producing sucrose from storage lipids during germination. Genetic analysis revealed that these mutants can be classified as carrying alleles at three independent loci, which we designated *ped1*, *ped2*, and *ped3*, (where *ped* stands for peroxisome defective). The *ped1* mutant lacks the thiolase protein, an enzyme involved in fatty acid β-oxidation during germination and subsequent seedling growth, whereas the *ped2* mutant has a defect in the intracellular transport of thiolase from the cytosol to glyoxysomes. Etiolated cotyledons of both *ped1* (Figure 1D) and *ped2* mutants have glyoxysomes with abnormal morphology.

III. Transport of storage proteins to protein storage vacuoles is mediated by large PAC (precursor-accumulating) vesicles.

Novel vesicles that accumulate large amounts of proprotein precursors of storage proteins were purified from maturing pumpkin seeds. These vesicles were designated precursor-accumulating (PAC) vesicles and have diameters of 200 to 400 nm. We characterized them to answer the question of how seed protein precursors are accumulated in the vesicles to be delivered to protein storage vacuoles. They contain an electron-dense core of storage proteins surrounded by an electron-translucent layer, and some vesicles also contained small vesicle-like structures. An immunocytochemical analysis revealed numerous electron-dense aggregates of storage proteins within the endoplasmic reticulum (Figure 2). It is likely that these aggregates develop into the electron-dense cores of the PAC vesicles and then leave the endoplasmic reticulum. Immunocytochemical analysis also showed that complex glycans are associated with the peripheral region of PAC vesicles but not the electron-dense cores, indicating that Golgi-derived glycoproteins are incorporated into the PAC vesicles. These results suggest that the unique PAC vesicles might mediate a transport pathway for insoluble aggregates of storage proteins directly to protein storage vacuoles.

IV. Vacuolar processing enzymes in protein-storage vacuoles and lytic vacuoles.

Vacuolar processing enzymes (VPEs), which are responsible for maturation of various vacuolar proteins belong to a novel family of cysteine proteinases. Molecular characterization of castor bean VPE revealed that the latent precursor of VPE (proVPE) is converted into an active VPE by self-catalytic proteolysis. Thus, no other factor is necessary to produce active VPE, and VPE itself is a key enzyme in determining the final conformation of the vacuolar proteins. The VPE-mediated system is widely distributed in various plant organs. The temporal and spatial expression of the VPE system has been examined with three Arabidopsis VPEs, αVPE, βVPE and γVPE. The *βVPE* gene is expressed in seeds, suggesting that βVPE plays a role in maturation of seed proteins in protein storage vacuoles. On the other hand, both the *αVPE* and *γVPE* genes are expressed in senescent tissues and their expression patterns are correlated with programmed cell death. The vegetative VPEs might regulate the activation of senescence-associated hydrolytic enzymes in the lytic vacuoles of cells preparing for death.

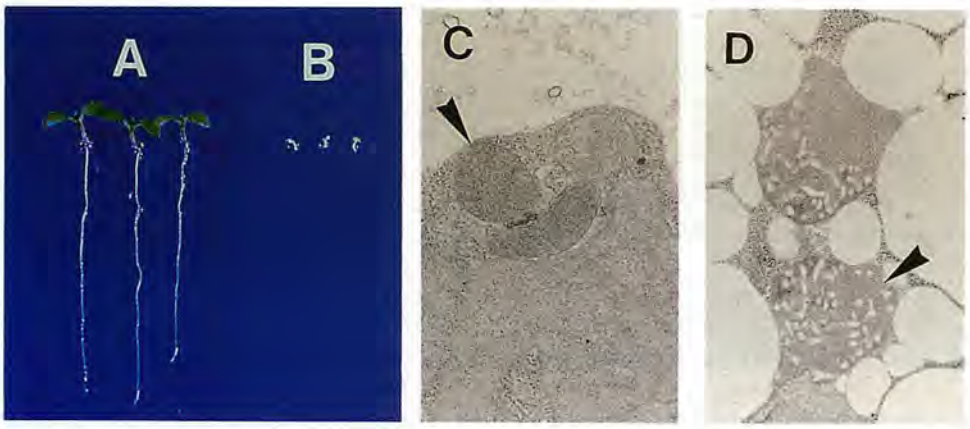

Figure 1. A microbody-defective mutant of Arabidopsis.

Wild-type *Arabidopsis* **(A)** can germinate and grow after supplying only water. In contrast, the *ped1* mutant **(B)**, which has a defect in glyoxysomal fatty acid β-oxidation, requires sucrose for post-germinative growth. Glyoxysomes in wild-type plant **(C; arrowhead)** are surrounded by a single membrane with approximately 1 μm in diameter, whereas glyoxysomes in the *ped1* mutant **(D; arrowhead)** become bigger, and contain tubular structures.

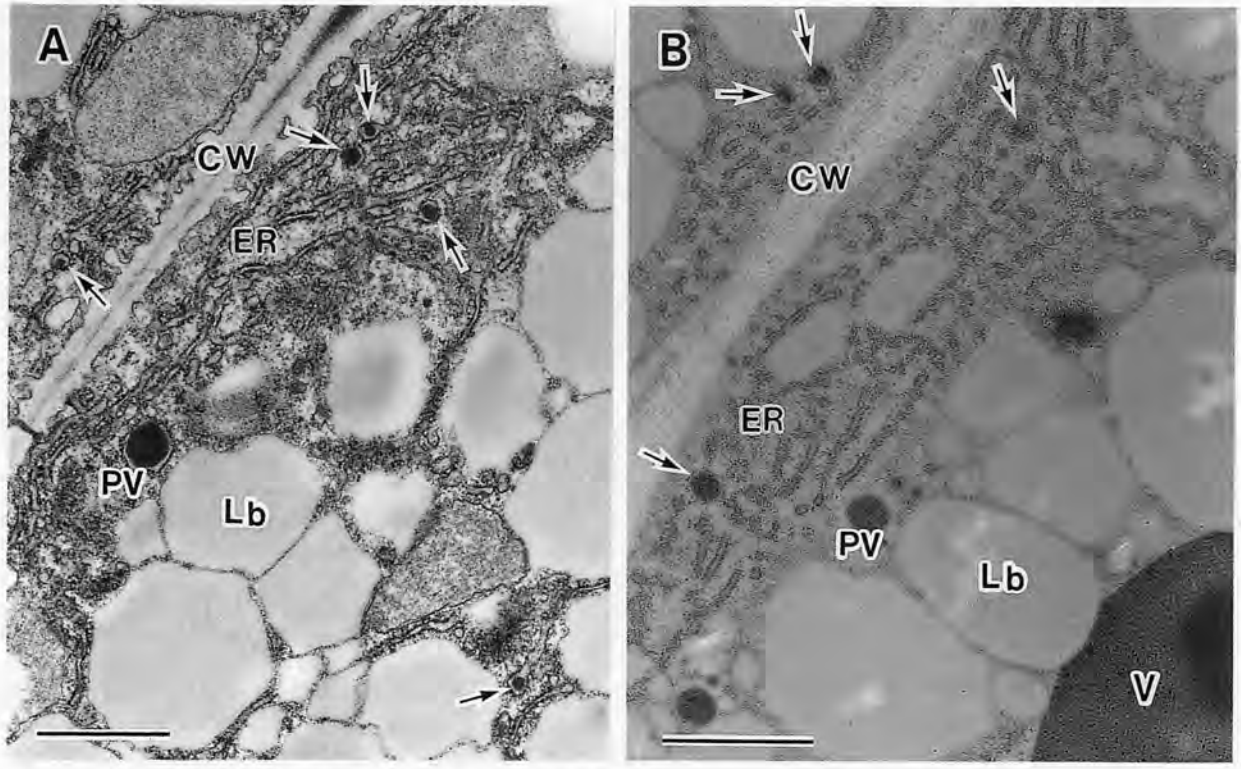

Figure 2. Electron microscopy of maturing pumpkin cotyledons showing numerous electron-dense aggregates within the ER. **(A)** Electron-dense core aggregates (arrows) formed within the ER in cells at the middle stage of seed maturation. The electron-translucent space between the aggregate and the ER membrane is loaded with ribosomes. PAC vesicles (PV) are also visible. **(B)** Immunoelectron microscopy with 2S albumin-specific antibodies. The aggregates within the ER (arrows) are labeled with gold particles. PAC vesicles (PV) and a protein storage vacuole (V) are also visible.
CW, cell wall; Lb, lipid body. Bars in (A) and (B) = 500 nm.

V. Role of molecular chaperones in organelle differentiation.

Molecular chaperones are cellular proteins that function in the folding and assembly of certain other polypeptides into oligomeric structures but that are not, themselves, components of the final oligomeric structure. To clarify the roles of molecular chaperones on organelle differentiation, we have purified and characterized chaperonin and Hsp70s and analyzed their roles in the translocation of proteins into chloroplasts. In addition to mitochondrial chaperonin10 homologues, we isolated a cDNA for chloroplastic chaperonin 10 homologues from *Arabidopsis thaliana*. The cDNA was 958 bp long and encoded a polypeptide of 253 amino acids. The deduced amino acid sequence showed that the protein contained an N-terminal chloroplast transit peptide and a mature region which was comprised of two distinct GroES-domains. The two halves of the Cpn20 show 42 % amino acid identity to each other. A Northern blot analysis revealed that the mRNA for the Cpn10 homologue was abundant in leaves and was increased by heat treatment. To examine the oligomeric structure of Cpn20, a histidine-tagged construct lacking the transit peptide was expressed in *E. coli* and purified by affinity chromatography. Gel filtration and cross-linking analyses showed that the expressed products form a tetramer. The expressed products can substitute for GroES to assist in the refolding of citrate synthase under non-permissive conditions. Further analysis on the subunit stoichiometry of the GroEL-Cpn20 complex revealed that the functional complex is composed of a GroEL tetradecamer and a Cpn20 tetramer.

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DIVISION OF BIOENERGETICS

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	Satsuki Okamoto

This division aims to elucidate the physiological role and mechanism of intracellular protein degradation in a lytic compartment. Up to recently, molecular biological studies on the topic of cellular regulation have focused on protein synthesis, and (the control of) gene expression. However, it is clear that cellular activity is maintained by a delicate balance between synthesis and breakdown of individual proteins. For the last few years, protein degradation has become a hot field in cell biology, because it plays critical roles in many aspects of cellular functions. Most of the selective protein degradation is believed to occur in the cytosol through the ubiquitin/proteasome system. In contrast, major bulk protein degradation takes place in the lysosome/vacuole. Non-specific protein turnover has essential roles under nutrient stress or for cell differentiation of eukaryotic cells. Until now, the molecular details of protein degradation in the lytic compartment remained to be uncovered due to the lack of a sensitive monitoring system, and the dynamic features of the lysosomal/vacuolar membrane system.

Autophagy is defined as the process of bulk degradation of cytoplasmic proteins or organelles in the lytic compartment and seems to be a ubiquitous and basic cellular activity in all eukaryotic cell types. Autophagy has been described mostly in higher eukaryotic cells by using electron microscopy. The lysosome/vacuole is a single membrane bound compartment containing various hydrolytic enzymes. Segregation of lytic enzymes from the cytosol requires an obvious cell biological process how to deliver proteins or organelles destined for degradation into the lumen of this compartment. Macroautophagy is the major route to the lysosome/vacuole under nutrient starvation conditions. This process involves the formation of a double membrane structure called an autophagosome (AP). The AP fuses with a primary lysosome and matures to be an autophagolysosome, then its contents are digested and recycled to be reused.

Autophagy has been studied extensively using various kinds of mammalian tissues and cultured cells. It is highly regulated by various factors such as amino acid or metabolites, hormones and growth factors, and second messengers. Because the modes of autophagy are so diverse and the physiological role and regulation of autophagy depend upon the cell type and physiological demands, it is difficult to present a general model for autophagy in higher eukaryotes. For this reason, it has been helpful to develop a simple system to elucidate the fundamental machinery of the autophagic process.

Yeast Induces Autophagy *as* Mammalian Cells

Recently we discovered yeast, *S. cerevisiae*, induces bulk protein turnover in the vacuoles under starvation conditions. This whole process corresponds to that of macroautophagy in higher eukaryotic cells. By electron microscopic analyses we succeeded in detecting double membrane structures in the cytoplasm enclosing a portion of cytosol. This autophagosome immediately fuses with vacuoles, delivering single inner membrane structures, autophagic body (AB) in the vacuole. When vacuolar proteinase activities are blocked genetically or by specific inhibitor ABs are accumulated in the vacuoles. They move around vigorously in the vacuoles by Brownian motion, and are easily detectable by light microscope. Thus we can follow the progression of autophagy as the accumulation of ABs in real time. Biochemical and immunoelectron microscopic analyses showed that starvation-induced sequestration is non-selective, that is, cytosolic enzymes and organelles are sequestered randomly to the vacuoles to be degraded.

Autophagy in yeast is a kind of general stress response to adverse environmental condition and is induced by various starvation conditions such as nitrogen, carbon, sulfate, and even amino acid depletion. Signal transduction of starvation for induction of autophagy is a key question to be solved. Recently we found rapamycin induces autophagy in the cell growing in a rich medium and that Tor, phosphatidylinositol kinase homologue plays essential role for the regulation of autophagy.

Another crucial and controversial question is the mechanism of formation of autophagosome. Still nothing is clear about the origin of the membrane. Freeze-fracture electron microscopy showed that autophagosomal membrane has quite characteristic feature and the density of intramembrane particles is extremely low as compared with other intracellular membrane.

Genetical dissection of autophagic process

Yeast has made great contribution to solve many fundamental problems in cell biology because of tractability of genetic and molecular biological techniques. In order to dissect the complex process of autophagy to its elementary steps we started to isolate mutants defective in the process of autophagy. Total 14 autophagy defective *agp* (Autophagy) mutants

were isolated. They cannot induce protein degradation upon shift from growth medium to the starvation medium. They grow normally in a rich medium, but start to die after 2 days in the starvation medium. This suggests that the autophagy is necessary for the maintenance of cell viability under starvation. Homozygous diploid of each *apg* mutant cannot undergo sporulation, indicating that this cell differenciation requires bulk protein degradation via autophagy.

APG genes are essential for Cvt pathway

One of vacuolar enzymes, aminopeptidase I, API is synthesized as a proform and sequestered to the vacuole directly from cytosol to the vacuole. All *apg* genes are necessary for this process. Electron microscopic analyses revealed that API first forms a complex in the cytosol and delivered to the vacuole by the distinct but topologically similar mechanism to the macroautophagy. Though this Cvt pathway is biosynthetic and constitutive, and kinetically quite different from the autophagy, but it must share most machinery with the autophagy.

Analyses of APG genes

Now we are focusing on characterization of *APG* genes, essential for autophagy. So far we have finished cloning and identification of twelve *APG* genes. *APG1* codes a novel Ser/Thr protein kinase essential for the induction of autophagy. This provides the first direct evidence for involvement of protein phosphorylation in the process of autophagy. Apg13p interacts with Apg1p and might function as a regulator of its kinase activity. So far most *APG* genes are novel except *APG6* and non-essential for vegetative growth. The *APG6* gene is allelic to *VPS30*, which is required for retrieval of the carboxypeptidase, CPY receptor to the late Golgi from the endosome. *APG14* encodes a novel hydrophilic protein of 40.5kDa. Subcellular fractionation experiments indicate that both Apg14p and Apg6p are peripheral membrane proteins and form a stable complex. The *apg14* mutant is normal for CPY sorting. While overexpression of Apg14p suppresses the autophagy defect of *apg6-1, APG14* does not function as a multicopy suppressor of the CPY sorting defect of this mutant. These results suggest that Apg6p has dual functions in autophagy and vacuolar protein sorting, and that Apg14p is specifically required for the autophagic process.

Using the assay system of autophagy we developed, we are studying genetic interaction among *APG* genes. Some *APG* gene products are starvation-inducible or change phosphorylation state by nutrient starvation. Analyses of intracellular localization of these Apgps may provide us specific marker to elucidate the membrane dynamics during autophagy.

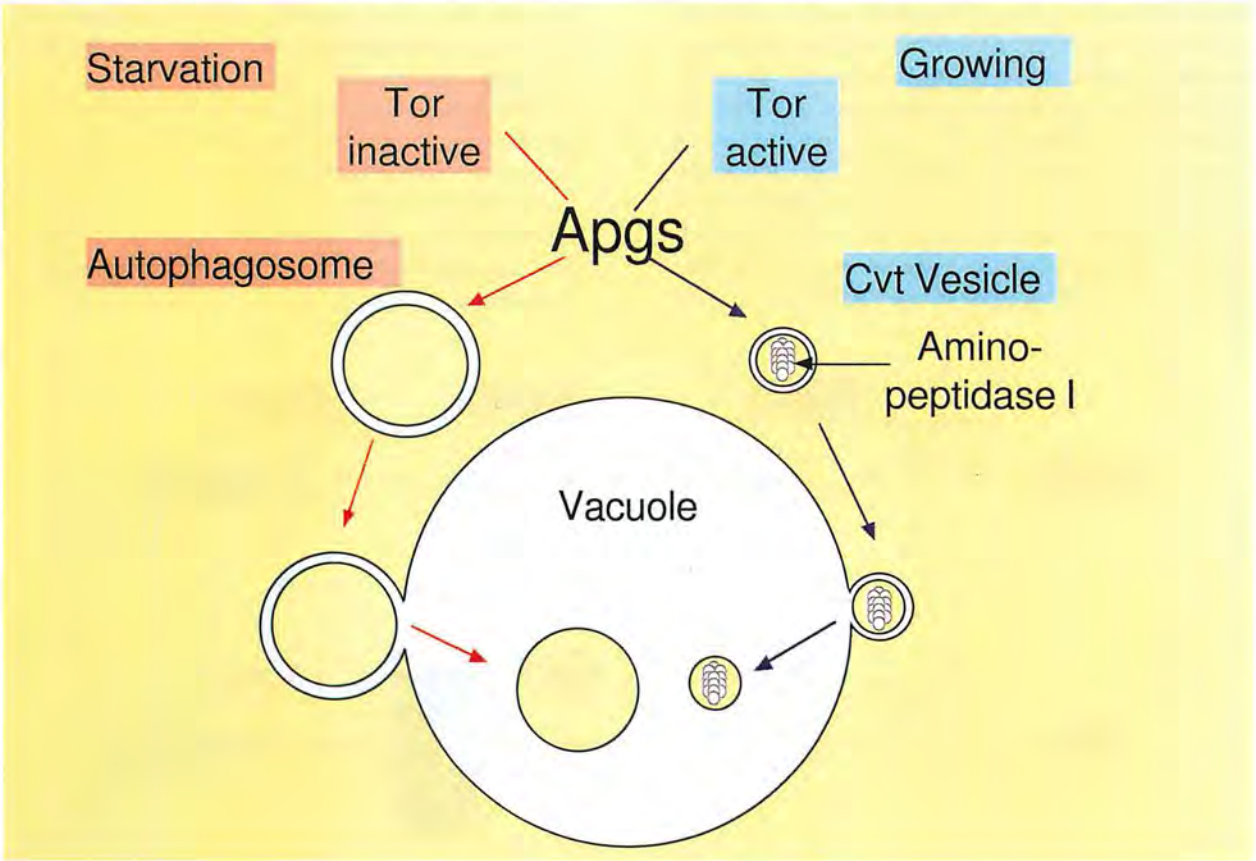

Fig. 1 Schema of autophagy in yeast. When yeast cells face to starvation, isolation membrane starts to enclose a portion of cytosol and form an autophagosome. Autophagosome fuses to the vacuolar membrane, delivering autophagic body in the vacuoles (Autophagic pathway). Under growing condition one of vacuolar enzymes, API is selectively sequestered to the vacuole (Cvt pathway). Two distinct pathways share most molecular machinery.

Novel protein conjugation system essential for autophagy

Recently we found that four *APG* genes are involved in a novel protein conjugation system essential for autophagy and the Cvt pathway. Apg12p is a 21kDa hydrophilic protein. Western blot analysis of HA-tagged Apg12p shows an additional band at 70kDa in addition to a band with the expected molecular mass. Sequence analysis of *APG5* shows that it encodes a hydrophilic protein of 34kDa. A western blot of Apg5p also shows the 70kDa band, suggesting covalent binding between Apg5p and Apg12p. Elimination of a single Gly at the C-terminus of Apg12p results in a complete loss of the higher molecular mass band. Similarly, substitution of Lys 149 of Apg5p, among 19 Lys residues, with Arg also abolishes this band. These results strongly suggest that the C-terminal Gly of Apg12p is conjugated to Lys149 of Apg5p via an isopeptide bond. Cells expressing these mutant forms of Apg12p or Apg5p are defective in autophagy and also Cvt pathway, indicating that the conjugate formation of Apg5p-Apg12p is essential for both pathways.

Furthermore, among the *apg* mutants, *apg7* and *apg10*, could not form the Apg5p-Apg12p conjugate. These two genes may encode enzymes required for the conjugate formation. From sequence data, Apg7p is predicted to be an E1 like activating enzyme for Apg12p. Recently, many ubiquitin-related molecules have been identified, and shown to function in various regulatory steps. However, Apg12p has no homology with ubiquitin or ubiquitin-related molecules. Apg12p is much larger than ubiquitin and seems to have a single target, Apg5p. The Apg5p-Apg12p conjugation reaction was reconstituted in vitro and shown to be ATP-dependent. Similarly, mutations in the ATP-binding site of Apg7p block conjugate formation. The function of this conjugate in the Apg and Cvt pathways is not known yet but that the conjugation may be necessary for autophagosome or Cvt vesicle formation.

Approach to mammalian autophagy

Then, there is no reason for us hesitating to challenge mammalian autophagy again, by taking advantage of identified genes involved in yeast autophagy. By searching databases, we have found several mammalian cDNA related to the yeast genes. Homologues of Apg12p and Apg5p are widely distributed from yeast to higher organisms. We cloned recently the human homologues of Apg12p and Apg5p and demonstrated a similar conjugation reaction in mammalian cultured cells. This result strongly suggests that the novel conjugation system is conserved in all eukaryotes. To elucidate roles of autophagy in development, differentiation, physiology, pathology in a multi-cellular system, we are making Apg12-deficient mice.

We also found that one of the Apgp homologues is specifically associated to the autophagosome membrane in human cultured cells. Post-translational processing of the molecule regulates the association. This is the first identified protein that is localized in mammalian autophagosomes. Yeast Csc1p is an AAA-type ATPase implicated in regulation of autophagy. We showed evidences that mouse SKD1 protein homologous to Csc1p plays a pivotal role on membrane transport via early endosomes in human cultured cells. This suggests involvement of endosomal system in the autophagic process.

Knowledge in yeast must give us key to uncover the mechanism of autophagy in higher eukaryotic organisms such as mammals. We are now developing an assay for monitoring autophagy in mammalian cultured cells to perform systematic evaluation of identified candidates for components in machinery of mammalian autophagy.

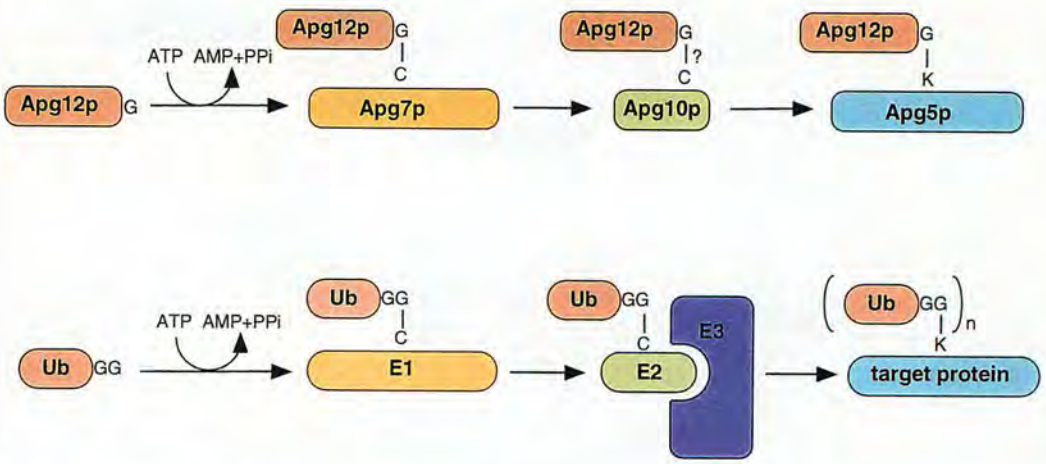

Fig. 2 The Apg12p conjugation system and ubiquitin system

Apg12p is activated by Apg7p in an ATP-dependent manner. After formation of a thioester with Apg7p, and then possibly with Apg10p, Apg12p is finally conjugated to Apg5p via an isopeptide bond (upper).

Although Apg12p has no homology to ubiquitin, the Apg12p conjugation system is quite similar to that of ubiquitin (lower) which is involved in selective proteolysis.

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DIVISION OF CELL PROLIFERATION (ADJUNCT)

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The aim of this new adjunct division, started in June 1998, is to understand the basic rules by which elaborate neural circuits develop and function. To address these questions, an effective approach is to investigate the whole of an easily-accessible nervous system that shares certain of the architectural and functional features of the more complex vertebrate brains. With less than 10^5 neurones, and subject to powerful molecular and genetic techniques, the brain of the fruit fly *Drosophila melanogaster* is a good model system for such a study, particularly because it contains neural arrangements, such as those in the olfactory and visual pathways, that are extraordinarily similar to equivalent parts of vertebrate brains.

I. Developmental neuroanatomy

A comprehensive and detailed anatomical knowledge of the brain is a prerequisite for 1) analysing the phenotypes of nervous system-related mutants, 2) identifying the cells that express the cloned genes, 3) understanding the way information is processed in the brain, and 4) devising computer models that simulate brain functions. In spite of the hundred years of efforts using Golgi and other anatomical techniques, however, the circuit structure of higher order associative regions of the brain is still essentially unresolved.

The GAL4 enhancer-trap system, which is widely used for mutagenesis and gene cloning of *Drosophila*, is also a powerful tool for obtaining a vast array of molecular markers that label specific subsets of brain cells. Forming a consortium with eight other *Drosophila* laboratories in Japan, we have established more than 4000 GAL4 strains. Different aspects of the morphology of the GAL4-expressing cells can be visualised by crossing these strains with the strains that carry various UAS-linked reporter genes, such as nuclear-specific UAS-*NLS-lacZ*, axon-targeting UAS-*tau*, cytoplasmic UAS-*lacZ* and UAS-*GFP*, and presynaptic site-specific UAS-*neuronal synaptobrevin-GFP*.

The screening of the GAL4 strains is being performed by observing the expression pattern in the adult brain. Useful strains will be selected to analyse the information pathway of different sensory modalities in the higher-order brain regions, as well as to study how the labelled neurones form their elaborate fibre connections during neurogenesis.

II. Functional neuroanatomy

With the GAL4-UAS system, it is possible to alter the normal cell functions by ectopically expressing developmental switch genes specifically in the GAL4-expressing cells. If this results in a change of certain behaviour of the animal, it is likely that these altered cells may play major roles in controlling that behaviour.

We use UAS-linked *transformer (tra)* gene, which acts early in the sex determination cascade, to alter the sex of the GAL4-expressing cells. The courtship behaviour of *Drosophila* is very different between male and female. Male flies actively try to attract females by presenting stereotyped steps of courtship, whereas females stay passive. Does this male-specific behaviour disappear, if particular brain cells are feminised?

Previous studies by other investigators showed that feminisation of the mushroom body neurones may make the male flies bisexual. Although this is an interesting phenomenon, the male-specific behaviour did persist in these experiments. To find the GAL4 strains that can really suppress male behaviour, we performed a much larger behavioural screening. For this purpose, we abandoned any assumption to preselect strains that drive *tra* gene in particular brain structures. Instead, we crossed all the available homozygous viable GAL4 strains with UAS-*tra*. Among the 446 strains tested so far, we found only two strains that show near-total suppression of male-specific courtship behaviour, regardless of the sex of the targets (Fig. 1).

The GAL4 expression patterns of these two strains, however, were not very specific. Rather, GAL4 is expressed in most of the brain cells. The results can be explained by assuming the following: 1: The courtship behaviour is controlled by various biochemical types of neurones, which may either be packed within a small brain area, or scattered in many regions; and 2: The male behaviour would manifest unless all the relevant neurones are female. Since these neurones may not share common enhancer activity, only GAL4 strains with near-ubiquitous expression pattern would be able to feminise all of these cells simultaneously.

III. Contribution to the science community

As the number of identified neurones grows, it becomes important to develop a system with which science community can easily access the record of complicated three-dimensional circuit structures. As a joint venture with German and US research groups, we maintain *Flybrain*, a web-based image database of the *Drosophila* nervous system (http://flybrain.nibb.ac.jp). Over 2000 images has already been stored and served worldwide.

Another database maintained here is *Jfly*, which is intended to help the exchange of information among Japanese-speaking *Drosophila* researchers (http://jfly.nibb.ac.jp). Archives of research-related discussions, images and experimental protocols, as well as meetings and job announcements, are provided.

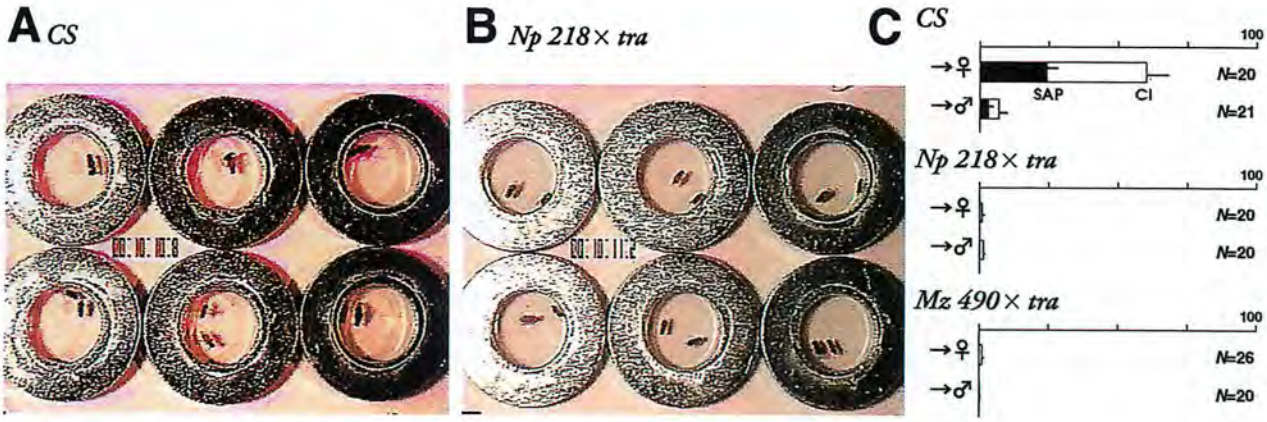

Fig. 1 Reduced male-specific activity caused by the ectopic expression of the *transformer* gene

(A) Wild-type (CS) male flies actively court females when they are put together in small chambers. Within ten minutes, most (in this case four out of six) males reach the final step of the courtship: copulation. Other males are chasing and courting their partners. (B) Males of the GAL4 enhancer-trap strain *Np218* crossed to UAS-*tra*, on the other hand, show no interests in females. (C) Quantitative comparison of the courtship behaviour. The duration of the time when a male fly engaged in any kind of the courtship-related behaviour (courtship index, CI), and the time when a male fly performed the courtship-specific wing vibration behaviour (sex appeal parameter, SAP), were recorded during the first ten minutes after the subject (male) and the target (either male or female) flies were put in the chamber. Wild type *(CS)* males spend long time courting females, but court males only occasionally. The males of the *Np218* and *Mz 490* strains crossed to UAS-*tra* spend only a very short period for courting both male and female.

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DIVISION OF CELLULAR COMMUNICATION (ADJUNCT)

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Institute Research Fellow: *Osamu Kagami*
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The research in this laboratory, started in November, 1996, is aimed at an understanding of the molecular mechanisms that regulate the assembly and function of cytoskeletal proteins. Current research effort is centered on the function of axonemal dyneins, microtubule-based motor proteins that produce force for flagellar beating. The organism employed is *Chlamydomonas*, a biflagellate green alga particularly suited for genetic and molecular biological studies.

A single flagellar axoneme contains at least eleven kinds of dynein heavy chains in inner and outer arms. To understand the specific function of each heavy chain, we have been isolating and characterizing mutants that lack different kinds of axonemal dyneins. Their motility phenotypes have indicated that different dynein species differ in function in a fundamental manner. For example, the outer-arm heavy chains are important for flagellar beating at high frequency, whereas the inner-arm heavy chains are important for producing proper waveforms. Indirect evidence also suggests that the force generation properties differ greatly among different heavy chains. We are currently trying to directly measure the force production by different dyneins using micro-physiological techniques.

The inner dynein arms are known to contain actin as a subunit. Hence the two independent motility systems of eukaryotes - the actin-based and microtubule-based motility systems - should somehow cooperate in the inner-arm dynein although the function of actin in dynein arms is totally unknown at present. Several years ago, we found that the mutant *ida5*, lacking four out of the seven subspecies of inner-arm dyneins, has a mutation in the actin-encoding gene. Intriguingly, *Chlamydomonas* has been known to have only a single gene of conventional actin, and the mutant *ida5* was found to express no conventional actin at all. On close inspection, the cytoplasm and axonemes of this mutant were found to contain a novel actin-like protein (NAP) which displays exceptionally low homology (64% in amino acid identity) to conventional actin. The mutant *ida5* is deficient in the formation of the fertilization tubule and thus has a low mating efficiency. However, it displays normal cell division and grows as rapidly as wild type, possibly because NAP can substitute for actin in important cellular functions. Thus conventional actin and NAP may overlap in some, but not all, cellular functions. It is interesting to note that NAP is expressed in significant amount only in the mutant lacking actin; i.e., the expression of NAP appears to depend on the presence of actin. We are currently investigating how such regulation takes place.

We have recently succeeded in transforming the mutant *ida5* with cloned actin gene and found that inner dynein arms become restored upon transformation. Transformation with the NAP-encoding gene is under way. Studies with artificially mutated actin gene will enable us to determine what functions are carried out by actin and NAP and, in particular, whether actin or NAP is really essential for cytokinesis, assembly and function of inner dynein arms, or other fundamental processes in *Chlamydomonas*.

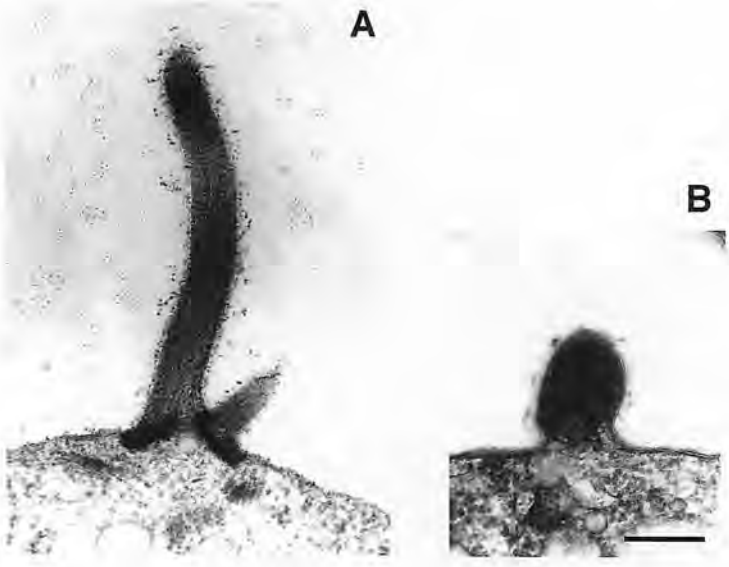

Fig. 1. Fertilization tubules in wild-type(A) and *ida5* (B) mt^+ gametes produced in response to a 1 hour exposure to 10 mM dibutyryl-cAMP and 1 mM IBMX. Bar, 0.3 μm. Wild-type fertilization tubules have been shown to contain F-actin bundles.

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DIVISION OF CELL FUSION (ADJUNCT)

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Cytokinesis in animal and some primitive eukaryotic cells is achieved by the progressive contraction of the cleavage furrow. The cleavage furrow contains a contractile apparatus, called the contractile ring, which is composed of a bundle of actin filaments that lies in the furrow cortex beneath the plasma membrane. It has been established that the contractile ring contracts as the result of interaction between actin filaments and myosin. However, little is known about process of its formation, mechanism that controls its formation, protein constituents, and its ultrastructure.The goal of our research is to solve these problems and thereby clarify the molecular mechanism of cytokinesis. For this purpose, we use three kinds of cells, namely, sea urchin egg, *Xenopus* egg, and the fission yeast *Schizosaccharomyces pombe*.

S. pombe is an excellent system to investigate the changes in the actin cytoskeleton during cell cycle since F-actin patches, F-actin cables and F-actin ring are only visible structures in the cell (Fig. 1). The F-actin ring is considered to correspond to the contractile ring in animal cells. It is formed during anaphase in this organism. In order to obtain basic features of the actin cytoskeleton in *S. pombe*, we investigated subcellular localization and interactions of actin and two actin cytoskeleton-related proteins, Cdc8 tropomyosin and actin-related protein 3 (Arp3), using specific antibodies and by gene disruption. Actin was localized to medial microfilamentous ring in the region of the septum during cytokinesis and to cortical patches by immunoelectron microscopy. F-actin cables were detected throughout the cell cycle by fluorescent staining with Bodipy-phallacidin. The cables were often linked to the patches and to the medial F-actin ring during its formation. Tropomyosin was localized to the F-actin ring and the cables. It was also distributed in the cell as patches, although colocalization with F-actin was not frequent. In *cdc8*^{ts} mutant cells, F-actin cables were not observed although the F-actin patches were detected and cell polarity was maintained. These observations suggest that the F-actin cables may be involved in the formation of the F-actin ring, and that tropomyosin plays an important role in organizing both the F-actin ring and the F-actin cable, but is not involved in the F-actin patch formation or maintenance of cell polarity.

Binding of Arp3 to actin was revealed by immunoprecipitation as well as by DNaseI column chromatography. Arp3 seemed to form a complex with several proteins in the cell extracts, as previously reported for other organisms. Arp3 was found to be concentrated in the medial region of the cell from early anaphase to late cytokinesis, however, it was not localized on the F-actin ring. Following *arp3* gene disruption, F-actin patches were delocalized throughout the cell and cells did not undergo polarized growth, suggesting that Arp3 influences the proper localization of the actin patches in the cell and thereby controls the polarized growth of the cell.

Next we investigated the role of myosin in cytokinesis of *S. pombe*. Recently, it has been shown by Kitayama et al. (J. Cell Biol. 137: 1309-1319, 1997) that Myo2, a type II myosin heavy chain plays a role in the F-actin ring formation in this organism. We found another myosin II called Myo3. Myo3 is the same protein as Myp2 reported independently by Bezanilla et al. (Mol. Biol. Cell, 8: 2693-2705, 1997). Overexpression of Myo3 in the cell leads to formation of aberrant F-actin ring, F-actin cable, and septum. We knocked out the myosin genes in *S. pombe*. Since Myo3 is not essential, but Myo2 is essential for its growth, we made a *myo2myo3* null strain containing pREP81-myo2 to control the expression of myo2. After shut off the myo2, no F-actin ring formation occurs during mitosis. Therefore, type-II myosin is necessary in the formation of the contractile ring in *S. pombe*.

We also concentrate our study on function of actin-regulatory proteins, including ADF/cofilin family pro-

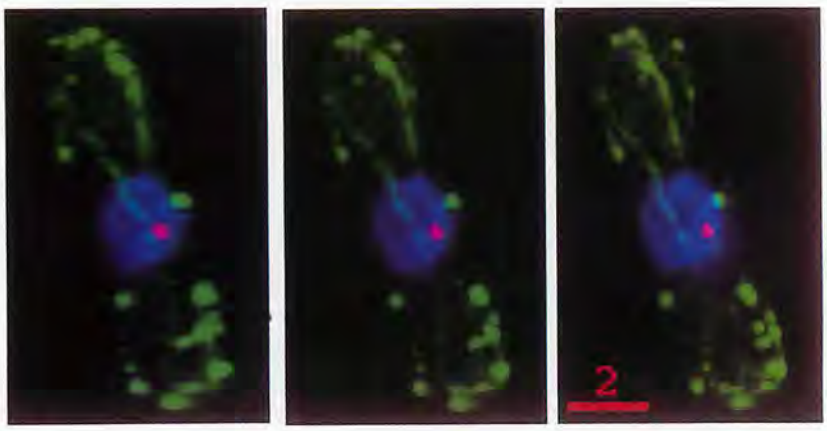

Fig. 1. 3-D images of an interphase *S. pombe* cell. Each image is rotated by 12 degree from the neighbor. Green, F-actin. Blue, DNA. Red, spindle pole body. Bar, 2 μm.

teins, during cytokinesis using *Xenopus* eggs and embryos. We found that ADF/cofilin family proteins are essential for cytokinesis (Abe, Obinata, Minamide, and Bamburg, J. Cell Biol. 132: 871-875, 1996). Recent studies revealed that ADF/cofilin accelerates turnover of actin filaments both in vitro and in vivo. Most recently, we found a novel actin-regulatory protein which induces disassembly of actin filaments cooperatively with ADF/cofilin. cDNA analysis revealed that this protein is a *Xenopus* homologue of yeast actin interacting protein 1 (AIP1). Thus, we designated this protein as *Xenopus* AIP1 (XAIP1). Purified XAIP1 itself binds to pure actin filaments to some extent, but it induces a rapid, drastic disassembly of actin filaments associated with cofilin. Microinjection of this protein into *Xenopus* embryos arrested development by the resulting actin cytoskeletal disorder. XAIP1 represents the first case of a protein cooperatively disassembling actin filaments with ADF/cofilin family proteins.

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DEPARTMENT OF DEVELOPMENTAL BIOLOGY

Chairman: Yoshitaka Nagahama

The Department is composed of three divisions and one adjunct division. Department members conduct molecular analysis on various aspects of developmental phenomena including: (1) differentiation and maturation of germ cells, (2) molecular basis of body plans, and (3) gene regulation in cell differentiation and growth.

DIVISION OF REPRODUCTIVE BIOLOGY

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	Ryo Horiuchi (Graduate University for Advanced Studies)
Monbusho Foreign Scientists:	*Allen Schuetz (Johns Hopkins University)*
	Srinivas K. Saidapur (Karnatak University)
Visiting Scientist:	*Graham Young (University of Otago)*

Gametogenesis of teleost fishes, as in other oviparous vertebrates, is controlled primarily by pituitary gonadotropins. In most cases, gonadotropins act via biosynthesis of gonadal steroidal hormones which, in turn, mediate various stages of gametogenesis. Using fish as a primary study model, our research focuses on (1) the identification of steroidal mediators involved in gonadal sex differentiation and gametogenesis, and (2) the mechanisms of synthesis and action of these mediators.

I. Endocrine regulation of oocyte growth and maturation

Two follicular steroidal mediators, estradiol-17β (oocyte growth) and 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP; oocyte maturation), were identified in several teleost fishes. Two-cell type models in which the thecal layer provides precursor steroids to the granulosa layer, have been proposed for estradiol-17β and 17α,20β-DP production. There is a distinct shift in expression of steroidogenic enzyme genes from cytochrome P450 aromatase (P450arom) to 20β-hydroxysteroid dehydrogenase (20β-HSD) in granulosa cells immediately prior to oocyte maturation. The presence of orphan nuclear binding sites in the medaka P450arom promoter and the similar expression patterns of FTZ-F1 and P450arom transcripts in ovarian follicles suggest that FTZ-F1 is involved in the transcriptional regulation of medaka P450arom during oocyte growth. The preovulatory surge of LH-like gonadotropin is responsible for the rapid expression of 20β-HSD mRNA transcripts in granulosa cells during oocyte maturation.

17α,20β-DP induces oocyte maturation by acting on a pertussis toxin-sensitive G-protein-coupled membrane receptor. The early steps of 17α,20β-DP action involve the formation of downstream mediator of this steroid, the maturation-promoting factor or metaphase-promoting factor (MPF) consisting of cdc2 kinase and cyclin B. 17α,20β-DP induces oocytes to synthesize cyclin B which activates a preexisting 35-kDa cdc2 kinase via phosphorylation of its threonine 161 by a threonine kinase (MO15), thus producing the 34 kDa active cdc2. A 54-kDa Y box protein and polyadenylation of cyclin B mRNA are thought to be involed in 17α,20β-DP-induced initiation of cyclin B mRNA translation.

Upon egg activation, MPF is inactivated by degradation of cyclin B. The availability of biologically-active goldfish cyclin B produced in *E. coli* and purified goldfish proteasomes allowed the role of the proteasome in the regulation of cyclin degradation to be examined for the first time. It was demonstrated that the 26S proteasome initiates cyclin B degradation through the first cut of its NH_2 terminus at lysine 57.

II. Endocrine regulation of spermatogenesis

The importance of gonadotropins and androgens for spermatogenesis is generally accepted in vertebrates; however, the mechanism of action of these hormones remains unresolved. Using an organ culture system for eel testes consisting of spermatogonia and inactive somatic cells, we have shown that the hormonal regulation of spermatogenesis in eel testes involves the gonadotropin stimulation of Leydig cells to produce 11-ketotestosterone (11-KT), a potent androgen in fish. In turn, 11-KT activates Sertoli cells to stimulate premitotic spermatogonia to complete spermatogenesis (Fig. 1). A subtractive hybridization method was used to clone genes that are expressed differentially in eel testes in the first 24 hr after gonadotropin treatment. Six down-regulated and two up-regulated cDNA clones were isolated. Two cDNA clones suppressed by gonadotropin stimulation exhibited ZP (zona pellucida sperm-binding protein)2- and ZP3-like structures. Transcripts of ZP2- and ZP3 are detected in immature testes and disappear immediately after HCG injection, suggesting that ZP2 and ZP3 may play important roles in the prevention of spermatogenesis in the eel. One of the up-regulated cDNAs was identified as coding the activin βB subunit. Activn βB mRNA transcripts were absent in testes prior to gonadotropin treatment, but were abundant in Sertoli cells in testes of eels treated with gonadotropin for 1-6 days. 11-KT induced a marked production of activin B in cultured testes, indicating that eel activin βB subunit production is largely for activin B formation. An androgen receptor localized in Sertoli cells. Addition of recombinant eel activin B to the culture medium induced proliferation of

to the culture medium induced proliferation of spermatogonia, producing late type B spermatogonia, within 15 days in the same manner as did 11-KT. Activin B also acts on spermatogonia to induce *de novo* synsthesis of G1/S cyclins (cyclins A, D, and E) and Cdks (cdc2, cdk2, and cdk4), leading to the initiation of mitosis (spermatogonial proliferation) (Fig. 1).

III. Endocrine regulation of gonadal sex differentiation and sex change

The initial differentiation and development of steroid-producing cells during gonadal sexual differentiation were examined by *in situ* hybridization and immunohistochemistry in tilapia, *Oreochromis niloticus*. Transcripts of P450arom became evident for the first time in the gonads of genetic females two weeks before morphological sex differentiation (Fig. 2). These gonads were also positive to antibodies

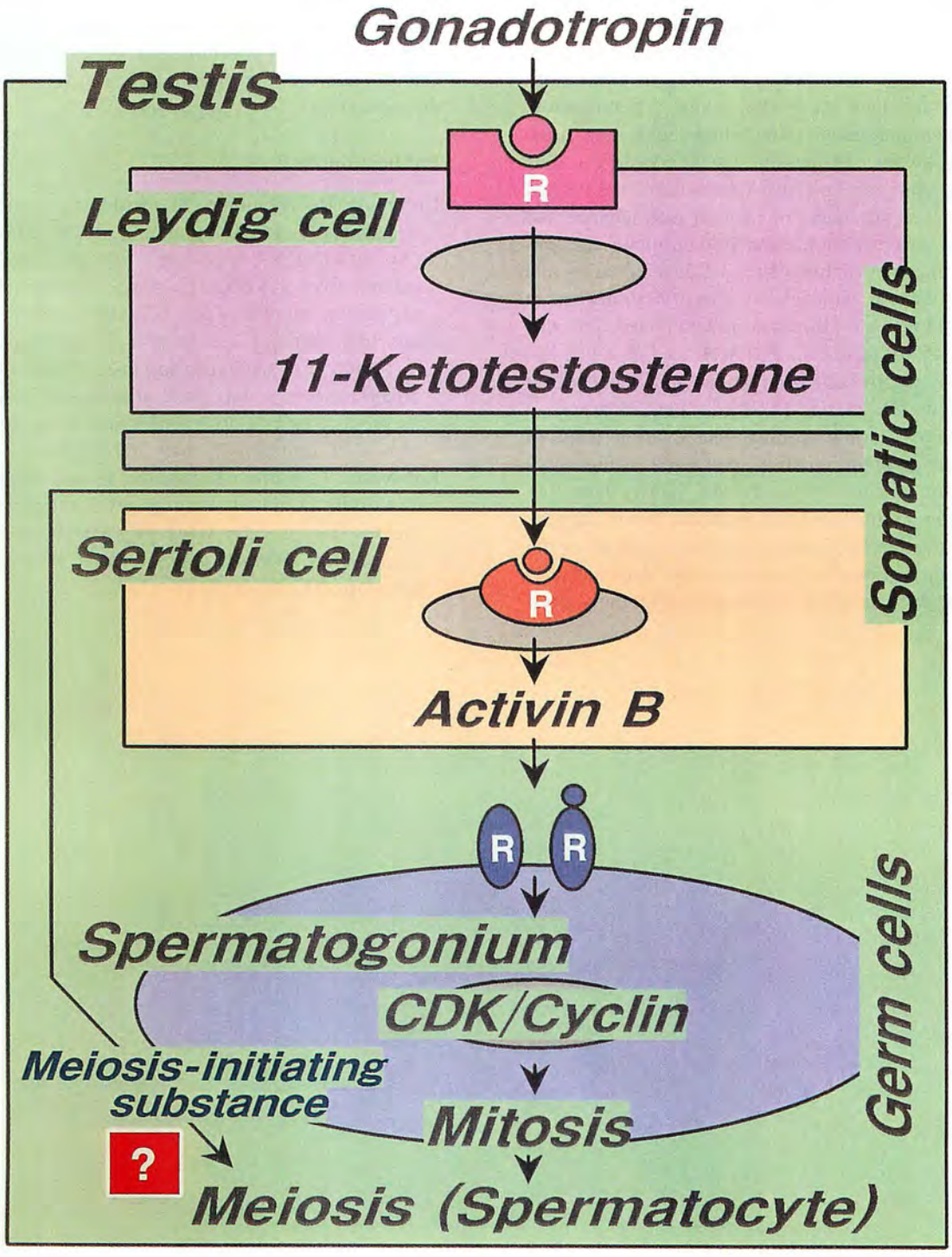

Fig. 1 Hormonal regulation of spermatogenesis initiation in the Japanese eel.

against P450scc, 3β-HSD, P450c17, and P450arom. Thus, steroid-producing cells in ovaries, but not testes, at the undifferentiated and differentiating stages express all of the steroidogenic enzymes required for estradiol-17β biosynthesis from cholesterol. These results strongly suggest that endogenous estrogens act as the natural inducers of ovarian differentiation in tilapia. In contrast, weakly positive reactions to P450scc, 3β-HSD and P450c17 antibodies appeared first in the testis at 30 days; however, staining intensity increased slowly during development of the testis. Therefore, the ability of steroid-producing cells to synthesize steroid hormones in the testes only appears at the time of testicular differentiation.

Sequential, protogynous hermaphroditism, i.e. female to male sex change, provides an ideal comparative model for studies on sexual differentiation. Fully-functional females are induced to develop functional testes by non-invasive social conditions, thus allowing simultaneous investigation into the normal endocrine requirements of ovarian maintenance and testicular differentiation. As steroid hormones are considered major mediators of sexual differentiation and gonadal function, molecular studies of key enzymes in the steroidogenic pathways were completed. Two different P450arom mRNAs as well as P450 11β-hydroxylase mRNA have been cloned and characterized throughout sex change in *Thalassoma duperrey*, a protogynous hermaphrodite. The ovarian form of P450arom is predominantly expressed in the ovary prior to the initiation of sex change. Shortly thereafter, expression diminished to undetectable levels suggesting that ovarian P450arom/estrogen is critical for the maintenance of ovarian function and/or detrimental to testicular differentiation. Although intriguingly complex and potentially relevant, the neural form of P450arom, named for its abundance in the brain, does not appear to be directly related to the gonadal restructuring seen during sex change. Nevertheless, its function and significance are being actively pursued. In contrast, P450 11β-hydroxylase is likely to be a major factor regulating the gonadal changes. P450 11β-hydroxylase mRNA was relatively abundant in the ovary; however, it was up-regulated concomitant with the onset of testicular differentiation indicating the importance of P450 11β-hydroxylase/11-KT to spermatogenesis and spermiation. Consequently, the switch from an ovary to a testis corresponds exceptionally well to a switch in the steroidogenic pathway of the gonad thus providing further evidence of the critical nature of steroid hormones in teleost sexual differentiation.

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Fig. 2 Expression of cytochrome P450 aromatase mRNA in the gonad of tilapia two weeks before morphological sex differentiation. Note strong signals in steroid-producing cells (arrows).

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DIVISION OF CELL DIFFERENTIATION

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Steroid hormones, which act as endocrine messengers, are mainly synthesized in steroidogenic tissues such as the gonads and adrenal cortex. Because of the pivotal function of sex steroids and corticosteroids secreted from these tissues, several groups have investigated the regulation of hormone production. Specifically, in the past decade, extensive research has been directed into the area of transcriptional regulation of genes implicated in steroidogenesis. As one of these studies, we identified Ad4BP/SF-1, which belongs to a nuclear hormone receptor family, as a major steroidogenic tissue-specific transcription factor. Further studies have suggested that this versatile transcription factor is central to the regulation of a reproductive behavior thorugh functioning in the pituitary gonadotroph and ventromedial hypothalamic nucleus in addition to the steroidogenic tissues. Based on these observation, our attention has been directed to understanding mechanisms underlying differentiation of the reproductive system and establishment of reproductive behavior of animals.

I. Transcriptinal control of genes necessary for gonadal differentiation.

Because of the fundamental and pivotal function of the gonads, extensive efforts have been made to characterize the differentiation processes including sex-dependent differentiation. By focusing on the steroidogenesis as one of the gonad specific functions, we identified a steroidogenic tissue specific transcription factor designated Ad4BP/SF-1. Recent studies with an antiserum to Ad4BP/SF-1 and a gene disrupted mice clearly demonstrated that the transcription factor is essential for differentiation of the steroidogenic tissues, the gonads and the adrenal cortex. Showing a good correlation with the observation, a particular cell population immunoreactive for Ad4BP/SF-1 (AGP, adreno-gonadal primordium) was identified to be present from the dorsal aorta to coelomic epithelia of rodent fetauses and succesively they were shown to give rise to two distinct primordia, the gonads and adrenal cortex. In the case of the gonadal primodium, it finally gives rise to the testis or ovary according to the sex chromosomal composition. These observations gave us many interesting issues as follows. What is the signal for the onset of the Ad4BP/SF-1 gene transcription in the particular cells comprising AGP? What is the mechanism underlying the separation of the AGP into the adrenal and gonadal primordia? How is the mechanism employed for sex differentiation of the gonads? Why dose such sex dependent differentiation not occur in the adrenal cortex? To address these issues, nuclear transcription factors (WT-1, SRY, SOX-9, DAX-1, Emx-2, and GATA-4, as well as Ad4BP/SF-1), all of which are critically implicated in gonadal and adrenocortical differentiation, havebeen underinvestigation from the molecular and morphological aspects.

As one of these efforts, we investigsted *Dax-1* gene regulation and identified a novel Ad4 site, which is recognized by Ad4BP/SF-1, by transient transfection and electrophoretic mobility shift assays. In addition, immunohistochemical analyses with antibodies specific for Dax-1 indicated the presence of immunoreactive cells in the steroidogenic tissues, pituitary gland and hypothalamus. Although the distributions of Dax-1 and Ad4BP/SF-1 were highly similar, they were not completely identical. Namely a certain population of cells immunoreactive for Ad4BP/SF-1 is negative for Dax-1 expression. Such inconsistent distributions between the two transcription factors were observed in all tisseus. To confirm the positive regulation of the *Dax-1* gene by Ad4BP/SF-1, the Dax-1 expression was investigated with the *mFtz-F1* gene disrupted mice. The Dax-1 expression was significantly impaired in the *mFtz-F1* gene disrupted mice (Fig. 1), confirming our understanding that Ad4BP/SF-1 controls the transcription of the *Dax-1* gene.

II. Implication of Ad4BP/SF-1 in strctural organization of spleen.

During characterization of the *mFtz-F1* gene disrupted mice, we were aware of the abnormal spleen of the *mFtz-F1* gene disrupted mice in addition to the tissues above (Fig. 2). The spleen has normally two main functions. The first is to provide a proper microenvironment to lymphoid and myeloid cells, while the second involves clearance of abnormal, damaged, and aged erythrocytes. Immuno-histochemical examination of the mammalian spleens confirmed the expression of Ad4BP/SF-1 in endothelial cells of the splenic venous sinuses and pulp vein. In the *mFtz-F1* gene disrupted mice, several structural abnormalities were detected in the spleen, including underdevelopment and nonuniform distribution of erythrocytes. Examination of the spleen of KO fetuses showed failure of development of certain tubular structures during embryogenesis. These structures are normally assembled by Ad4BP/SF-1 immunoreactive cells, and most likely form the vascular system during later stages of development. Other structural abnormalities

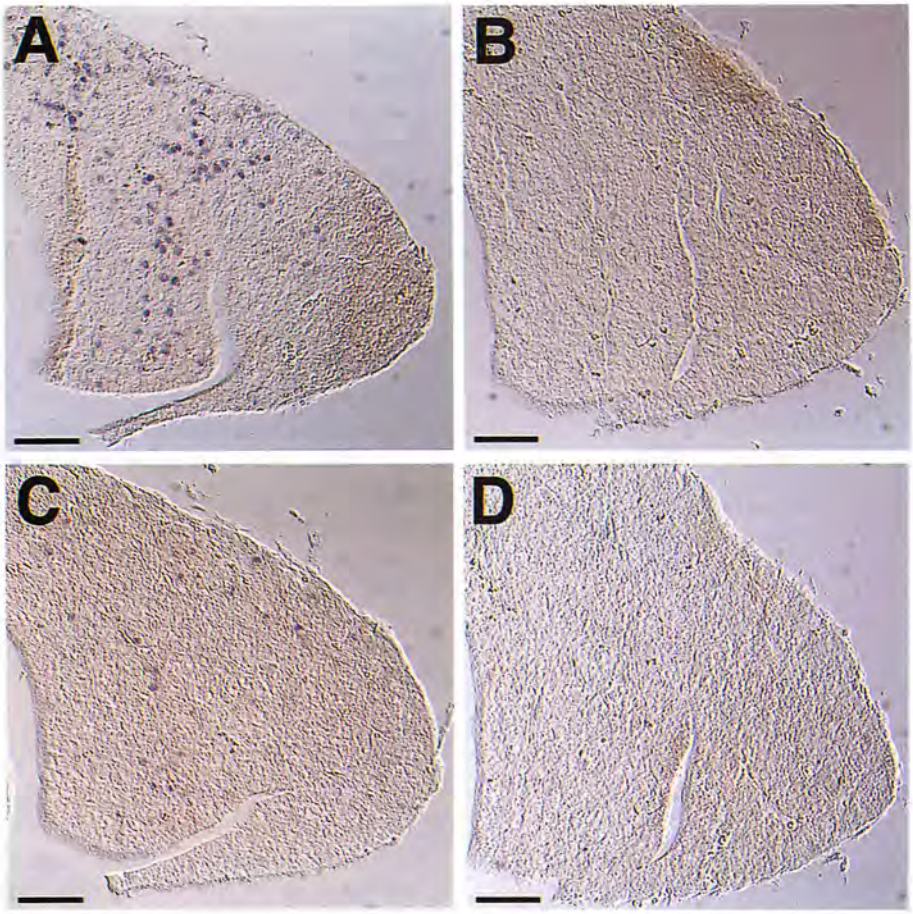

Fig. 1, Immunohistochemical detection of Dax-1 in the pituitary. Sections of the pituitary glands from the newborn were prepared from wild-type (A and C) and *mFtz-F1* gene disrupted (B and D) mice. They were stained with anti-Ad4BP/SF-1 antisera (A and B) or anti-Dax-1 antibodies (C and D) as described. Bars; 50 μm.

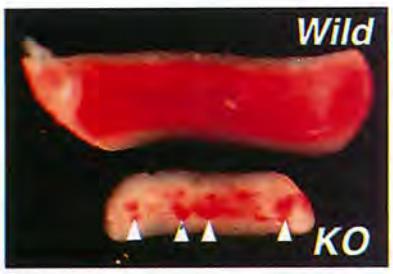

Fig. 2, Comparison of macroscopic features of spleens of wild-type (Wild) and *mFtz-F1* gene disrupted (KO) newborn mice. Arrowheads show red spots characteristic of the KO spleen.

in the spleen of the *mFtz-F1* gene disrupted mice included defects in the tissue distribution of type IV collagen, laminin, c-Kit, and vimentin. These morphological defects in the vascular system were associated with a decrease in the proportion of hematopoietic cells although differentiation of these cells was not affected significantly. A high number of abnormal red blood cells containing Howell-Jolly bodies were noted in the *mFtz-F1* gene disrupted mice, indicating impaired ability for clearance due to the affected splenic vascular system. We also detected the presence of an mRNA encoding cholesterol side chain cleavage P450 in the spleen, resembling the findings in steroidogenic tissues such as the gonads and adrenal cortex. The mRNA transcript was not involved in splenic structural defects as it was detected in the spleens of both normal and the *mFtz-F1* gene disrupted mice, indicating that the regulatory mechanism of the *P450* gene in the spleen is different from that in steroidogenic tissues. Our results indicate a lack of the *mFtz-F1* gene in mice is associated with structural and functional abnormalities of the splenic vascular system (In press, Blood, 1999).

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DIVISION OF MORPHOGENESIS

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Early embryogenesis is regulated by a number of endocrine and paracrine factors that mediate cell-to-cell communications. Particularly, polypeptide growth factors are believed to be essential for the regulation of cell proliferation, differentiation and apoptosis in morphogenic events during early development. The most striking feature of growth factor is that their functions are extremely well conserved among animal species, which allows us to investigate developmental mechanisms governed by growth factors using multiple model animals, such as mouse, zebrafish, *Xenopus, Drosophila, C. elegans* etc. To understand molecular basis of morphogenesis, we focus on a group of growth factors Transforming growth factor-β (TGF-β) superfamily and investigate molecular mechanisms of their actions in early embryogenesis. Our goal is to unveil species-specific and conserved mechanism of early development.

I. The role of BMP in ectodermal pattern formation.

Bone morphogenetic proteins belonging to the TGF-β superfamily have critical roles in the determination of cell fate during early development. It is widely accepted that ventrally expressed BMPs are essential for epidermal differentiation. Overexpression BMP in dorsal blastomeres of *Xenopus* laevis results in the loss of neural tissues including head and eyes. To investigate whether BMP can determine epidermal differentiation by acting directly on ectoderm or acting through the conversion of mesodermal cell fate, we performed bead transplantation experiment using zebrafish as a model. BMP-soaked beads were transplanted into presumptive neural region of early gastrula (shield stage) embryo and marker gene expression was examined. The result indicated that BMP induces epidermal fate and inhibits neural fate, acting directly on ectoderm (Figure 1). We also found that action range of BMP in zebrafish ectoderm is rather restricted, denying the possibility of relay mechanism of signal propagation.

Neural induction takes place because BMP activity is inhibited by organizer factors emanated from Spemann organizer. Such organizer factors include Chordin, Noggin and Follistatin. Chordin and Noggin have been shown to bind BMPs directly and thus inhibit their epidermal inducing activity. We have been able to show that Follistatin can also bind BMPs directly albeit with a low affinity and high dissociation constant.

II. The role of XIAP and Dlx5 in BMP signaling

TAK1, a member of the MAP kinase kinase kinase family, and its activator, TAB1, participate in the bone morphogenetic protein (BMP) signaling pathway involved in mesoderm induction and patterning in early *Xenopus* embryos. However, the events leading from receptor activation to TAK1 activation remain to be identified. A yeast interaction screen was used to search for proteins that function in the pathway linking the receptors and TAB1-TAK1. The human X-chromosome-linked inhibitor of apoptosis protein (XIAP) was isolated as a TAB1-binding protein. XIAP associated not only with TAB1 but also with the BMP receptors in mammalian cells. Injection of XIAP mRNA into dorsal blastomeres enhanced the ventralization of *Xenopus* embryos in a TAB1-TAK1-dependent manner. Furthermore, a truncated form of XIAP lacking the TAB1-binding domain partially blocked the expression of ventral mesodermal marker genes induced by a constitutively-active BMP type I receptor. Taken together, we identified that XIAP participates in the BMP signaling pathway as a positive regulator linking the BMP receptors and TAB1-TAK1.

Recently, we isolated a murine homeobox-containing gene, distal-less 5 (mDlx5), as a BMP-inducible gene in osteoblastic MC3T3-E1 cells. Stable transfectants of MC3T3-E1 that overexpress mDlx5 mRNA showed increase in various osteogenic markers; a 4-fold increase in alkaline phosphatase activity, a 6-fold increase in osteocalcin production and appearance in mineralization of extracellular matrix. Furthermore, mDlx5 was induced orthotopically in mouse embryos treated with BMP-4 and in fractured bone of adult mice. Consistent with these observations, we also found that injection of mDlx5 mRNA in dorsal blastomeres enhanced the ventralization of *Xenopus* embryos. Our results indicated that mDlx5 is

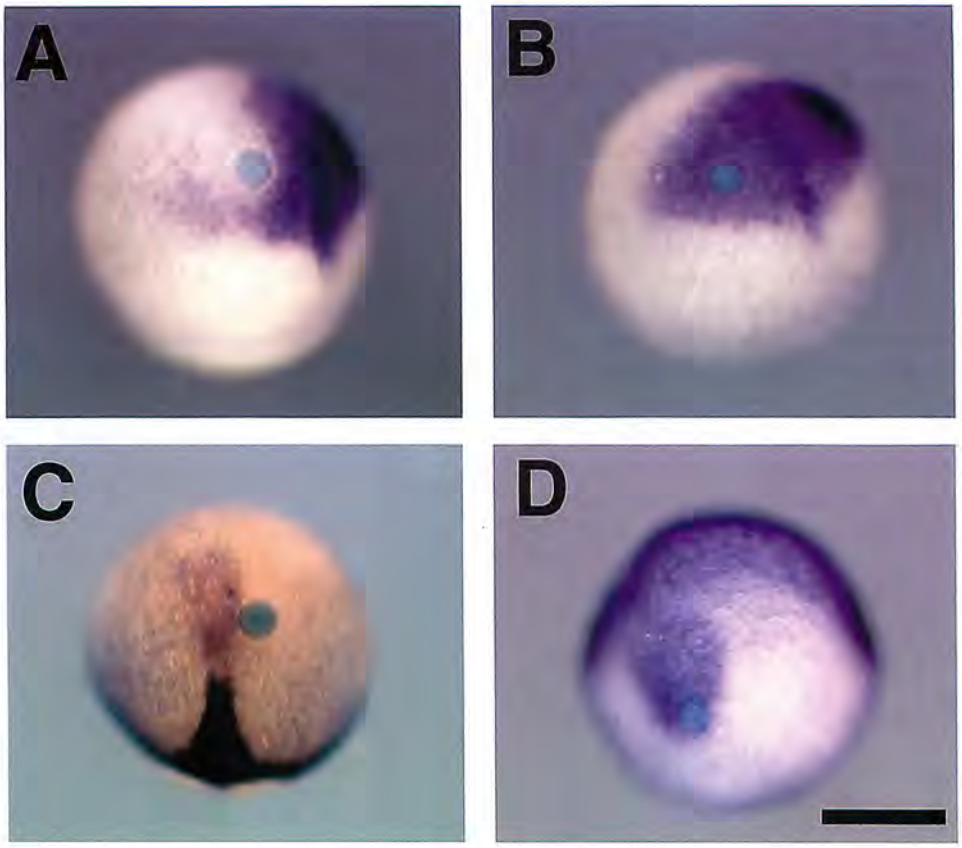

Figure 1 Effect of BMP in ectodermal patterning. (A) BMP-soaked bead in neural region suppressed a neural marker otx-2 expression. (B) Control bead soaked in BSA does not affect otx-2 expression. (C) BMP-bead transplantation into shiled stage embryos does not perturb expression of mesodermal marker genes goosecoid (light brown) and no tail (dark brown). (D) BMP-bead ectopically induces an epidermal marker gata-3.

a target gene of the BMP signaling pathway and acts as an important regulator of both osteogenesis and dorso-ventral patterning of embryonic axis.

III. Eary pattern formation of the peripheral nervous system in *Drosophila*

Large mechanosensory bristles (macrochaetes) develop in fixed numbers at constant positions on the adult notum of *Drosophila melanogaster*. This pattern formation of macrochaetes has provided an ideal model system for studying two-dimensional patterning. Precise positioning of macrochaetes depends on the complex expression pattern of proneural genes in the wing disc. Prepattern genes are thought to regulate these expression of proneural genes. A proneural cluster of dorsocentral bristles forms adjacent to the dorsal side of *wingless* (*wg*)-expressing cells in the notum region of the wing imaginal disc. It has been shown that *wg* activity is required for these structures to form. However, the restriction of this proneural cluster to the dorsal posterior side of the *wg* expression domain in the anterior compartment of the wing imaginal disc has suggested that Wg signaling itself is insufficient to establish the dorsocentral proneural cluster. Some factor(s) from posterior side must participate in this action in cooperation with Wg signaling. We have examined the role of Dpp (*Drosophila* BMP2/4 homolog) signaling in dorsocentral bristle formation by either ectopically activating or conditionally reducing Dpp signaling. Ubiquitous activation of Dpp signaling in the notum region of the wing imaginal disc induced additional dorsocentral proneural cluster all along the dorsal side of the *wg* expression domain, and altered *wg* expression. Conditional loss-of-function of Dpp signaling during disc development resulted in the inhibition of dorsocentral proneural cluster formation and expansion of the *wg* expression domain. These results indicate that Dpp signaling has two indispensable roles in dorsocentral bristle formation, induction of the dorsocentral proneural cluster in cooperation with Wg signaling and restriction of the *wg* expression domain in the notum region of the wing imaginal disc.

IV. TGF-β family in nematode

Nematode *C. elegans* provides powerful genetical approaches to understand the role of TGF-β family ligands and their signaling mechanism. We have identified a new member of the TGF-β superfamily, CET-1, from *C. elegans*, which is expressed in the ventral nerve cord and other neurons. *cet-1* null mutants have shortened bodies and male tail abnormal phenotype resembling *sma* mutants. Overexpression experiments demonstrated that *cet-1* function requires wild type

sma genes, suggesting that CET-1 functions as ligand in *sma* pathway. Interestingly, CET-1 appears to affect body length in a dose dependent manner. Heterozygotes for *cet-1* displayed body lengths ranging between null mutant and wild type, and overexpression of CET-1 in wild type worms elongated body length close to *lon* mutants. Epistasis analysis concerning male sensory ray pattern revealed that *mab-21* lies downstream and is negatively regulated by the *cet-1/sma* pathway. Our results show that *cet-1* controls diverse biological processes during *C. elegans* development probably through different target genes.

Recently, almost entire genome sequence of *C. elegans* was published. The sequence information, therefore, allows us comprehensive approach in gene expression analysis. To identify the target genes of *cet-1/sma* pathway and understand the molecular basis of the regulation of body length, we performed a differential hybridization between wild-type and *cet-1/sma* mutants, using a high-density filter on which over 7,000 different cDNAs were arrayed. We are now analyzing functions of candidate genes whose expressions are up- or down-regulated in *cet-1/sma* mutants.

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DIVISION OF DEVELOPMENTAL BIOLOGY (ADJUNCT)

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Availability of sugars and the inter-organ transport and distribution of sugars are essential in the growth and development of the plant body. Expression of a variety of plant genes is regulated, either positively or negatively depending on the gene, by the level of sugars at the sugar-importing sink sites as well as at the sugar-exporting source sites of the plant body. Thus sugars are not only important as sources for cellular energy and the synthesis of macromolecules but also as a signal controlling the growth and development of plants by changing the pattern of gene expression. Our research attention is focused to eludicate the mechanisms involved in the regulation of gene expression in response to sugars, especially the activation of gene expression by increased-levels of sugars, and the role of such regulation in the organ development in plants. In addition, mechanisms involved in the accumulation of storage proteins in plant vacuoles are also studied.

I. Molecular and genetic approaches for the analysis of the sugar-signalling during growth and development of *Arabidopsis thaliana*.

During the growth of plants, new organs develop as carbohydrate sink, and many vegetative organs shows sink to source transition during their maturation. Many aspects of the organ development in higher plants are thought to be affected, to some degree, by the levels of sugars. Sugars seem to have influence on the meristematic transition in long day plants from vegetative to reproductive growth. To obtain insights into the role of sugar-regulated gene expression in the growth and organ development in plants, we are screening for mutants of Arabidopsis with defects or anomalies both in the sugar-regulated gene expression and in the developmental processes such as leaf development and the determination of the flowering time. To aid this purpose, we have established more than 7,000 independent lines of Arabidopsis plants transformed with T-DNA containing multiple copies of the enhancer sequence. We have identified more than 20 of mutant lines with defects in the development of leaves or anormalies in the flowering time, which also show the altered patterns of the expression of sugar-inducible genes, such as β-amylase gene (*Atβ-Amy*) (Fig. 1). The mutants were named as *uns* (*unusual sugar response*) after their abnormal sugar responses with the gene expression.

II. Regulatory factors involved in the sugar-inducible expression of plant genes

Expression of genes coding for sporamin and β-amylase, two major proteins of the storage roots of sweet potato, is inducible by high levels of sugars in various vegetative tissues. The GUS reporter genes under the control of the promoters of these genes are also inducible by sugars in leaves of transgenic tobacco plants, and these fusion genes are expressed in tubers of transgenic potato plants. Although the induction of expression of these fusion genes requires the activity of hexokinase, phosphorylation of hexose by hexokinase is not sufficient to cause the induction. The induction requires Ca^{2+}-signalling and the activity of protein kinase.

Eight different cDNAs for the isoforms of calcium-dependent protein kinase (CDPK) were isolated from leaves of tobacco, and transcripts of two of them were found to be increased upon treatment of leaves with various metabolizable sugars. Antibodies against a fragment of one of these isoforms cross-reacted strongly with the 57 kDa-protein in the soluble fraction from the young leaves. The level of this 57 kDa-protein decreased significantly as leaf matures, while the level of this 57-kDa protein in mature leaves increased significantly after the treatment of leaves with sugars. The sugar-induction of the 57-kDa protein occured preceding the induction of expression of the β-amylase:GUS reporter gene. In addition, a 54 kDa-protein with autophosphorylation acitivity in the plasma membrane of mature leaves also increased significantly upon treatment of leaves with sugars. This protein was purified to about 1,000-fold compared to the crude extract. It phosphorylated histone IIIS in a Ca^{2+}-dependent manner and cross-reacted with an antibody against CDPK of *Arabidopsis thaliana*. These results suggest the possible involvement of CDPKs in the sugar-inducible gene expression and the development of leaves.

Selected Publications:

Iwata, Y., Kuriyama, M., Nakakita, M., Kojima, H., Ohto, M. and Nakamura, K., (1998) Characterization of a calcium depedent protein kinase of tobacco leaves that is asociated with the plasma membrane and is inducible by sucrose. *Plant Cell Physiol.* 39(11): 1176-1183.

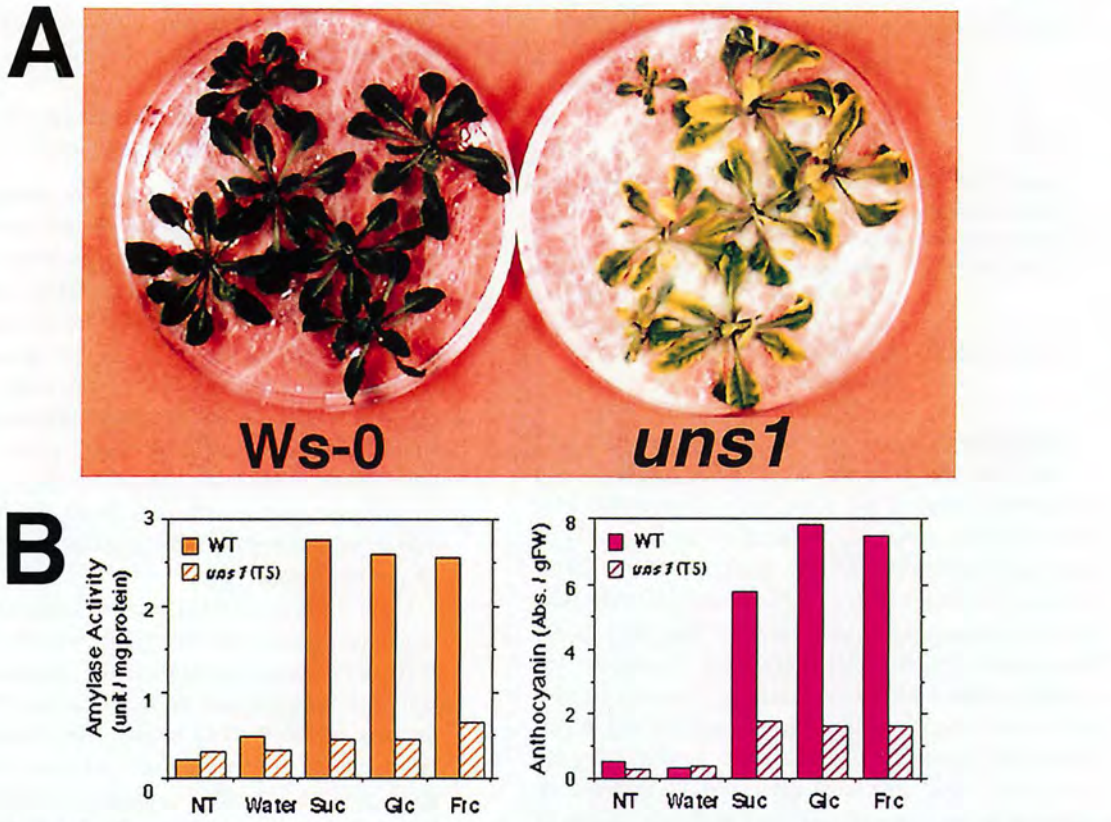

Fig.1 Phenotypes of wild type and homozygous mutant plants (*uns1*) grown for 3 weeks. The chlorophyll content of the mutant plants (Fig.1A, right) is much lower than that of wild type plants (Fig.1A, left). Mutant plants also showed the reduced levels of the sugar-inducible increase of β-amylase (Fig.1B, left) and anthocyanin (Fig.1B, right) when leaf explants were treated with high levels (5%) of sugars.

DEPARTMENT OF REGULATION BIOLOGY

Chairman: Norio Murata

The Department is composed of two regular divisions and two adjunct divisions. The study of this department is focused on the molecular mechanisms for the development of central nervous systems in mice, chickens and *Drosophila*, and also on the molecular mechanisms for the response of plants toward external environments, such as light, temperature and salinity.

DIVISION OF MOLECULAR NEUROBIOLOGY

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JST Technical Staff:	*Masae Mizoguchi*
	Megumi Goto
	Minako Ishida (Jan. 1, 1999 ~)

** to Washington University School of Medicine (Oct. 1, 1998 ~)*
*** to NIBB Center for Transgenic Animals and Plants (Sept. 1, 1998 ~)*
**** from Institute for Zoology, University of Heidelberg*

We have been studying the molecular and cellular mechanisms underlying the development and functioning of the vertebrate central nervous system. We are currently searching for and analyzing the functions of molecules involved in various cellular events in brain morphogenesis and brain function, such as generation of neuroblasts, their migration to form the laminar structure and various nuclei, elongation and path-finding of neural processes, the formation and refinement of specific connections between neurons, and also synaptic plasticity. We have been using various techniques including molecular biology (*e.g.* cDNA cloning, site-directed mutagenesis), biochemistry (protein purification, carbohydrate analysis), immunological methods (monoclonal-antibody production), neuroanatomy, cell and organotypic culture (immortalized cell-line production), and embryo manipulation (classical embryology, gene transfer with viral vectors, and gene targeting).

I. Molecular mechanism of retinotectal projection

A. Topographic projection

Topographic maps are a fundamental feature of neural networks in the nervous system. Understanding the molecular mechanisms by which topographically ordered neuronal connections are established during development has long been a major challenge in developmental neurobiology. The retinotectal projection of lower vertebrates including birds has been used as an readily accessible model system. In this projection, the temporal (posterior) retina is connected to the rostral (anterior) part of the contralateral optic tectum, nasal (anterior) retina to the caudal (posterior) tectum, and likewise dorsal and ventral retina are connected to the ventral and dorsal tectum, respectively. Thus, images received by the retina are precisely projected onto the tectum in a reversed manner. In 1963, Sperry proposed that topographic mapping could be guided by complementary position labels in gradients across pre-and postsynaptic fields. Although this concept is widely accepted today, and Eph receptor tyrosine kinases and their ligands were recently identified as candidates for such positional labels, the molecular mechanism of retinotectal map formation remains to be elucidated.

In 1993, we began to screen for topographic molecules which show asymmetrical distribution in the embryonic chicken retina. In the first-round screening, using a cDNA subtractive hybridization technique, we discovered several distinct transcripts which were topographically expressed along the nasotemporal (anteroposterior) axis in the retina. Among these, two winged-helix transcriptional regulators termed CBF-1 and CBF-2 were expressed in the nasal and temporal retina, respectively, and our misexpression experiments using a retroviral vector suggested that these two transcriptional factors direct the retinal ganglion cell axons to project to the appropriate tectal targets along the anteroposterior axis.

In 1997, to further search for topographic molecules in the embryonic retina, we performed a large-scale screen using a new cDNA display system called Restriction Landmark cDNA Scanning (RLCS) (Fig. 1A). A number of molecules displaying various asymmetrical expression patterns along the nasotemporal axis or dorsoventral axis in the retina have been identified. These included already known topographic molecules such as EphA3, CBF-2, etc. expressed along the nasotemporal axis, and ephrin-B2, EphB3, etc. expressed along the dorsoventral axis.

In 1998, we continued our efforts to identify all of the topographic cDNA clones and examine their expression patterns during development (Fig. 1B). Furthermore, with respect to the topographic molecules which might have important roles in formation of the retinotectal map and/or the neural network in the retina, we have started over- and misexpression experiments using viral vectors and *in ovo* electroporation.

B. Layer-specific projection

After reaching their appropriate target zones along the rostrocaudal and dorsoventral axes of the tectum, retinal axons begin to seek their appropriate termination sites among 15 distinct laminae within the tectum, of which only three or four receive retinal projections. The molecular and cellular bases of such

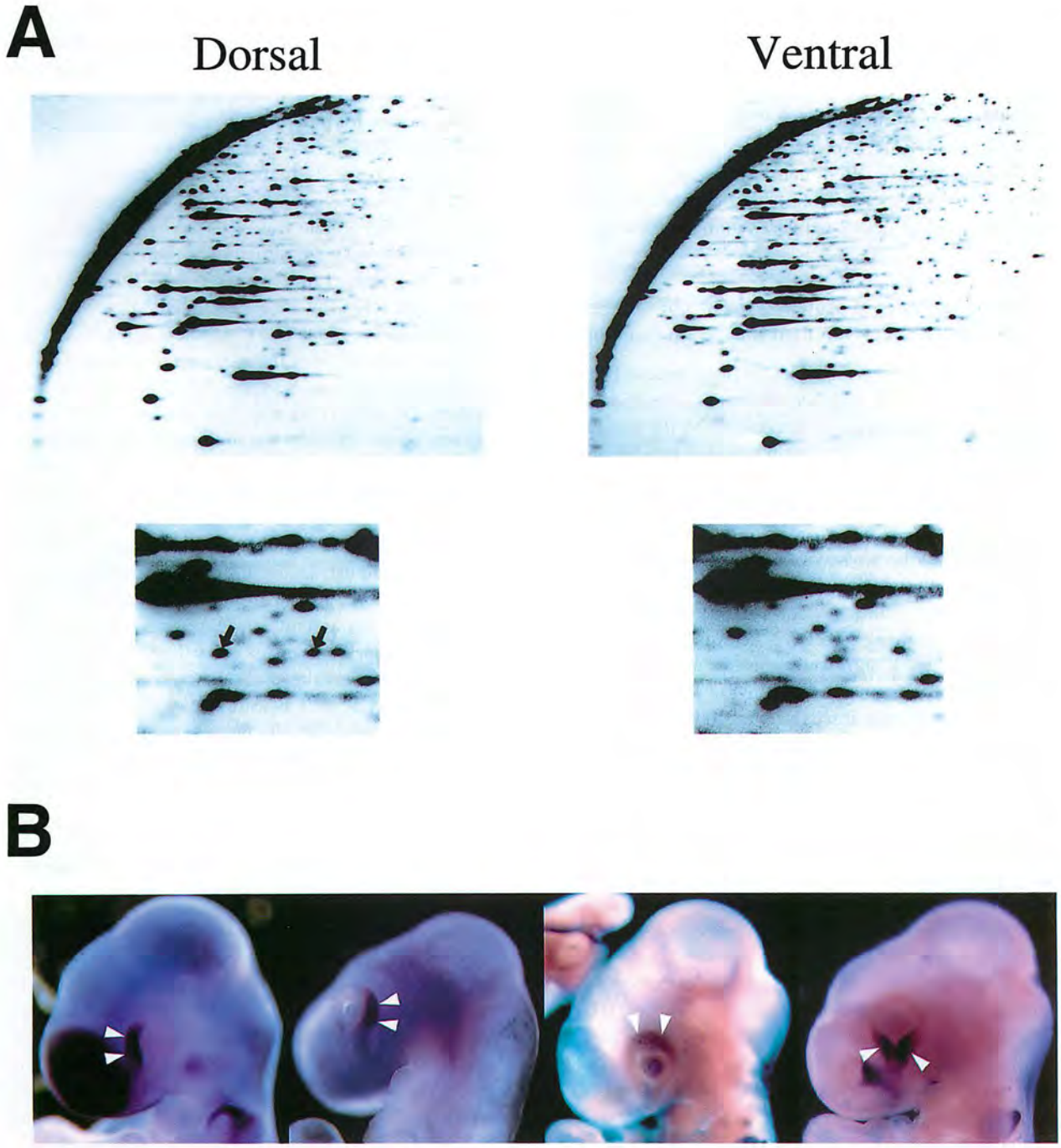

Fig. 1 Genes expressed in a region-specific fashion in the chick retina

(A) RLCS profiles for the dorsal and ventral retina. The lower panels are enlargements of corresponding regions of the top pictures. Spots indicated by arrows in the left panel are those of dorsal-specific expression. (B) Region-specific gene expression in the retina. Whole-mount *in situ* hybridization showed that these topographic genes were specifically expressed in the nasal, temporal, dorsal, or ventral region of the retina, respectively.

discrete target choice are poorly understood.

In 1994, we screened for monoclonal antibodies that recognize one of these retinal termination laminae. Among these, we found three clones TB5, TB2 and TB4, which labeled laminae B, D and F, respectively. cDNA cloning and immunochemical analysis revealed that the TB4 antigen molecule was ezrin, a cytoskeletal-membrane linker molecule belonging to the ezrin-radixin-moesin family. Ezrin was selectively expressed in a subset of retinal ganglion cells that project to the lamina F. Similar subset-selective expression and resultant lamina-selective distribution of ezrin were also observed in the lamina-specific central projections from the dorsal root ganglia. The staining pattern for TB4 in the dorsal root ganglia and spinal cord indicated that high expression of ezrin was restricted to cutaneous sensory neurons, but not muscle sensory neurons. Since ezrin modulates cell morphology and cell adhesion profiles by linking specific membrane proteins with the cytoskeleton, it was sug-

gested that ezrin may be involved in the formation and/or maintenance of lamina-specific connections for neuronal subsets in the visual and somatosensory systems.

We expect that our studies will lead to elucidation of the molecular mechanism underlying formation of the retinotectal projection, and ultimately to uncover the basic principles for establishing complicated but extremely precise neural networks in the nervous system.

II. Functional roles of protein tyrosine phosphatase ζ and γ in brain development and brain function

Protein tyrosine phosphorylation plays crucial roles in various aspects of brain development. The level of tyrosine phosphorylation is determined by the balance between the activities of protein tyrosine kinases and protein tyrosine phosphatases. Many types of receptor-like protein tyrosine phosphatases (RPTP) have been cloned and characterized. In 1994, we found that PTPζ/RPTPβ, a nervous system-specific RPTP, is expressed as a chondroitin sulfate proteoglycan in the brain. An RNA splice variant corresponding to the extracellular region of PTPζ is secreted as a major proteoglycan in the brain known as 6B4 proteoglycan/phosphacan. The extracellular region of PTPζ consists of a carbonic anhydrase (CAH)-like domain, a fibronectin type III (FN-III)-like domain and a serine-glycine-rich region, which is considered to be the chondroitin sulfate attachment region. PTPζ has another family member, RPTPγ, which also contains CAH-like and FN-III-like domains. We found that RPTPγ has four splice variants including an extracellular secreted form. However, they are not synthesized as proteoglycans and are expressed in various tissues including the brain, kidney, lung and heart.

In 1996, we found that PTPζ binds pleiotrophin/heparin-binding growth-associated molecule (HB-GAM) with high affinity (Kd=0.25 nM). The chondroitin sulfate portion of PTPζ is essential to achieve high affinity binding between PTPζ and pleiotrophin, and removal of chondroitin sulfate chains results in a decrease in the binding affinity (Kd=13 nM). This is the first demonstration that chondroitin sulfate plays an important regulatory role in growth factor signaling.

In the embryonic rat brain, pleiotrophin is localized along the radial glial fibers, a scaffold for neuronal migration. On the other hand, PTPζ is expressed in the migrating neurons, suggesting that the ligand-receptor relationship between PTPζ and pleiotrophin plays a role in migration of neurons during brain development. Thus, we examined the roles of pleiotrophin-PTPζ interaction in neuronal migration using the glass fiber assay and Boyden chamber cell migration assay. Pleiotrophin on the substratum stimulated migration of cortical neurons in both assays. Polyclonal antibodies against the extracellular domain of PTPζ, 6B4 proteoglycan (a secreted extracellular form of PTPζ) and sodium vanadate (a protein tyrosine phosphatase inhibitor) added to the culture medium strongly suppressed pleiotrophin-induced neuronal migration. These results suggested that PTPζ is involved in neuronal migration as a neuronal receptor for pleiotrophin distributed along radial glial fibers.

Furthermore, to study the physiological functions of PTPζ *in vivo*, we generated PTPζ-deficient mice in which the PTPζ gene was replaced by the *LacZ* gene by homologous recombination in mouse embryonic stem (ES) cells. First, we examined the cell types expressing PTPζ by investigating the expression of *LacZ* in heterozygous PTPζ-deficient mice. Throughout development from the early stage of embryogenesis, LacZ staining was restricted to the nervous system. On embryonic day 12.5 (E12.5), *LacZ* staining was observed in the forebrain, midbrain, hindbrain and spinal cord (Fig. 2A). Examination of the cerebral cortex at higher magnification indicated that subsets of neurons including pyramidal neurons expressed *LacZ* (Fig. 2B). At the early postnatal stages, subsets of neurons and astrocytes in the brain including pyramidal cells in the hippocampus expressed *LacZ* (Fig. 2C). Both neurons and astrocytes were positive for *LacZ* in primary culture of cells from the fetal cerebral cortex. From these results, we concluded that many neurons as well as astrocytes express PTPζ. We are currently studying the phenotype of homozygous mutant mice using biochemical, anatomical, physiological and ethological techniques, and various abnormalities have been found.

III. Functional roles of subfamily 2 sodium channels

Voltage-gated sodium channels (NaChs) are responsible for the depolarizing phase of action potentials in excitable cells and are essential for many physiological functions. Cloning of NaChs revealed marked conservation in the primary structures that underlies their functional similarity. Thus, all NaChs cloned had been grouped into a single gene family. However, recently, novel NaChs, human Nav2.1, mouse Nav2.3 and rat SCL11/Na-G, were cloned from inexcitable cells such as glial cells. These molecules closely resemble each other but are divergent from the previously cloned sodium channels including the regions involved in activation, inactivation and ion selectivity. Thus, these molecules have been grouped into a new subfamily of sodium channels (subfamily 2). To date, subfamily 2 channels have not been expressed in a functionally active form using *in vitro* expression systems, and therefore the functional properties of these NaChs are not yet clear. To clarify the cells expressing subfamily 2 sodium channels and their physiological functions *in vivo*, we planned to generate knock-out mice deficient in channel genes.

We successfully produced mutant mice in which the mNav2.3 gene was replaced with the *LacZ* or *neo* gene by gene targeting. Using these mice, we found that mNav2.3 gene expression was restricted to the

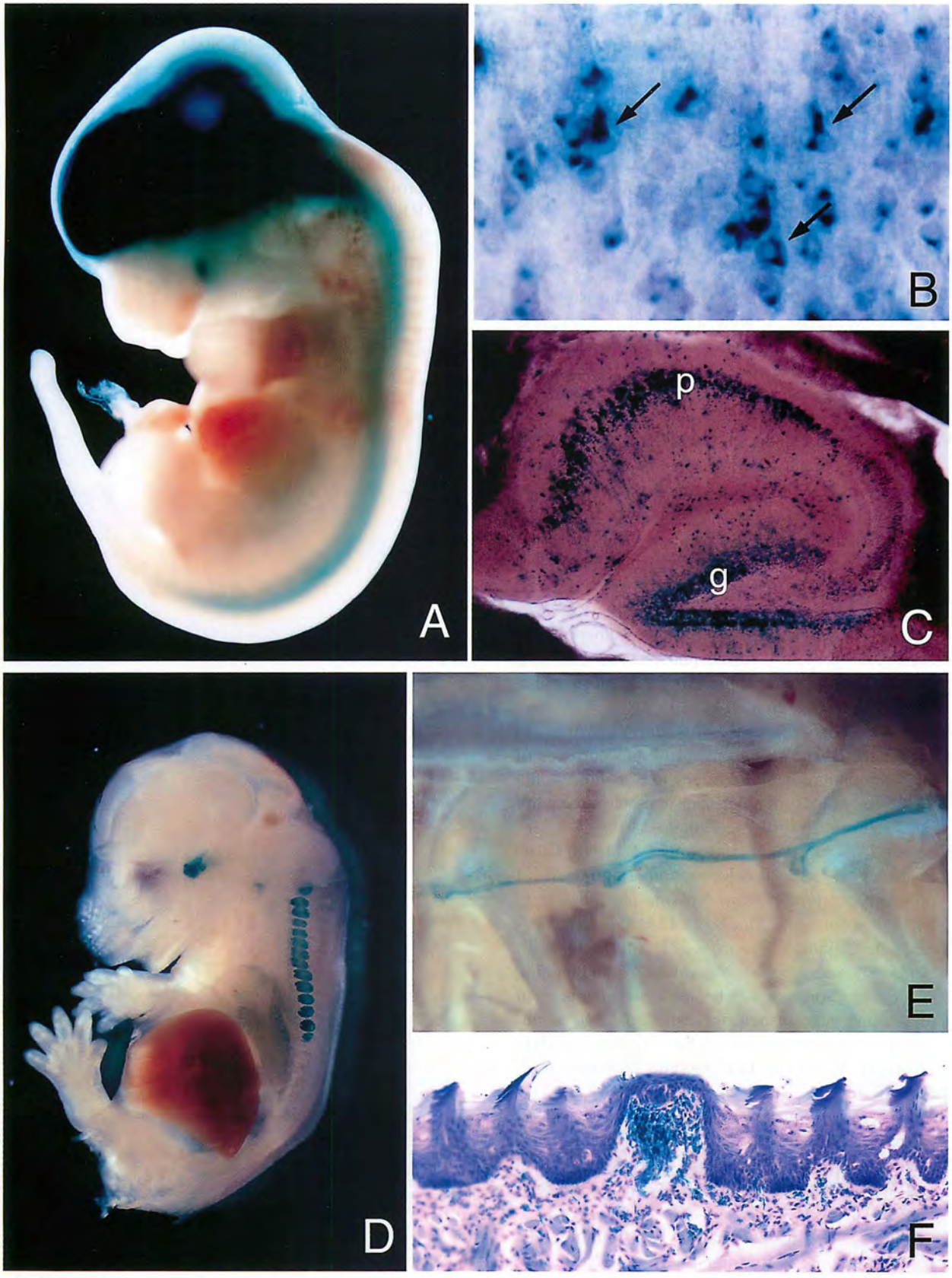

Fig. 2 *LacZ* expression in PTPζ$^{+/-}$ mice (A-C), and in mNav2.3$^{+/-}$ mice (D-F)

(A) E12.5 whole embryo, (B) P7 cerebral cortex section, and (C) section of adult hippocampus. (D) E15 whole embryo, (E) adult sympathetic nerve and ganglia, and (F) section of adult tongue. In (B), the section was counterstained with cresyl violet, and the arrows indicate the pyramidal cells. In (C), p and g indicate the pyramidal cell layer and granule cell layer, respectively. In (F), the section was counterstained with cresyl violet.

dorsal root ganglion (DRG) and lung during the embryonic stage (Fig. 2D). During the postnatal period, in addition to these tissues, Schwann cells in the sensory afferent nerve fibers (Fig. 2E, F) and a subset of neurons in the central nervous system were positive for mNav2.3 expression. We are currently examining the phenotypes of homozygous mNav2.3-deficient mice to gain insight into the physiological functions of this channel.

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DIVISION OF CELLULAR REGULATION

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The research efforts of this division are aimed at developing a full understanding of the molecular mechanisms by which plants are able to acclimate to and tolerate stresses that arise from changes in environmental conditions, with particular emphasis on temperature stress and salt stress. In 1998, we made significant progress in the following areas, using higher plants and cyanobacteria as our experimental materials.

I. Enhancement of the stress tolerance of plants by genetic engineering of the synthesis of glycinebetaine

Glycinebetaine (hereafter abbreviated as betaine) is a compatible solute that is found in a number of halotolerant species of plants and bacteria. It has been implicated in the protection of cellular functions against high concentrations of salt and other types of environmental stress. To examine the effects of betaine *in vivo* on the protection against high-salt stress, we transformed *Arabidopsis thaliana*, which does not normally accumulate betaine, with the *codA* gene for choline oxidase (which catalyzes the conversion of choline to betaine) from *Arthrobacter globiformis*. Transformed *Arabidopsis* plants were able to synthesize betaine and to tolerate high-salt stress. Moreover, transformation with the *codA* gene enhanced the protection of the photosynthetic machinery against low-temperature photoinhibition and accelerated the recovery of the machinery from photo-induced damage. Betaine also accumulated in the seeds of the transformed plants. Such seeds were more tolerant than seeds of wild-type plants to low temperatures during imbibition and germination. The growth of young seedlings of transformed plants was also accelerated at low temperatures. Furthermore, transformation with the *codA* gene significantly enhanced the tolerance of seeds to high temperatures during both imbibition and germination (Fig. 1), and young seedlings also exhibited enhanced tolerance to high temperatures. Thus, accumulation of betaine increased the tolerance of *Arabidopsis* to various stresses, namely, high-salt stress, light stress, low-temperature stress and high-temperature stress.

We also introduced the *codA* gene into rice plants by *Agrobacterium*-mediated transformation, aiming to enhance the tolerance of this important crop to salt stress. Transformed rice plants accumulated betaine and, as a consequence, exhibited tolerance to high-salt stress. During treatment with 0.15 M NaCl (high-salt stress), growth of both wild-type and transformed plants was inhibited and obvious damage, such as wilting, bleaching of chlorophyll and necrosis, was visible. After removal of the salt stress, transformed plants began to grow again at the normal rate after a significantly shorter time than the wild-type plants. The photosynthetic machinery of the transformed plants was more tolerant to salt stress and low-temperature stress than was that of the wild-type plants. Our results in *Arabidopsis* and rice demonstrate the potential usefulness of the *codA* gene in the genetic engineering of stress tolerance in a wide variety of agronomically important crops.

II. Important roles of polyunsaturated membrane lipids in stress tolerance

In studies conducted prior to 1998, we succeeded in inactivating genes for fatty-acid desaturases by targeted mutagenesis in the cyanobacterium *Synechocystis* sp. PCC 6803 and we produced several mutant strains in which the fatty acids of the membrane lipids contained abnormal numbers of unsaturated bonds. By comparing various properties of these strains, we demonstrated that polyunsaturated membrane lipids are important in the ability of the photosynthetic machinery to tolerate low temperatures. In order to extend this finding by an alternative approach, we introduced the *desA* gene for the Δ12 fatty-acid desaturase of *Synechocystis* sp. PCC 6803 into the wild-type strain *Synechococcus* sp. PCC

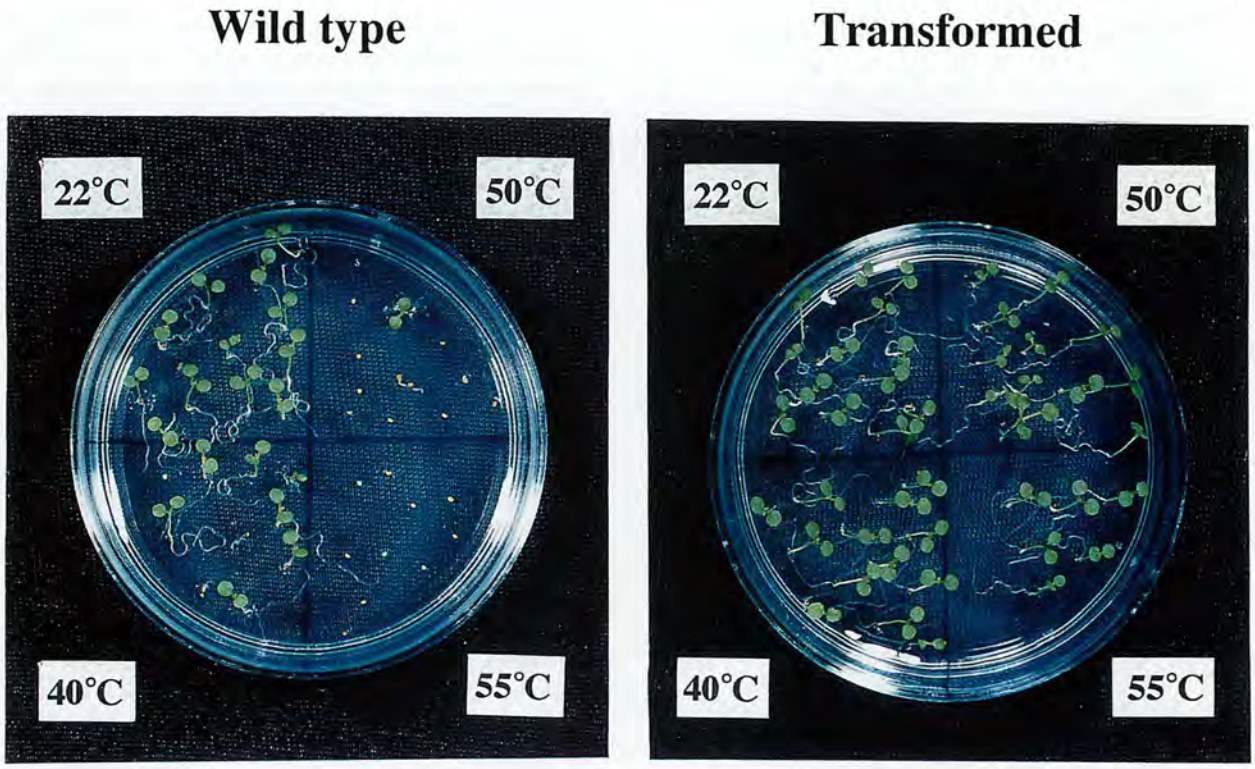

Fig. 1. Effects of high temperature during imbibition on the subsequent germination of seeds from wild-type and transformed plants of *Arabidopsis thaliana*. Dry seeds were allowed to imbibe water at 22, 40, 50 and 55°C for 60 min. Then seeds were inoculated on plates of MS medium. After incubation at 4°C for 2 days, the seeds were incubated at 22°C. Three days later, after germination had occurred, plates were photographed. Each plate was 9 cm in diameter.

7942, which normally contains monounsaturated fatty acids and no polyunsaturated fatty acids. We designated the transformed strain *desA*$^+$. The transformation resulted in replacement of half of the monounsaturated fatty acids in the membrane lipids by diunsaturated fatty acids. Comparison of *desA*$^+$ cells with wild-type cells revealed that the increased number of double bonds in the membrane lipids enhanced the ability of cells to resist photoinhibition at low temperatures by accelerating the recovery of the photosystem II complex from photoinhibitory damage. Our findings indicate that polyunsaturated membrane lipids are important in protecting the photosynthetic machinery from damage caused by strong light at low temperatures.

The D1 protein at the catalytic center of the photosystem II complex of *Synechococcus* sp. PCC 7942 exists as two isoforms, designated D1:1 and D1:2, and transfer of cells to low temperatures results in the replacement of D1:1, the prevailing form, by D1:2. The extent of such replacement in *desA*$^+$ cells at low temperatures was greater than that in wild-type cells. It seems likely that polyunsaturated membrane lipids facilitate the exchange of isoforms of the D1 protein and, in this way, they maintain the photochemical activity of the photosystem II complex at low temperatures.

We investigated the role of polyunsaturated membrane lipids in the tolerance of the photosynthetic machinery to high-salt stress by comparing the *desA*$^-$*/desD*$^-$ mutant of *Synechocystis* sp. PCC 6803, which contained monounsaturated fatty acids, with the wild-type strain, which contained a full complement of polyunsaturated fatty acids. The oxygen-evolving activity of *desA*$^-$*/desD*$^-$ cells was more sensitive to high-salt stress than was that of wild-type cells. Moreover, the activity of the Na^+/H^+ antiport in *desA*$^-$*/desD*$^-$ cells was suppressed to a greater extent than that of the antiport in wild-type cells under high-salt stress. These observations suggest that polyunsaturated membrane lipids might stimulate the activity and/or the synthesis of the Na^+/H^+ antiport system and protect the photosynthetic machinery against salt-induced inactivation.

III. Molecular mechanisms of the protection of the photosynthetic machinery against high-temperature stress

The oxygen-evolving machinery of the photosystem II complex is extremely susceptible to inhibition at high temperatures. We have been studying the molecular mechanisms that underlie the stabilization of the photosynthetic machinery against the heat-induced inactivation in the cyanobacterium *Synechococcus* sp. PCC 7002. Biochemical investigations of thylakoid membranes allowed us to identify two proteins, cytochrome c_{550} and PsbU, as factors that stabilize the oxygen-evolving machinery at high temperatures. To elucidate the role of PsbU *in vivo*, we inactivated the *psbU* gene in *Synechococcus* sp. PCC 7002 by tar-

geted mutagenesis. Mutated cells were not only unable to increase the thermal stability of their oxygen-evolving machinery but they were also unable to develop cellular thermotolerance upon acclimation to high temperatures. These results suggest that PsbU might play an important role in enhancing the thermal stability of the oxygen-evolving machinery at high temperatures and, moreover, that the stabilization of the machinery might be crucial for the acquisition of cellular thermotolerance.

IV. Stress-dependent enhanced expression of cytochrome c_M and suppression of the expression of cytochrome c_6 and plastocyanin

Cytochrome c_M is a *c*-type cytochrome with a molecular mass of 8 kDa. We previously identified and cloned the *cytM* gene for cytochrome c_M from *Synechocystis* sp. PCC 6803. We cloned homologs of the *cytM* gene from other cyanophytes and a prochlorophyte, providing evidence that suggests that the *cytM* gene might be distributed universally in oxygenic photosynthetic prokaryotes. Northern blotting analysis revealed that the *cytM* gene of *Synechocystis* sp. PCC 6803 is barely expressed under normal growth conditions. However, expression of the gene was induced when cells were exposed to low temperature and/or high-intensity light. By contrast, the expression of the *petJ* gene for cytochrome c_6 and expression of the *petE* gene for plastocyanin, which are electron carriers that transport electrons from the cytochrome b_6/f complex to the photosystem I complex, were suppressed at low temperatures and also under high-intensity light. These observations suggest that regulation of the expression of the *cytM* gene might be the mirror image of regulation of the *petJ* and *petE* genes under the stress conditions examined.

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DIVISION OF BIOLOGICAL REGULATION AND PHOTOBIOLOGY (ADJUNCT)

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Plants use light as an environmental factor which controls their development as well as their other physiological phenomena. Phytochrome and blue-light receptors, such as cryptochrome and phototropin (NPH1), are main photoreceptors for plant photomorphogenesis. The goal of our research is to clarify the signal transduction pathways of photomorphogenesis. We are focusing our study on chloroplast photorelocation movement which is thought to be one of the simplest phenomena in this field. We use the fern *Adiantum* gametophytes as a model plant not only because the gametophytes are very sensitive to light, but also because the organization of the cells is very simple and easy to manipulate. We also use *Arabidopsis* for mutant screening to clarify the genes regulating chloroplast photorelocation.

I. Cloning and characterization of blue-light photoreceptors in the fern *Adiantum capillus-veneris*

1-1 Cryptochromes

We have described many blue-light-induced photomorphological responses in gametophytes of the fern *Adiantum capillus-veneris*. As the first step in understanding the molecular mechanisms of these various blue-light responses in *Adiantum*, we isolated blue-light photoreceptor-related genes as homologues of *Arabidopsis* cryptochrome1.

We cloned five different genes, which are designated as *Adiantum* cryptochrome1, 2, 3, 4, and 5 (*CRY1, 2, 3, 4,* and *5*), from a genomic DNA library. The N-termini of the five genes showed remarkable homology to *Arabidopsis* cryptochrome1, as well as to other cryptochromes which have already been identified. However, these genes have every distinct C-termini from each other.

To analyze the correlation between blue-light-induced responses and cryptochromes, we investigated the expression pattern of these genes in various developmental stages and also in different light conditions using RNA blot analysis and competitive PCR analysis.

The results showed *CRY1, CRY2* and *CRY3* to have similar expression patterns. Transcripts of these genes were elevated after spores imbibed water in the dark, but after that the level of these transcripts did not vary much. The character of this expression pattern is very similar to those of previously characterized plant cryptochromes. *CRY4* and *CRY5* showed unique light-regulated expression patterns. *CRY4* was down regulated by light throughout the life cycle of *Adiantum*. The cDNA expression level was reduced by approximately 100 times when dark imbibed spores were irradiated with red light, which caused spore germination. *CRY5* expression was induced rapidly after light exposure and kept increasing for 12 hours. The expression level was reduced for the next 12 hours and after that it did not vary much.

The major change in *CRY4* and *CRY5* expression level occurred before spore germination, so transcripts of these two genes might act somewhat in the process of light dependent germination of *Adiantum*.

1-2 Phototropin

NPH1 (phototropin) is another blue-light photoreceptor recently isolated in higher plants, which is a flavin binding protein with a light sensitive protein kinase activity. By RT-PCR with primers designed from highly conserved sequence motifs in NPH1 photoreceptors, we cloned a cDNA fragment from *Adiantum*. The RT-PCR experiment suggested that the accumulation of mRNA for the *Adiantum* NPH1 is down regulated by light. The structural and biochemical characterization of *Adiantum* NPH1 is in progress.

II. Chloroplast relocation

2-1 *Arabidopsis*

Chloroplasts accumulate at cell surface under weak light and escape from strong light to optimize photosynthesis. Which photoreceptors are active in chloroplast relocation, however, is not known.

We are studying light-induced chloroplast relocation in *Arabidopsis* leaves. In a light-adapted mesophyll cell, chloroplasts spread over the cell surface, but in a dark-adapted cell they escape from the cell surface. When a cell is partly irradiated with blue light at a strength of $30Wm^{-2}$, chloroplasts move out from the irradiated spot. In comparison, chloroplasts accumulate in a spot irradiated with blue light at a strength of $3Wm^{-2}$. Simultaneous irradiation with red light increased the sensitivity to the blue light, although the red light irradiation alone did not induce chloroplast relocation.

Photoreceptor mutants, such as *cry1, cry2, cry1cry2* double mutant and *nph1*, were demonstrated to be normal for blue-light-induced chloroplast relocation for both weak and strong blue light, meaning thet CRY1, CRY2 and NPH1 are not the photoreceptors for chloroplast relocation. To find the photoreceptor for blue-light-induced chloroplast relocation, we are screening mutants from T-DNA tagging lines. Identification of the mutated genes of *Arabidopsis* mutants is now in progress.

2-2 *Adiantum*

Mutants of chloroplast relocation movement were screened and analyzed is *Adiantum* gametophytes.

Mutants which were deficient either in high fluence response or in low fluence response were obtained independently, meaning that the signal transduction pathways of both responses are different.

Publication:

Nozue, K., Kanegae, T., Imaizumi, T., Fukada, S., Okamoto, H., Yeh, K.C., Lagarias, J.C. and Wada, M. (1998) A phytochrome from the fern *Adiantum* with features of the putative photoreceptor NPH1. *Proc. Natl. Acad. Sci. USA*, **95**, 15826-15830.

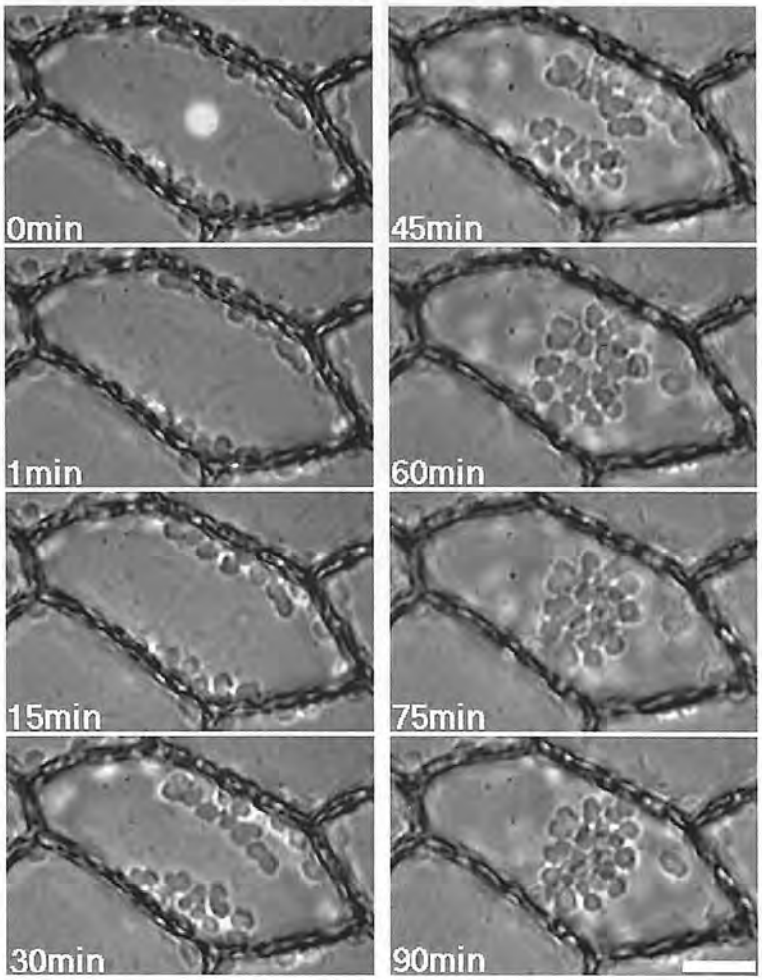

Figure 1. Serial photographs of chloroplast relocation in a dark-adapted (for 2 days) prothallial cell induced by one min illumination with a red microbeam (10 μm in diameter) of 30 Wm^{-2}. Chloroplast moved toward the beam after the beam was switched off over 70 min and then dispersed. Bar, 20 μm.

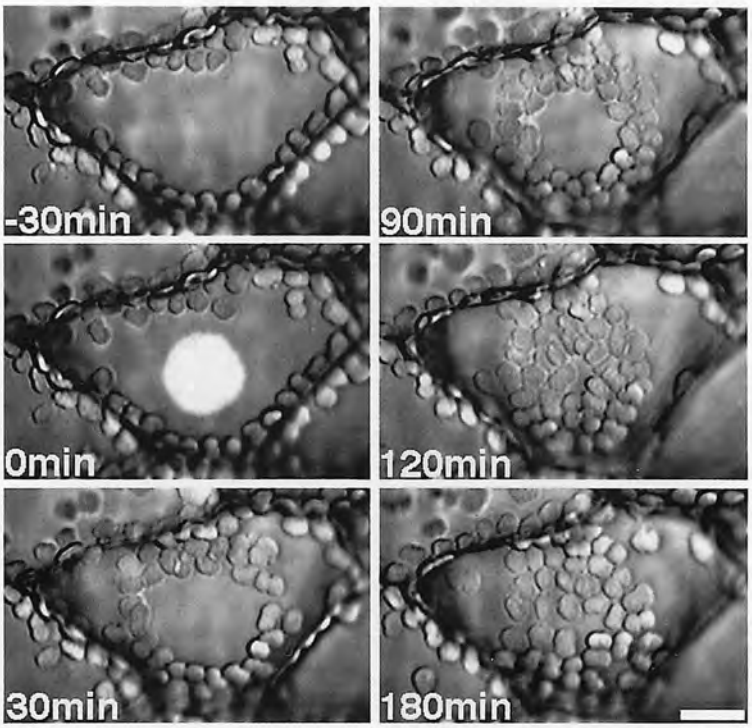

Figure 2. Serial photographs of chloroplast relocation in a dark-adapted prothallial cell induced by continuous blue microbeam irradiation (10 Wm^{-2}, 27 μm in diameter). The illumination spot is shown only time 0 but the light was given from time 0 to 90 min. The numbers shown in the photographs are the time after onset of blue light irradiation. Chloroplasts move toward the beam, but they can not get into the beam irradiated area until the microbeam was switched off. Bar, 20 μm.

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The vertebrate nervous system contains a large number of neurons that are connected to each other by specific axonal projections. We are interested in how the complex but precise neuronal network, which is indispensable for functioning of the nervous system, is constructed during development. The goal of our research in this division is to elucidate the cellular and molecular mechanisms underlying the neuronal network formation in vertebrates, with current focuses on the mechanisms of axon guidance and neuronal migration. Our laboratory was just started in April 1998 by two of the members and others joined thereafter. Plans of research but not results of experiments will be described below.

I. Mechanism of Axonal Guidance

During development of the nervous system, growing axons are guided through specific pathways to correct targets. Our research interest focuses on the cellular and molecular mechanisms of axon guidance in the vertebrate nervous system. We are particularly interested in how guidance mechanisms operating in different phases of axonal growth are assembled to generate the complex but precise axonal wiring patterns in the brain. To address these issues, we are first trying to understand axonal wiring patterns during development with reference to the structural organization of the brain. Using whole-mounted preparations of embryonic rat brains, we will reveal whole axonal trajectories by labeling with neuronal tracers, such as DiI, or by immunohistochemical staining with antibodies against molecular markers expressed in a subset of neurons. We will next explore the axonal guidance mechanisms generating these wiring patterns. Following questions will be addressed. 1) What structures along the pathway have key roles in axonal guidance? 2) What guidance cues exist in these structures? Are these attractive cues or repulsive cues? Are these short-range cues or long-range cues? 3) What molecules are responsible for these cues? 4) How do multiple guidance mechanisms work in concert to generate specific wiring patterns? To answer these questions, we use neuroanatomical techniques, in vitro culture techniques including dissociated cell, explant and whole-mount cultures, and biochemical and molecular biological techniques.

II. Mechanism of Neuronal Migration

A variety of neurons migrate from their birthplace to the position where they finally settle. We are also interested in the mechanisms underlying the neuronal migration in vertebrate central nervous system, in particular, how neurons are guided through specific routes to final positions. To address these issues, we are planning to develop in vitro culture systems that reconstruct migratory events occurring in vivo.

LABORATORY OF GENE EXPRESSION AND REGULATION

Head: Takashi Horiuchi

The laboratory consists of four regular divisions and conducts research into regulatory mechanisms of gene expression in higher plants and animals.

DIVISION OF GENE EXPRESSION AND REGULATION I

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The main interest of the group is in understanding the biology of the dynamic genome, namely, genome organization and reorganization and its impact on gene expression and regulation. Although there are many elements affecting organization and reorganization of the genomes, we are currently focused on mobile genetic elements in general and plant transposable controlling elements in particular.

I. Identification and characterization of mutable alleles in the Japanese morning glory

The Japanese morning glory (*Pharbitis nil or Ipomoea nil*) is a traditional horticultural plant in Japan, and extensive physiological and genetical studies on the plant have been conducted. A number of mutants related to the color and shape of its flowers and leaves have been isolated since the 17th century, and more than 200 genetic loci including about 20 mutable loci have been assigned to one of the 10 linkage groups. We have identified that the mutable *flecked* allele bearing white flowers with colored flecks is the *DFR-B* gene having the *En/Spm*-related transposable element *Tpn1* inserted into its second intron. The non-autonomous *Tpn1* element carries a genomic DNA segment containing at least four exon sequences encoding part of a HMG-box sequence. Spliced hybrid transcripts containing the *DFR-B* exon(s) and the *HMG* exons from *Tpn1* were detected in the flower buds of the *flecked* line, and they were polyadenylated within *Tpn1*. Thus *Tpn1* can be regarded as a specialized transducing transposon carrying a part of the genomic sequence for a HMG-box (Takahashi et al. (1999) Mol. Gen. Genet. in press).

The plants with the recessive mutable *speckled* allele, in the presence of the dominant *Speckled-activator*, produce colorless flowers with fine and round colored spots distributed over the corolla, while plants carrying the *speckled* allele without active *Speckled-activator* bear pale yellow flowers. Currently, we are analyzing the *CHI* gene containing another *En/Spm*-related element *Tpn2* in the mutable *speckled* lines. We are also characterizing not only other mutable alleles such as *purple-mutable* but also stable alleles displaying white flowers.

II. Identification of the mutable alleles in the common morning glory

The mutable a^{flaked} line of the common morning glory (*Pharbitis purpurea* or *Ipomoea purpurea*) also bears white flowers with colored flakes and sectors (**Fig. 1**). The mutable a^{flaked} allele is known to exhibit incomplete dominance. Interestingly, not only intensely colored flakes but also white spots and sectors were often observed in lightly colored flowers of the morning glory with the heterozygous state A/a^{flaked}. We showed that the mutable a^{flaked} allele is caused by insertion of a new transposable element, *Tip100*, into the *CHS-D* gene intron. *Tip100* is 3.9 kb long and belongs to the *Ac/Ds* family. Although the timing and frequency of the flower variegation vary in different lines (**Fig. 1A** and **B**), they carry the identical mutable allele (**Fig. 1E**).

It has been postulated that the timing and the frequency of the variegation are determined by the active state of another genetic element *modulator*. It was further postulated that some lines bearing white flowers must be a *modulator* inactive line whereas other white-flower lines must be deficient at the A locus, (**Fig. 1C** and **D**), because in the presence of active *modulator* the latter can produce variegated flowers while the former still display white flowers. Indeed, the structure of the *CHS-D* gene in one of the former lines we analyzed was found to be indistinguishable from that in the mutable a^{flaked} lines (**Fig. 1E**) whereas a white-flower line deficient at the A locus carries double insertions of *Tip100* (**Fig. 1F**). Presumably, excision of one of the two copies of *Tip100* from the *CHS-D* gene in the latter line appears to be insufficient to restore the *CHS-D* function and probably both elements are rarely excised in the same tissue.

III. Characterization of the genes for anthocyanin pigmentation in morning glories.

As an initial step to characterize the genes for anthocyanin pigmentation in morning glories, we sequenced segments of about 17 kb genomic DNA containing three *DFR* genes in the Japanese and common morning glories. The three *DFR* genes in both plants are arranged in tandem array, and all of them comprise six exons with identical intron positions. Their *DFR-B* genes, which carry longer introns than the *DFR-A* and *DFR-C* genes, are responsible for pigmentation in flowers, stems and coloring leaves. Indeed, the *DFR-B* gene of the common morning glory is expressed extensively in the young buds of pigmented flowers, considerably in stems, and moderately in sepals and leaves, whereas the *DFR-A* and *DFR-C* genes were expressed scarcely but significantly in the young flower buds and stems. Several novel mobile element-like sequences of around 200 bp were found in the

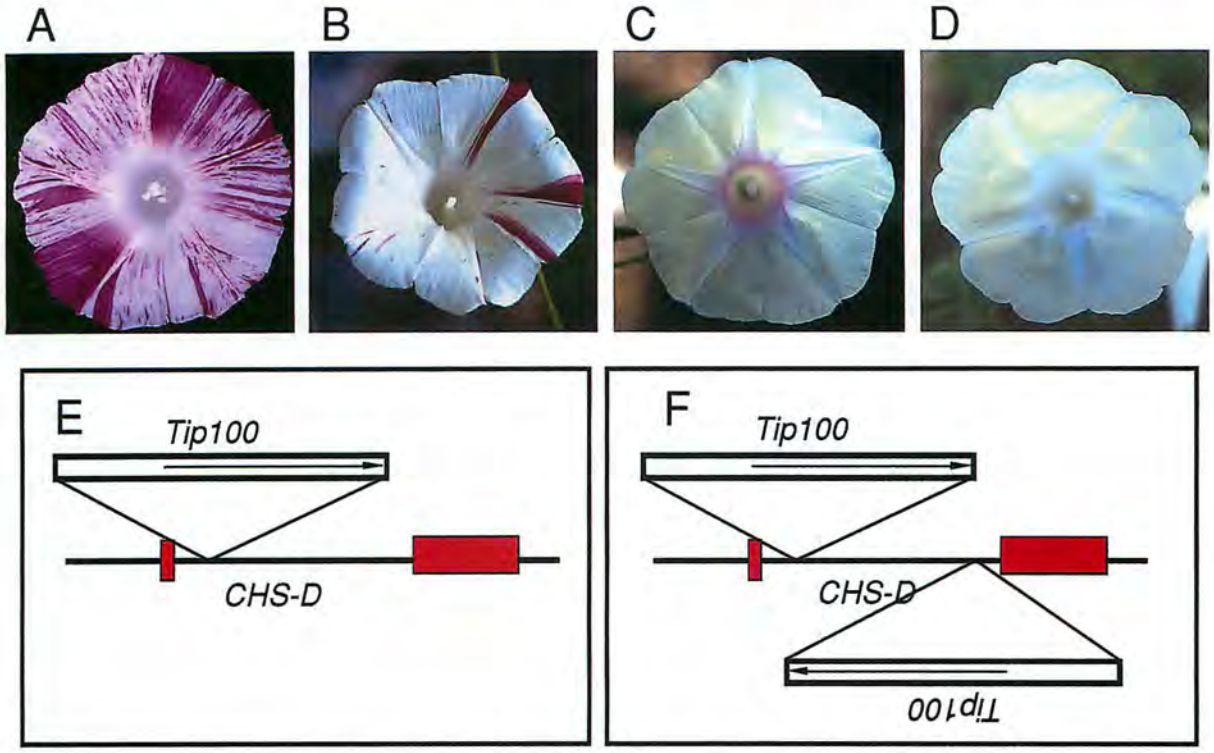

Fig. 1 The mutable lines of the common morning glory: heavily (A) and lightly (B) variegated lines; white-flower lines (C and D); structures of the *CHS-D* genes in the lines A, B and C (E) and the line D (F).

genomic *DFR* regions.

A phylogenetic tree indicated that each *DFR* gene in the Japanese morning glory is most closely related to the corresponding *DFR* gene in the common morning glory and that the *DFR-B* gene is the most diversified gene among the three *DFR* genes. At least two gene duplication processes must be involved in generating three tandem copies from a single copy, and significant sequence divergence in both exons and introns of the duplicated *DFR* genes might occur during evolution of the genomes of these morning glories. Since homologies between the corresponding *DFR* genes extend beyond the exon sequences to introns and intergenic sequences, the results can be interpreted to mean that duplications and major divergence occurred prior to speciation of the Japanese and common morning glories (Inagaki et al. (1999) Gene in press).

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DIVISION OF GENE EXPRESSION AND REGULATION II

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Homologous recombination which may occur in all organisms apparently involves exchange between two parent-derived chromatids plus the repair of DNA damage induced by physical and chemical reagents. As deduced from our analyses of recombination hotspots of *E. coli* and *S. cerevisiae*, in particular the activity related to DNA replication fork blocking events, the physiological function of homologous recombination, especially in normally growing cells is better understood. In 1998, work on the two following interrelated subjects have advanced our knowledge of related factors.

I. Molecular mechanism of expansion/contraction of ribosomal RNA gene repeats in *S. cerevisiae*.

HOT1 is a mitotic recombinational hotspot in the yeast *S. cerevisiae* and was first identified by Keil and Roeder (1984). HOT1 stimulates both intra- and inter-chromosomal recombination nearby regions when inserted at a non-rDNA location. HOT1 was originally cloned on a 4.6 kb *Bgl*II-A fragment which locates in rRNA repeated genes (about 150 copies) on chromosome XII. A single rRNA unit consists of two transcribed 35S and 5S rRNA genes and two non-transcribed regions, NTS1 and NTS2, the former is between 3'-ends of 35S and 5S rRNA genes, and the latter is 5' ends of these two genes. The HOT1 DNA fragment contains the NTS1, 5SRNA and NTS2 region but it was later found to be composed of two non-contiguous cis-elements, E and I, located in NTS1 and NTS2, respectively. Because E and I positionally and functionally overlapped with the enhancer and initiator of the 35S rRNA transcription, respectively, Roeder's group suggested that transcription by RNA polymerase I, initiated at the 35S rRNA promoter site may stimulate recombination of the downstream region, thereby revealing Hot1 activity.

The NTS1 has a site at which the replication fork is blocked and we termed this site SOG, but later called RFB (replication fork barrier). By assaying activity for various DNA fragments derived from the NTS1 and cloned on plasmids, we determined the minimal region, about 100 bp long, located near or within the enhancer region of the 35S rRNA transcription and essential for blocking replication fork advancing in a direction opposite that for transcription. The RFB sequence has no homology to any other known sequence and has no characteristic structure such 2-fold symmetry, repeated structure, etc.; hence, *trans*-factor(s) may have a role in blocking the fork. Interestingly, this region is included in one of two *cis*-elements required for a recombinational hotspot, Hot1, activity.

To investigate functional relationships between the fork blocking activity in RFB and the hotspot activity in HOT1, we first isolated mutants defective in Hot1 activity and examined whether these mutations would also affect RFB activity. Using a colony color sectoring assay method, we isolated 23 Hot defective mutants from approximately 40,000 mutagenized colonies. Among these Hot$^-$ mutants, one proved to be a *rad52* mutant; the other 4 mutants lose fork blocking activity. Genetic analysis of these mutants revealed that all four were recessive for the RFB phenotype and defined one complementation group. This mutation was designated *fob1* (fork blocking function) and one of the *fob1* mutants, *fob1-4*, was further analyzed. First, from yeast cDNA bank, we cloned *FOB1* gene by selecting a DNA fragment, which had suppressive activity for Hot deficiency of the mutant. The minimal *FOB1* plasmid was shown to complement both the Hot$^-$ and RFB$^-$ phenotype of the *fob1-4* mutant, suggesting that both phenotypes are caused by a mutation in the *FOB1* gene. DNA sequencing of the *FOB1* gene revealed that the putative Fob1 protein consists of 566 amino acids and has a molecular mass of 65,000 daltons. This gene has no homology with any DNA sequence registered in Genbank. Sequencing of the *fob1-4* mutant gene revealed two mutational changes in the open reading frame, one is non-sense (amber) and other is a miss-sense mutation. The amber mutation may account for the two defective phenotypes of the *fob1-4* mutant and why it is non-leakyness.

Because there is an extraordinary functional similarity between factors required for the hotspot activity in *E. coli* and *S. cerevisiae*, in both organisms ds-break and repair would occur when the DNA replication fork is arrested at fork blocking sites and probably was spontaneously blocked.

Next we investigated relationship between *FOB1* gene and expansion/contraction of rRNA gene (rDNA) repeats in collaboration with the laboratory of Dr. Masayasu Nomura in Univ. of California (Irvine). As described above, *S. cerevisiae* carries approximately 150 copies of rRNA gene in tandem repeats. It was found that the absence of an essential subunit of RNA polymerase I (PolI) in *rpa135* deletion mutants triggers a gradual decrease in rDNA repeat number to about one-half the normal level. Reintroduction of the missing *RPA135* gene induced a gradual increase in repeat number back to the normal level . Gene *FOB1* was shown to be essential for both the decrease and increase of rDNA repeats (Fig.1). Thus, DNA replication fork blockage appears to stimulate recombination and play an essential role in rDNA expansion/contrac-

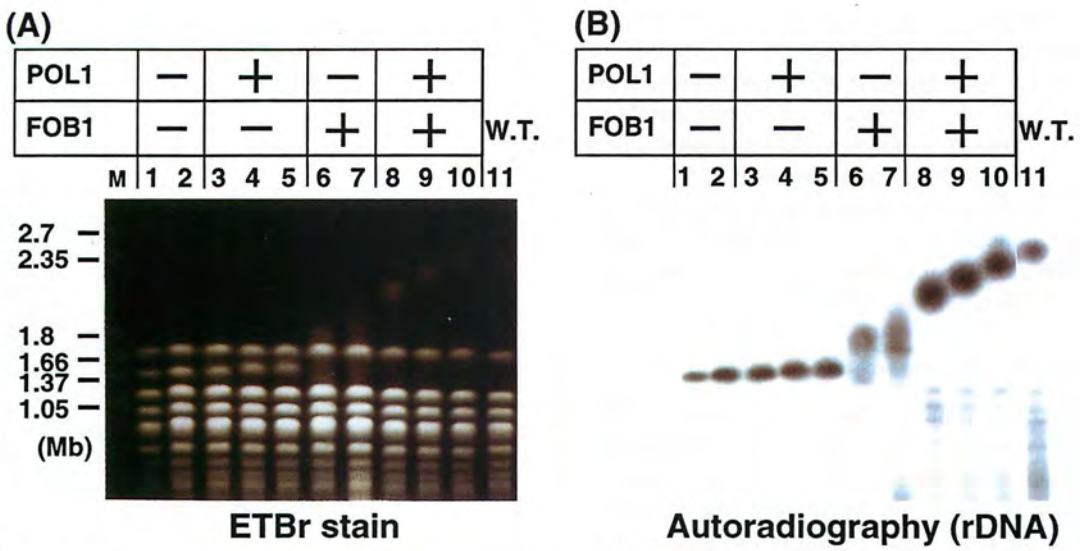

Figure 1. Increase of the size of chromosome by Fob1 and PolI proteins. (by Pulse-field gel electrophoresis)

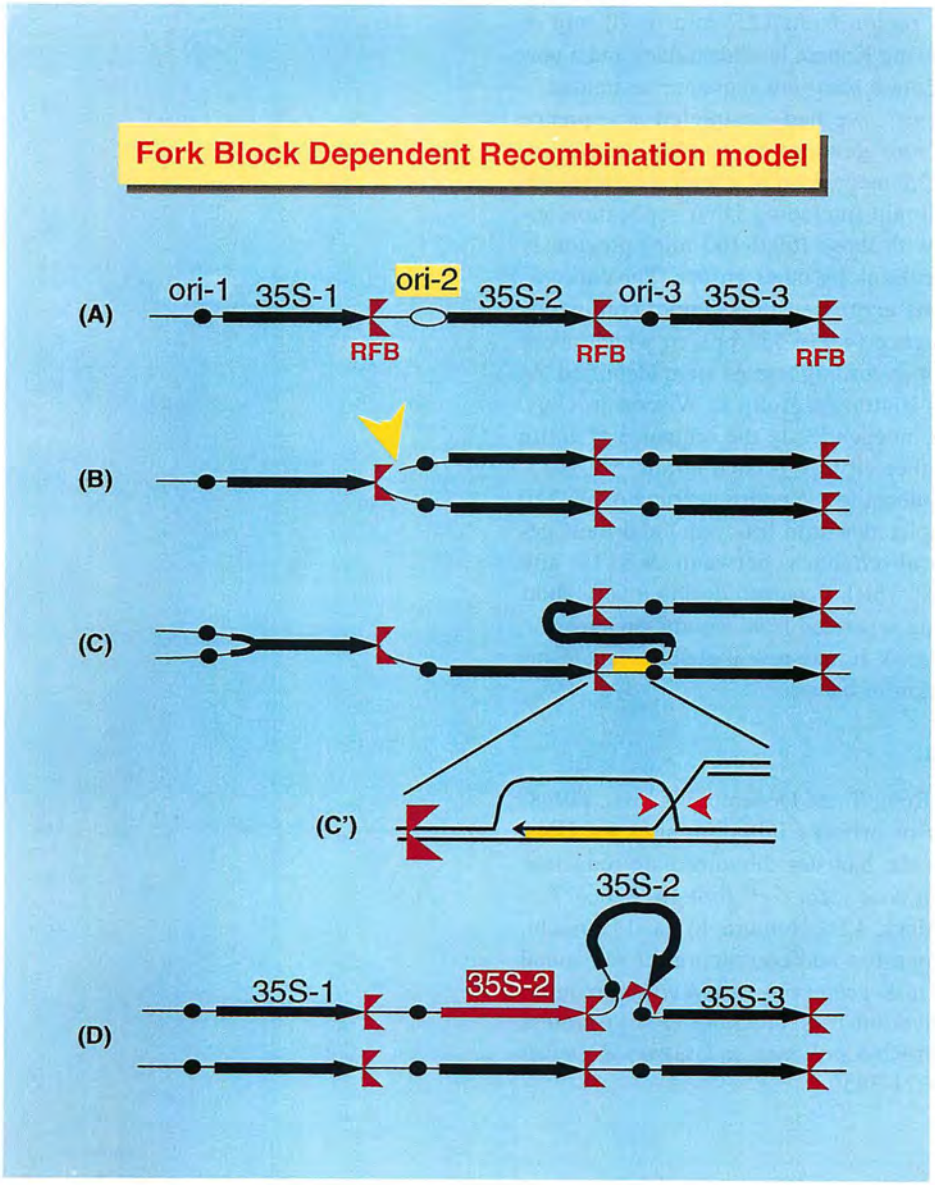

Figure 2. Fork block-dependent recombination model for rDNA expansion/contraction.

tion and sequence homogenization, and possibly, in the instability of repeated sequences in general (ex. expansion of triplet repeats). RNA polymerase I, on the other hand, appears to control repeat numbers, perhaps by stabilizing rDNA with the normal repeat numbers as a stable nucleolar structure.

We propose a model, shown in Fig.2, we call the fork block dependent recombination model to show how the blocked replication fork changes the copy number of rDNA.

II. The *E. coli* genome project

E. coli genome project in Japan was started in 1989, the objective being to analyze, in an independently living organism, nucleotide sequences from 0 min in a clockwise direction. In April 1995, the DNA sequences of the 0-12.7 min region in the genetic map were determined and they were published or registered. At that time I (T. H.) was appointed leader of the Japan *E. coli* genome project and a new group of researchers was reorganized to analyze DNA sequences of the region from 12.7 min to 70 min of W3110 strain, using Kohara lambda clones and a new protocol, including a shot-gun sequence technique.

In January 1997, we had completed a sequence analysis of *E. coli* genome by combining our sequence data of 2.2 mega base of a region corresponding to 12.7-69.0 min (including DNA replication terminus region) with those (69.0-100 min) previously registered in GenBank by other groups. The entire *E. coli* genome was expressed in a single, continuous nucleotide sequence (4,636,552 bp), in which about 4.300 putative open reading frames were identified. At this same time, Blattner's group in Wisconsin Univ. had determined independently the sequence of entire genome of another closely related strain, MG1655. Now we are sequencing the entire genome of W3110 (it will be completed within this year) and their genomic structural changes between W3110 and MG1655 strains, which occurred during a very short period after being separated from a common ancestor, are being compared. It may reveal about mechanisms of micro-evolution in bacteria.

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DIVISION OF SPECIATION MECHANISMS I

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Our research goal is to understand mechanisms underlying evolution of the nervous system. In order to approach this question, we are currently focusing on two systems.

I. Evolution of cytokine receptor families in the immune and nervous systems

It has been recognized that cytokines, defined as inter-cellular mediators in the immune system, have a variety of roles in the nervous system as well. One such a factor, LIF (leukemia inhibitory factor) known also as CDF (Cholinergic Differentiation Factor), is a pleiotropic factor which shows a remarkable repertoire of activities from embryonic stem cells to neurons (Yamamori, T., 1996). Recent study have revealed that CDF/LIF and its receptors belong to the IL-6 family and the receptor family.

Based on Bazan's model which predicted the cytokine receptor family as a member of immunoglobulin super gene family (1990) and the model of the interaction among the members of the IL-6 family (ligand) and the IL-6 receptor family (Taga and Kishimoto, 1992; Stahl and Yancopoulos, 1993), we proposed that the evolution of the IL-6/class IB receptor family may have occurred in at least two major steps (Yamamori and Sarai, 1994). Firstly, binding subunits of an IL-6 receptor and for a CDF/LIF receptor evolved and secondly, a third binding subunits of a CNTF receptor evolved. Our model predicts that the binding subunits generally determine the specificity of the receptors and it is possible that novel members of the cytokine family and their receptors exist in the nervous system. In order to prove this hypothesis, we are currently working to examine the expression pattern of the family in the nervous system.

II. Gene expression and cerebellar long-term plasticity

In order to know roles of the genes involved in long-term memory, we choose the cerebellum as a model system. In the cerebellum the conjunctive stimuli of parallel fibers and a climbing fiber to a Purkinje cell induce prolonged reduction of a synaptic efficacy between the paralleled fiber to the Purkinje cell (LTD; long-term depression, Ito et al., 1982).

Previously, we examined the expression of 10 immediate early genes (IEGs) including all the known Fos and Jun family in cerebellar slices under the pharmacological condition that cause long-term desensitization of the Purkinje cell to AMPA (a glutamate analogue). Among the IEGs examined, Fos and Jun-B were predominantly induced under the conjunctive condition (Nakazawa et al., 1993).

Recently, we have examined Jun-B expression in vivo under a conjunctive protocol of AMPA, a pharmacological substitute for parallel fiber stimulation, and climbing fiber stimulation via electric Inferior Olive stimulation. June-B are predominantly induced around the local area where the AMPA and climbing fiber stimulation were conjunct (Yamamori et al., 1995). These results suggest that the coincidence mechanism may exist at gene expression level and lead to a cerebellar long-term plasticity (Fig. 1).

Toward further confirming this hypothesis, we are currently identifying several molecules which are induced in Purkinje cells 3 hours after the conjunction in collaboration with Dr. Ryoji Yano's group (Brain Science Institute, RIKEN). One of these genes is currently further being characterized. We are also examining the expression of the genes under different types of, cognitive and procedural, learning tasks.

During our attempt to isolate LTD-related genes, we accidentally found repetitive genes which were specifically expressed in the rat brain (Bsr: brain specific repetitive gene). To our surprise, the genes are only found in the rattus but no other species so far examined including murine species. Although we do not know the function of this new type of gene at the moment, it may play some important role in the rat brain and we hope further characterization of the gene reveals it.

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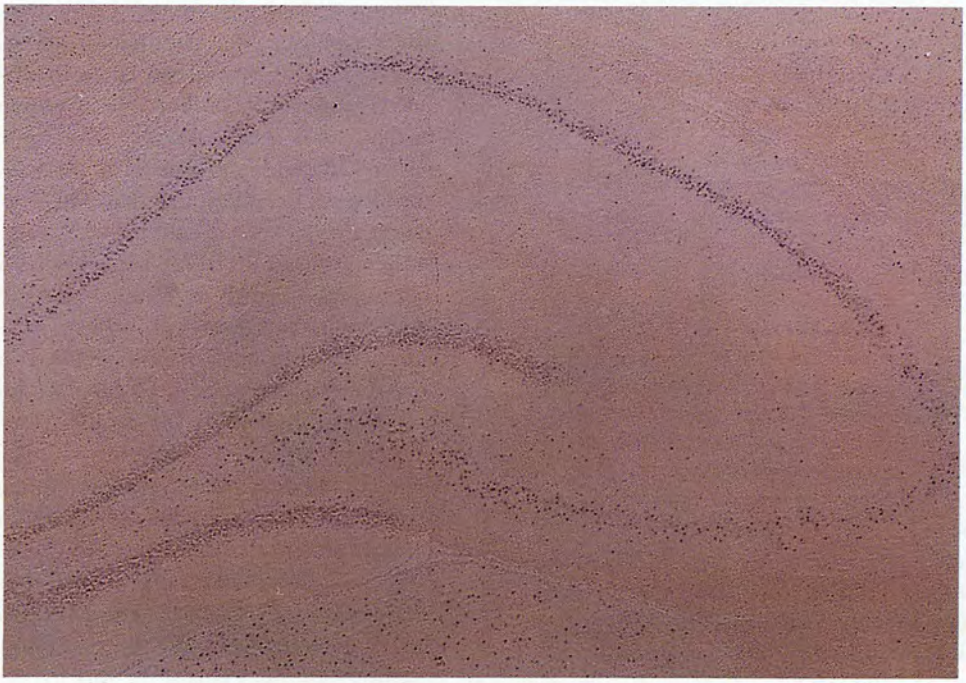

Fig. 1 Expression of Bsr in the rat hippocampus. In situ hybridization with an antisense probe of Bsr.

DIVISION OF SPECIATION MECHANISMS II

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All living organisms evolved from a common ancestor more than 35 billion years ago, and accumulated mutations on their genomes caused the present biodiversity. The traces of evolutionary processes are remained in the genomes of extant organisms and we can infer (1) the phylogenetic relationships of organisms and (2) the genetic changes having caused the phenotypic evolution by comparing the genomes of different organisms. The inferred phylogenetic relationships give important insights on problems in various fields of evolutionary biology and our group is now focusing on biogeography, evolution of morphological traits and systematics in wide range of taxa. On the phenotypic evolution, we are especially interested in the morphological evolution and aim to explore genetic changes led the evolution of plant body plan. We selected *Arabidopsis* (angiosperm), *Gnetum* (gymnosperm), *Ginkgo* (gymnosperm), *Ceratopteris* (pteridophyte), and *Physcomitrella* (bryophyte) as models to compare the genetic cascades regulating morphogenesis especially in the reproductive organs and shoot apical meristem of land plants.

I. Molecular phylogeny of plants and lichen

(a) Phylogeny of maple trees and their disjunct distribution between North America and eastern Asia. The maple tree genus, *Acer* is the largest genus in broad-leaved deciduous trees and contains about 200 species. The delimitation of the genus is clear but the intrageneric classification was controversial because of homoplasies in morphological characters. The phylogenetic relationship in *Acer* was inferred based on chloroplast DNA restriction site polymorphisms with 17 restriction endonucleases and previously proposed intrageneric classifications were evaluated. Based on the estimated evolutionary rate of chlotoplast DNA, divergence of eastern Asian and North American species in two different sections were estimated to have taken place in late Miocene. In consideration with previous data, multiple migrations and disjunctions are likely to have formed the eastern Asian and North American similar but disjunct distribution.

(b) Phylogeny of *Coriaria* and the biogeography. *Coriaria* is distributed in four separate areas in the world, which is the most conspicuous disjunct distribution in flowering plants. The phylogenetic relationships of 12 *Coriaria* species collected from the representative disjunct areas were inferred by comparing the combined data set of *rbcL* (a large subunit of ribulose 1,5-bisphosphate carboxylase / oxygenase) and *matK* (maturase K) genes. The estimated divergence time between the Eurasian species and other species distributed in the Southern Hemisphere was estimated as 63 and 59 million years ago using *rbcL* and *matK* molecular clocks, respectively. These results do not support the previously proposed hypotheses to explain the disjunct distribution based on the continental drift, but suggest that the distribution pattern was formed by several geographical migrations and separations in the Cenozoic.

(c) Phylogeny of lady ferns. The lady fern group, Physematieae is one of five tribes in the Dryopteridaceae and contains about 700 species distributed mainly in temperate forests. The classification of the group is controversial and nucleotide sequences of the chloroplast gene *rbcL* from 42 species of the fern tribe Physematieae (Dryopteridaceae) were analyzed to provide insights into the inter and intra generic relationships and the generic circumscriptions of the group. Phylogenetic relationships of several enigmatic genera were revealed.

(d) Other phylogenetic studies

The Hydrostachiacear and the Podostemaceae have unusual morphology in angiosperms and their phylogentic relationships are controversial. During the course of field expedition to Madagascar (August to September, 1998), more than 10 species of *Hydrostachis* and three species of Podostemaceae were collected and the phylogenetic analyses are in progress. Molecular phylogeny of *Myelochroa* (lichen), *Diplazium* (ferns), major groups in bryophytes, and the Droseraceae (carnivorous plants) are also in progress.

II Evolution of body plan in plants

(a) Evolution of reproductive organs. A flower is the most complex reproductive organ in land plants and composed of sepals, petals, stamens, and gynoecium (s). Female haploid reproductive cells are covered with a sporangium (nucellus) and two integuments, and further enclosed in a gynoecium. Male haploid reproductive cells (pollens) are covered with a sporangium (pollen sack). On the other hand, gymnosperms and ferns have simpler reproductive organs than angiosperms and lack sepals and petals. Female sporangia (nucellus) of gymnosperms are covered with only one integument. Sporangia of ferns have no integuments and are naked on the abaxial side of a leaf.

The development of floral organs is mainly regulated by the members of the MADS gene family whose members are transcription factors containing

the conserved MADS and K domains. MADS genes of angiosperms are divided into more than 10 groups based on the gene tree.

What kind of changes of the MADS genes caused the evolution of the complex reproductive organs in the flowering plant lineage? To address the function of the MADS genes in a plant with primitive reproductive structures lacking specialized floral organs, MADS genes were newly sought from the fern *Ceratopteris richardii*. A MADS gene tree, incorporating the seed plant and fern MADS genes, revealed that the known fern MADS genes form only three gene groups. The number is considerably less than the number of MADS gene groups in seed plants. The expression patterns of five genes, representing all three fern MADS gene groups, during sporophyte and gametophyte development were examined. In flowering plants, some MADS genes are expressed in specific floral organ primordia as homeotic selector genes, while other MADS genes are expressed in both reproductive and vegetative organs. Like the latter type of flowering plant MADS genes, most of the fern MADS genes are expressed in both reproductive and vegetative organs. The ubiquitously-expressed MADS genes may be more primitive than the reproductive organ-specific MADS genes. If so, it is likely that some of the MADS genes were co-opted as homeotic selector genes of specialized reproductive organs and their expression restricted to specific floral organ primordia, events that occurred after the divergence of ferns and angiosperms. To verify the hypothesis, analyses of MADS genes in gymnosperms (*Gnetum* and *Ginkgo*) with more complexed reproductive organs than ferns, but simpler ones than angiosperms, are in progress. The restriction of MADS gene expression may have been caused by the evolution of other genes that regulate the MADS genes. *LEAFY* gene is one of the regulators of *Arabidopsis* floral homeotic MADS genes. The *LEAFY* gene homolog of *Gnetum* has been cloned and the characterization is in progress. The roles of MADS genes in non-flowering plants are mysterious and characterization of MADS gene functions in the moss *Physcomitrella patens* will give insights for the point.

(b) Establishment of tagged mutant library of the moss *Physcomitrella patens*. Mosses have the different body plan from flowering plants. Leafy shoots of mosses are similar to the ones of angiosperms, but develop in the gametophytic generation instead of the sporophytic generation as angiosperms. Organs of mosses are much simpler than flowering plants. For example, the leaves are composed of one layer of cells. Therefore, the body plan of mosses may be regulated by different genes from angiosperms. In addition to analyses of the homologs of angiosperm genes governing morphogenesis, it is necessary to screen specific genes in the moss. We established enhancer and gene trap lines and tagged mutant libraries of *Physcomitrella patens* to clone genes related to leafy shoot development. *P. patens* is known by its high rate of homologous recombination and suitable for molecular biological analyses using the gene targeting. Our libraries should be also useful for other purposes.

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Wolf, P.G., Pryer, K.M., Smith, A.R. and Hasebe, M.

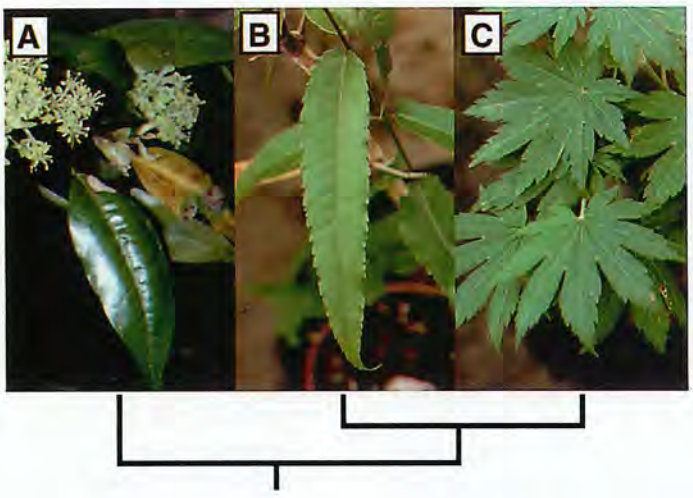

Fig. 1. Phylogeny of *Acer*. (A) *A. albopurpurascens*, (B) *A. laevigatum*, (C) *A. pseudosieboldianum*. (A) and (B) have been classified in the same group because of their peculiar simple leaves in maple trees. Phylogenetic tree based on RFLPs of chloroplast genome revealed that species with simple leaves (A and B) are polyphyletic and (B) is more closely related to the species with palmate leaves (C).

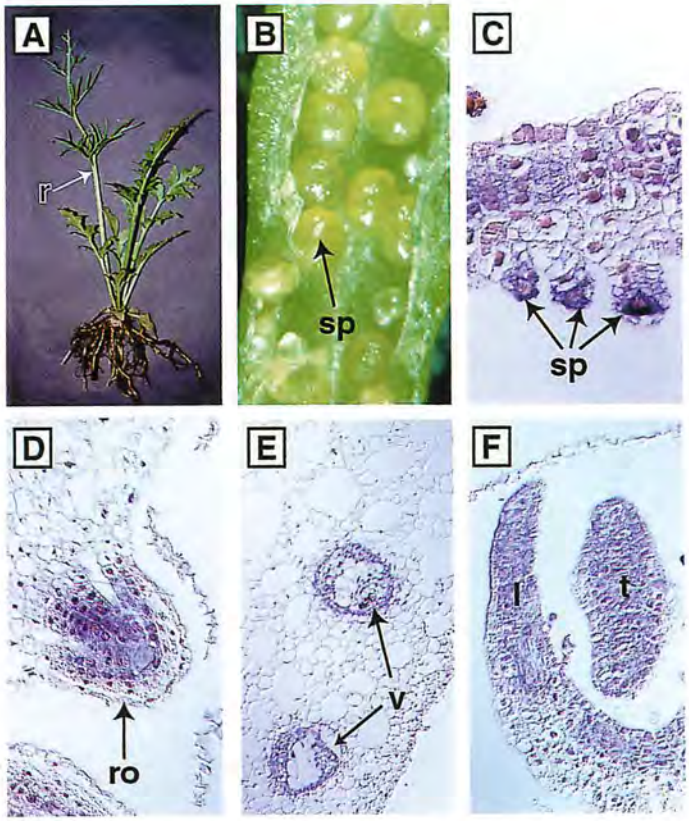

Fig. 2. Patterns of CMADS1 RNA expression in sporophyte tissues as detected by in situ hybridization. MADS genes of *Ceratopteris richardii* are expressed in both reproductive (C) and vegetative (D, E, and F) tissues. (A) *C. richardii* sporophyte. A whole plant with reproductive (r) and vegetative leaves. (B) Naked sporangia (sp) on the abaxial surface of a reproductive leaf. (C) Longitudinal section of a sporophyll showing sporangia (sp). (D) Longitudinal section of the growing tip of a root (ro). (E) Transverse section of a petiole showing vascular bundles (v). (F) A longitudinal (l) and a transverse (t) section of two young vegetative leaves.

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RESEARCH SUPPORT

TECHNOLOGY DEPARTMENT

Head: Hiroyuki Hattori

Common Facility Group
Chief: Kazuhiko Furukawa

Reseach Support Facilities
Mamoru Kubota (Subunit Chief)
Shoichi Higashi (Subunit Chief)
Tomoki Miwa (Subunit Chief)
Chieko Nanba
Toshiki Ohkawa
Kaoru Sawada
Makiko Itoh (Technical Assistant)
Keiko Suzuki (Technical Assistant)
Misayo Masuda (Technical Assistant)

Radioisotope Facility
Yousuke Kato (Subunit Chief)
Yoshimi Matsuda (Subunit Chief)
Naoki Morooka
Takayo Itoh (Technical Assistant)

Center for Analytical Instruments
Sonoko Ohsawa
Yumiko Makino
Tomoko Mori
Takeshi Mizutani
Hatsumi Moribe (Technical Assistant)

Glassware Washing Facility
Masayo Iwaki
(Kazuhiko Furukawa)
(Toshiki Ohkawa)

Research Support Group

Cell Biology Group
Maki Kondo (Subunit Chief)
Yukiko Kabeya
Katsushi Yamaguchi

Developmental Biology Group
Hiroko Kobayashi (Unit Chief)
Chiyo Takagi
Sanae Oka
Naomi Sumikawa

Regulation Biology Group
Hideko Nonaka
Akiko Oda
Shigemi Takami

Gene Expression and Regulation Group
Sachiko Tanaka
Koji Hayashi
Yasushi Takeuchi
Hideko Utsumi

The Technology Department is a supporting organization for researchers and research organization within the NIBB. The Department develops and promotes the institute's research activities and at the same time, maintains the research functions of the institute.

The department is organized into two groups: one, the Common Facility Group, which supports and maintains the institute's common research facilities and the other, the Research Support Group, which assists the research activities as described in individual reports.

Technical staffs participate, through the department, in mutual enligtement and education increase their capability in technical area. Each technical staff is proceeded to the fixed division usually and they support the various research with their special biological and biophysical techniques.

The Department hosts an annual meeting for technical engineers who work in various fields of biology at universities and research insititutes throughout Japan. At this meeting, the participants present their own activities and discuss technical problems. The Proceedings are published soon after the meeting.

RESEARCH SUPPORT FACILITY

Head of Facility: *Yoshinori Ohsumi (April 1, 1998-)*
Associate Professor: *Masakatsu Watanabe*
Research Associates: *Yoshio Hamada, (Tissue and Cell Culture)*
Atsushi Ogiwara (Computer)
Post Doctral Fellow: *Mineo Iseki [1] (Large Spectrograph) (-October 31, 1998)*
Technical Staffs: *Mamoru Kubota (Large Spectrograph)*
Chieko Nanba (Plant Culture, Farm, Plant Cell)
Toshiki Ohkawa (Computer)
Kaoru Sawada (Tissue and Cell Culture)
Tomoki Miwa (Computer)
Sho-ichi Higashi (May 1, 1998-) (Large Spectrograph)
Makiko Ito (Large Spectrograph)
Misayo Masuda (Computer)
Keiko Suzuki (Plant Culture, Farm, Plant Cell)

I. Facilities

1. *The Large Spectrograph Laboratory*

This laboratory provides, for cooperative use, the Okazaki Large Spectrograph (OLS), which is the largest spectrograph in the world, dedicated to action spectroscopical studies of various light-controlled biological processes.The spectrograph runs on a 30kW Xenon arc lamp and has a compound grating composed of 36 smaller individual gratings. It projects a spectrum of a wavelength range from 250nm (ultraviolet) to 1,000nm (infrared) onto its focal curve of 10m in length. The fluence rate (intensity) of the monochromatic light at each wavelength is more than twice as much as that of the corresponding monochromatic component of tropical sunlight at noon (Watanabe et al., 1982, *Photochem. Photobiol.*, 36, 491-498).

A tunable two-wavelength CW laser irradiation system is also available as a complementary light source to OLS to be used in irradiation experiments which specifically require ultra-high fluence rates as well as ultra-high spectral-, time-and spatial resolutions.It is composed of a high-power Ar-ion laser (Coherent, Innova 20) (336.6-528.7 nm, 20W output), two CW dye lasers (Coherent, CR599-01) (420-930nm, 250-1000mW output), A/O modulators (up to 40MHz) to chop the laser beam, a beam expander, and a tracking microbeam irradiator (up to 200mm s^{-1} in tracking speed, down to 2mm in beam diameter) with an infrared phase-contrast observation system.

2. *Tissue and Cell Culture Laboratory*

Various equipments for tissue and cell culture are provided. This laboratory is equipped with safely rooms which satisfy the P2/P3 physical containment level. This facility is routinely used for DNA recombination experiments.

3. *Computer Laboratory*

To meet various computational needs and to provide means of electronic communication, many kind of computers are equipped: UNIX servers and engineering workstations (Sun Ultra Enterprise server, SPARC stations, IRIS machines, NEWS machines, etc.), and some personal computers (Macintosh's and Windows machines). All of these machines, as well as almost every PC in each laboratory, are connected each other with local area networks, which are also linked to the high performance multimedia backbone network of Okazaki National Research Institutes.

The Computer Laboratory provides various computational services to the institute members: file servers for Macintosh and NetWare users, print servers for PC and UNIX users, computational servers that provides sequence analyses, database retrievals, homology search services, and so on. Providing network communication services is also an important task of this laboratory. We maintain the World-Wide Web server that contains the NIBB home pages (URL is http://www.nibb.ac.jp).

4. *Plant Culture Laboratory*

There are a large number of culture boxes, and a limited number of rooms with environmental control for plant culture. In some of these facilities and rooms, experiments can be carried out at the P1 physical containment level under extraordinary environments such as strong light intensity, low or high temperatures.

5. *Experimental Farm*

This laboratory consists of two 20 m^2 glass-houses with precise temperature and humidity control, three green houses (each 6 m^2) at the P1 physical containment level, a small farm, two greenhouses (45 and 88 m^2) with automatic sprinklers, two open aquariums (30 and 50t) and several smaller tanks. The laboratory also includes a building with office, storage and work space.

6. *Plant Cell Laboratory*

Autotrophic and heterotrophic culture devices and are equipped for experimental cultures of plant and microbial cells. A facility for preparation of plant cell cultures including an aseptic room with cleanbenches, is also provided.

7. *Laboratory of Stress-Resistant Plants*

This laboratory was founded to study transgenic plants with respect to tolerance toward various environmental stresses. It is located in the Agricultural Experimental Station of Nagoya University (30km from the National Institute for Basic Biology). The laboratory provides a variety of growth chambers that precisely control the conditions of plant growth and facilities for molecular biological, and physiological evaluations of transgenic plants.

The laboratory is also a base of domestic and inter-

national collaborations devoted to the topic of stress-resistant transgenic plants.

II. Research activities

1. *Faculty*

The faculty of the Research Support Facility conducts its own research as well as scientific and administrative public services.

(1) Photobiology: photoreceptive and signal transduction mechanisms of phototaxis of single-celled, flagellate algae are studied action spectroscopically (Watanabe 1995, *In* CRC Handbook of Organic Photochemistry and Photobiology) by measuring computerized-videomiceographs of the motile behavior of the cells at the cellular and subcellular levels (Matsunaga et al., 1998). Photo-receptive and signal transduction mechanisms of algal gametogenesis are also studied by action spectroscopy.

(2) Developmental Biology: Notch is an integral cell surface membrane protein that is known to play a key role in developmental cell-cell interactions in *Drosophila*, particularly in lateral specification of neural versus epidermal cell fates, a process described thus far only in invertebrates. It is thought to act by a direct signaling pathway rather than through one of the classical signal transduction cascades. The mammalian genome is known to contain three Notch homologues but their developmental significance is not clear. To investigate their role in mammalian development, we have sequenced the murine Notch 2 cDNA, determined the primary sequence of its protein, and have investigated its genomic organization. We are now attempting to produce a mutant in which the ankyrin repeat region of Notch 2 is replaced by lacZ. Analysis of the mutant phenotype will provide us with insights about the significance of the repeat in Notch 2 signal transduction in relation to its developmental importance.

(3) Computational Biology: Today, many kinds of genome structures of various organisms have been revealed. Using these genomic sequence data, we performed a comprehensive sequence analysis especially for *Bacillus subtilis*. Microbial genomics has entered a new era of functional analysis, and bioinformatics is also shifting to support the functional analysis. We have developed a knowledge base system for the transporter system of B. subtilis. The system not only represents functional information but also helps to discriminate transporter genes in newly determined genomic sequences.

2. *Cooperative Research Program for the Okazaki Large Spectrograph*

The NIBB Cooperative Research Program for the Use of the OLS supports about 30 projects every year conducted by visiting scientists including foreign scientists as well as those in the Institute.

Action spectroscopical studies for various regulatory and damaging actions of light on living organisms, biological molecules, and organic molecules have been conducted (Watanabe, 1995, *In* CRC Handbook of Organic Photochemistry and Photobiology).

Publication List:

I. Faculty

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Atsushi Ogiwara (1998). Systematic Classification of *B. subtilis* Genes and Construction of a Knowledge Base to Represent Functional Information. *Proc. Genome Informatics 1998*, 310-311.

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Mimuro, M., Tamai, N., Murakami, A., Watanabe, M., Erata, M., Watanabe, M.M., Tokutomi, M. and Yamazaki, I. (1998) Multiple pathways of the excitation energy flow in the phtosynthetic pigment system of a cryptophyta, Cryptomonas sp. (CR-1). *Phycological Res.*, **46**, 155-164.

II. Cooperative Research Program for the Okazaki Large Spectrograph

Furukawa, T., Watanabe, M. and Shihira-Ishikawa, I. (1998). Green- and Blue-Light-mediated chloroplast migration in the centric diatom, *Pleurosira laevis*. *Protoplasma*. **203**, 214-220.

Furusawa, Y., Quintern, L.E., Holtschmidt, H., Koepke, P. and Saito, M. (1998). Determination of erythema-effective solar radiation in Japan and Germany with a spore monolayer film optimized for the detection of UVB and UVA -results of a field campaign. *Appl. Microbiol Biotechnol.* **50**, 597-603.

Matsunaga, S., Hori, T., Takahashi, T., Kubota, M., Watanabe, M., Okamoto, K., Masuda, K. and Sugai, M. (1998). Discovery of signaling effect of UV-B/C light in the extended blue~UV-A-type action spectra for step-down and step-up photophobic responses in the unicellular flagellate alga *Euglena gracilis*. *Protoplasma* **201**, 45-52.

Munakata, N., Ono, M. and Watanabe, S. (1998). Monitoring of solar-UV exposure among schoolchildren in five japanese cities using spore dosimeter and UV-coloring Labels. *Jpn. J. Cancer Res.* **89**, 235-245.

Shinomura, T., Hanzawa, H., Schäfer, E. and Furuya, M. (1998). Mode of phytochrome B action in the photoregulation of seed germination in *Arabidopsis thaliana*. *Plant J.* **13**, 583-590.

Takeuchi, Y., Murakami, M., Nakajima, N., Kondo, N. and Nikaido, O. (1998). The photorepair and photoisomerization of DNA lesions in etiolated cucumber cotyledons after irradiation by UV-B depends on wavelength. *Plant Cell Physiol.* **39**, 745-750.

Torikai, A. and Hasegawa, H. (1998). Wavelength effect on the accelerated photodegradation of polymethylmethacrylate. *Polym. Degradn. Stab.* **61**, 361-364.

Torikai, A., Kobatake, T. and Okisaki, F. (1998). Photodegradation of polystyrene containing flame retardants. *J. Appl. Polym. Sci.* **67**, 1293-1300.

Torikai, A. (1998) Wavelength sneistivity in the photodegradation of polymethylmehtacrylate: Accelerated degradation and gel formation. In "Science and Technology of Polymers and Advanced Materials". (Prasad, P. N. et al. ed.), Plenum Press, New York, pp. 581-586.

RADIOISOTOPE FACILITY
(Managed by NIBB)

Head (Professor, concurrent post): Shigeru Iida
Associate Professor: Kazuo Ogawa
Technical Staff: Kazuhiko Furukawa (Radiation Protection Supervisor), Yosuke Kato (Radiation Protection Supervisor), Yoshimi Matuda (Radiation Protection Supervisor), Naoki Morooka
Supporting Staff: Takayo Ito, Risako Shirai

I. Research Supporting Activity

Technical and Supporting Staffs of this facility are serving the purchase of radioisotopes from JRA (Japan Radioisotope Association) and the transfer of radioisotope wastes to JRA. The physical maintenance of the controlled areas where radioisotopes are used by the registered users of NIBB (National Institute for Basic Biology) and NIPS (National Institute for Physiological Science) for research is also one of our business.

This facility consists of four controlled areas: Center, NIBB-sub, LGER (Laboratory of Gene Expression and Tegulation)-sub, and NIPS-sub. Users going in and out the controlled areas counted by the monitoring system are 8,482 in 1998. This count is comparable to that (8,922) in 1997. The items in each controlled area is presented in Figure 1.

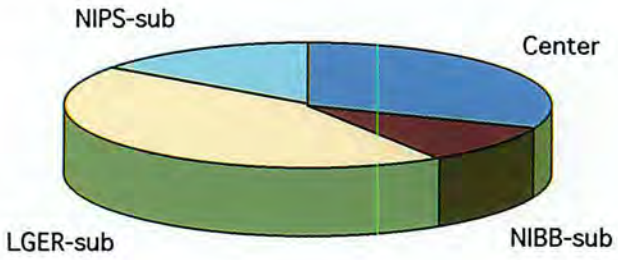
II. Academic activity

Academic activity by Associate Professor is focused on the analysis of the structure and function of a dynein motor protein. Dyneins are a group of microtubule-activated ATPases that serve to convert chemical energy into mechanical energy and divided into axonemal and cytoplasmic dyneins. Figure 2 shows the localization of two isoforms of dynein in the outer arms of sperm axonemes (Ogawa et al., 1977) and the mitotic apparatus of cleaving egg (Mohri et al., 1976) visualized by anti-axonemal dynein (Fragement A) antibodies.

The native dyneins are very large and range in molecular mass up to 1 to 2 mega Da. They are complex proteins containing heavy, intermediate, and light chains defined by the molecular mass. Our prensent project is the molecular cloning of polypeptides contained in outer arm dynein of sea urchin sperm flagella to understand the mechanism how dynein interacts with microtubules, resulting in producing the force.

Outer arm dynein consists of two heavy chains with ATPase activity. The motor activity is closely related to this polypeptide. The first successful molecular cloning of this huge polypeptide (520 kDa) was performed in our laboratory in 1991. Since then cDNA clones for axonemal and cytoplasmic dyneins have been isolated in a variety of organisms. The sequences of heavy chains, without exception, contain four P-loop motives referred to as ATP-binding sites in the midregion of the molecules. Figure 3A and B draw the structure of heavy chain deduced from the amino acid sequence (Ogawa, 1992). Taking the recent works by Koonce et al. (1998) and Vallee et al. (1998) into consideration, this model might be seen as depicted in Figure 3C. In particular, Vallee et al. (1998) have described the importance of a hairpin structure formed between M and C domains which binds to microtubules and presented a novel mechanism for dynein force production different from that of myosin and kinesin.

Outer arm dynein contains three intermediate chains (IC1, IC2, and IC3) that range in molecular mass from 70 to 120 kDa. IC2 and IC3 were cloned by Ogawa et al. (1995) and contain the WD repeats in the carboxy-terminal halves of the molecules. By contrast, IC1 is



	Actual counts
Center	2,628
NIBB-sub	843
LGER-sub	3,739
NIPS-sub	1,273
Total	8,483

Figure 1. Percentage of users going in and out the cntrolled areas during April to December, 1998.

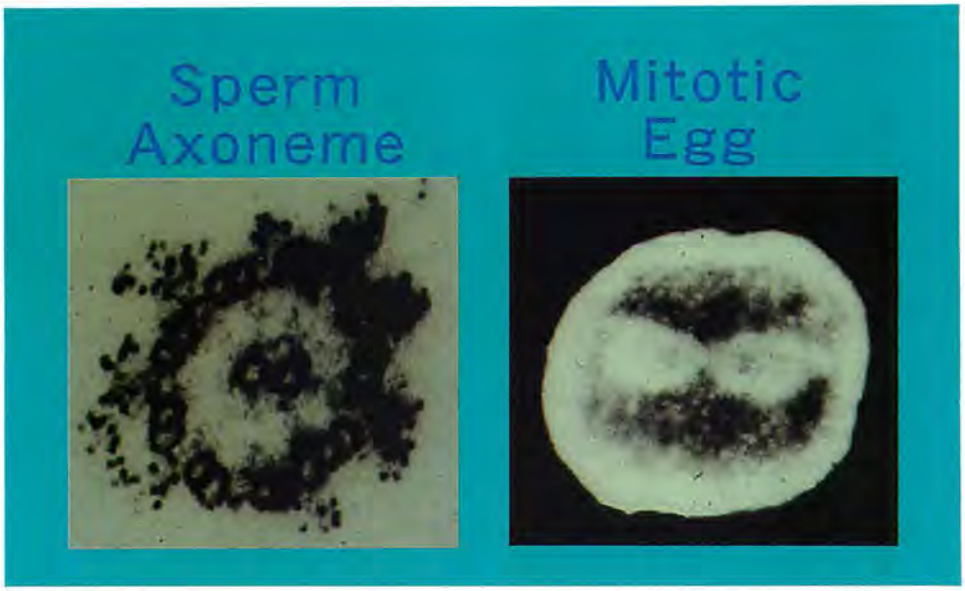


Figure 2. Localization of two dynein isotypes on outer arm of sperm axonemes and mitotic apparatus of cleaving egg.

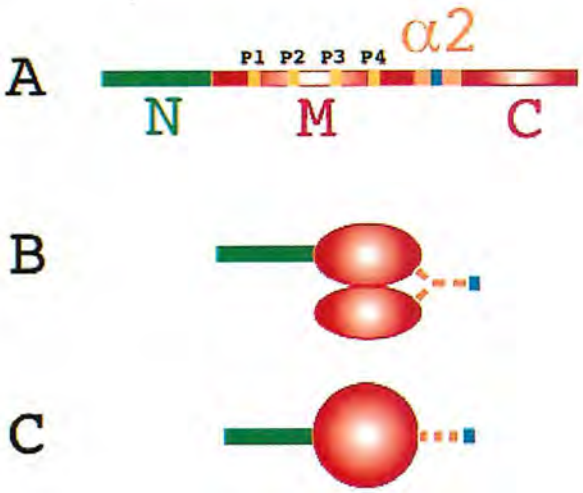


Figure 3. Structure of dynein heavy chain. A; Analysis of amino acid sequence of heavy chain reveals that it consists of three major domains referred to as N, M, and C from the N-terminus. B; M and C domains make larger domain (motor domain) by intramolecular association. C, According to Koonce et al. (1998) showthat recombinant motor domain would be spherical. Vallee et al. (1998) propose that α2 region corresponds to the B-link which is the stalk projected from the globular head structure of dynein, by demonstrating that the recombinant α2 actually binds to microtubules.

not a member of the WD family. IC1 has a unique sequence such that the N-terminal part is homologous to the sequence of thioredoxin, the middle part consists of three repetitive sequences homologous to the sequence of NDP kinase, and the C-terminal part contains a high proportion of negatively charged glutamic acid residues (Ogawa et al., 1996). Thus, IC1 is a novel dynein intermediate chain distinct from IC2 and IC3 and may be a multifunctional protein.

Six light chains with molecular masses of 23.2, 20.8, 12.3, 11.5, 10.4, and 9.3 kDa are associating with outer arm dynein. We have already isolated cDNA clone of five LCs. LC1 (23.2 kDa) and LC3 (12.3 kDa) are highly homologous to mouse Tctex2 and Tctex1, respectively. These mouse proteins are encoded by the *t* complex region that is involved in transmission ratio distortion (TRD), male sterility and the development of germ cells. Our finding raises the possibility that axonemal dynein proteins are involved in this phenomenon. TRD may be caused by the dysfunction of multiple axonemal dynein proteins.

Publication List:

O. Kagami, M. Goto, Y. Makino, H. Mohri, R. Kamiya, and K. Ogawa. A dynein light chain of sea urchin sperm flagella is a homolog of mouse Tctex1, which is encoded by a gene of the *t* complex sterility locus. Gene 211 (1998) 383-386.

THE CENTER FOR ANALYTICAL INSTRUMENTS
(Managed by NIBB)

Head of Facility: *Tetsuo Yamamori*
Technical Staffs: *Akio Murakami (-Apr. 30, 1998)*
Sonoko Ohsawa
Tomoko Mori (Jun.1, 1998 -)
Yumiko Makino
Takeshi Mizutani
Technical Assistant: *Hatsumi Moribe*

The Center, equipped with various types of instruments, serves for general use and supports biochemical structural analyses of biomolecules of proteins, nucleic acids and lipids. Amino acid sequence analysis, amino acid analysis, and chemical syntheses of peptides and DNA/RNA are carried out to support researchers in NIBB and NIPS. On June 1 in 1998, the Center merged with the facility of Molecular Biological Analysis in the Laboratory of Gene Expression and Regulation. Consequently, instruments transferred to the Center are Particle Delivery System, DNA Sequencers, DNA Synthesizer, Plasmid Isolation Systems, etc. Newly installed instruments are Fluorescence Bio Imaging Analyzer and DNA Sequencer (ABI 310). Some of instruments of the Center are opened to researchers outside the Institute.

Figure 1. Amino acid sequence analysis of protein by protein sequencer.

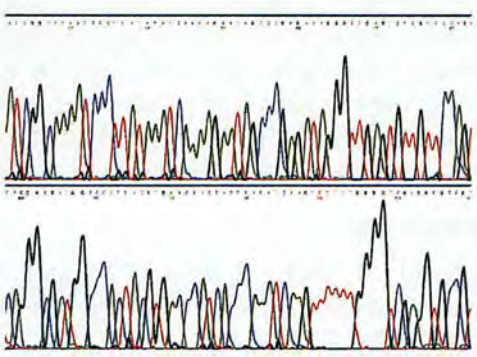
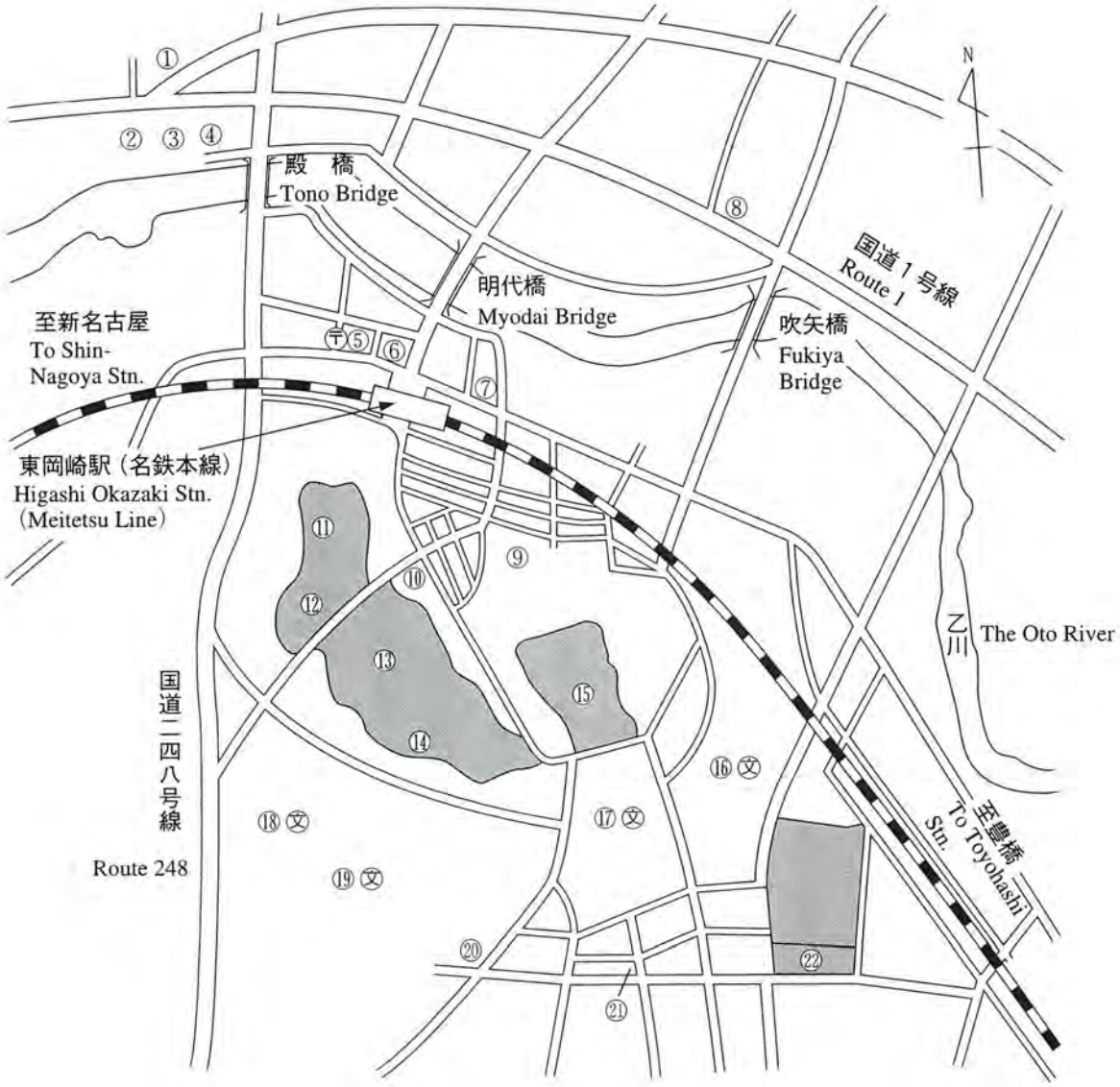


Figure 2. DNA sequencing electropherogram.

Representative instruments are listed below.

Protein Sequencers (ABI 494, ABI 473A)
Amino Acid Analyzer (Hitachi L8500A)
Peptide Synthesizers (ABI 433A, ABI 432A)
Plasmid Isolation Systems (Kurabo PI-100 Σ)
DNA Sequencers (ABI 377, 373S, ABI 310)
DNA/RNA Synthesizers (ABI 394, ABI 392)
Thermal Cyclers (Perkin Elmer PJ-9600, Takara TP-300)
Integrated Thermal Cyclers (ABI CATALYST Turbo 800)
Particle Delivery System (Bio-Rad BiolisticPDS-1000/He)
Gas Chromatograph (Shimadzu GC-14APF-SC)
Glycoprotein Analysis System (Takara Glyco-Tag)
High Performance Liquid Chromatographs (Shimadzu LC-10AD, 6AD)
Integrated Micropurification System (Pharmacia SMART)
Flow Cytometer (Coulter EPICS XL)
NMR Spectrometer (Bruker AMX-360wb)
EPR Spectrometer (Bruker ER-200D)
GC/Mass Spectrometer (JEOL DX-300)
Inductively Coupled Plasma Atomic Emission Spectrometer (Seiko SPS1200A)
Spectrofluorometers (Hitachi 850, Shimadzu RF-5000)
Spectrophotometers (Hitachi 330, Hitachi 557, Varian Cary 5G, Perkin Elmer Lambda-Bio)
Microplate Luminometer (Berthold MicroLumat LB 96P)
Time-resolved Fluorescence Microplate Reader (Pharmacia DELFIA Research)
Microplate Readers (Corona MTP-120, MTP-100F)
Spectropolarimeter (JASCO J-40S)
FT-IR Spectrophotometer (Horiba FT-730)
Laser Raman Spectrophotometer (JASCO R-800)
Biomolecular Interaction Analysis Systems (Pharmacia BIAcore 2000, Affinity Sensors IAsys)
Bio Imaging Analyzers (Fujifilm BAS2000)
Fluorescence Bio Imaging Analyzer (Takara FMBIO II)
Electrophoresis Imaging Systems (PDI Discovery Series, BIOIMAGE)
Microscopes (Carl Zeiss Axiophot, Axiovert)
Microscope Photometer (Carl Zeiss MPM 03-FL)



① 名鉄岡崎ホテル
Meitetsu Okazaki Hotel

② 岡崎城
Okazaki Castle

③ 岡崎ニューグランドホテル
Okazaki New Grand Hotel

④ 岡崎グランドホテル
Okazaki Grand Hotel

⑤ 郵便局
Post Office

⑥ 銀行
Bank

⑦ スーパーマーケット“ユニー”
Super Market "Uny"

⑧ 市役所
Okazaki City Office

⑨ 六所神社
Rokusho Shrine

⑩ 岡崎国立共同研究機構（看板）
Signboard of Okazaki National Research Institutes

⑪ 基礎生物学研究所
National Institute for Basic Biology

⑫ 生理学研究所
Nationai Institute for Physiological Sciences

⑬ 管理局
Administration Bureau

⑭ 分子科学研究所
Insutitute for Molecular Science

⑮ 三島ロッジ・岡崎コンファレンスセンター
Mishima Lodge · Okazaki Conference Center

⑯ 三島小
Mishima Elementary School

⑰ 岡崎高校
Okazaki High School

⑱ 愛教大附中
Jr. High School affiliated to Aichi Univ. of Education

⑲ 竜海中
Ryukai Jr. High School

⑳ 山手センターマーケット
Yamate Supermarket

㉑ 薬局
Pharmacy

㉒ 山手ロッジ
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