

RESEARCH SUPPORT

CENTER FOR TRANSGENIC ANIMALS AND PLANTS

Head: Masaharu Noda

Associate Professor: Eiji Watanabe
 Supporting Staff: Mie Yasuda (Sept. 1, 1999~)

I. Research supporting activity

NIBB Center for Transgenic Animals and Plants, established in April, 1998, aims to support researches in NIBB using transgenic and gene-targeting techniques. We are now planning on the construction of new facilities.

The expected activities of the Center at present are as follows:

1. Provision of information, materials and techniques to researchers.
2. Equipment of various instruments to analyze mutant animals and plants.
3. Development of novel techniques related to transgenic and gene targeting technology.

II. Academic activity

We are studying the functional role of subfamily 2 channels in collaboration with Division of Molecular Neurobiology. Subfamily 2 channels are a group of voltage-gated sodium channels (NaChs) that generate action potentials in electrically excitable cells such as neurons and muscle cells. Comparing with the other NaChs, this channel species has unique amino acid sequences in the regions, which are known to be involved in ion selectivity and voltage-dependent activation and inactivation, suggesting that subfamily 2 channels must have specific functional properties. To clarify the functional role *in vivo*, the subfamily 2 channel-deficient mice were generated by gene targeting. We are now examining the physiological phenotypes.

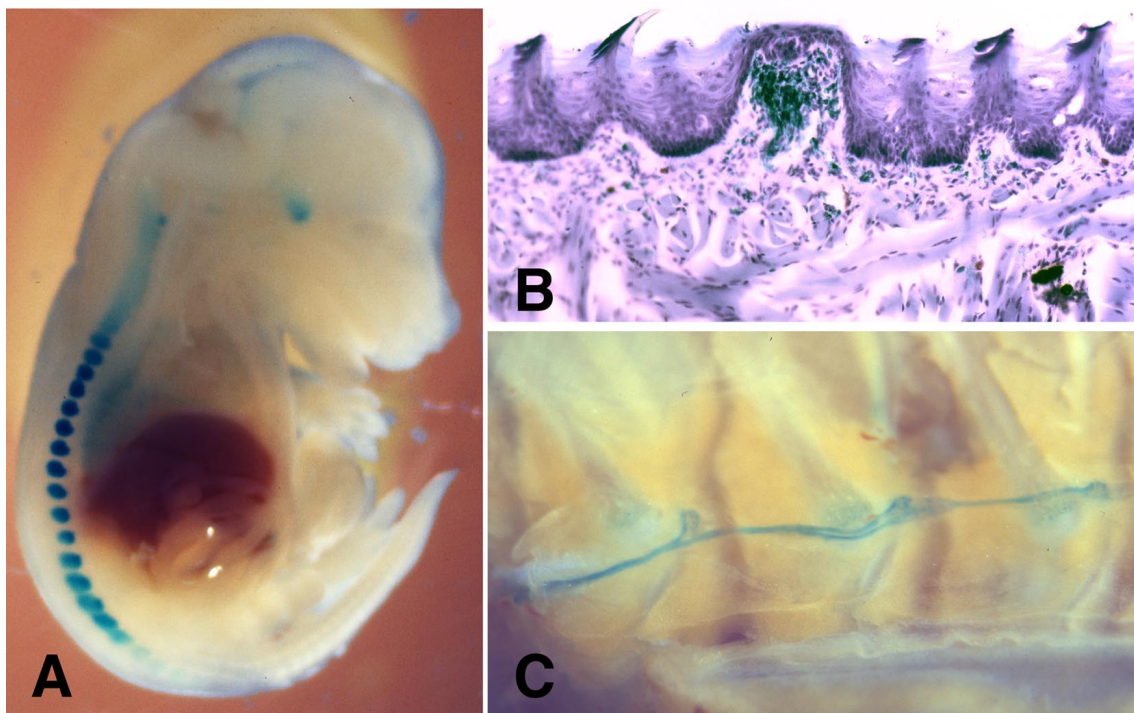


Fig. 1 *LacZ* expression in *mNaG*^{+/-} mice (A-C)

The expression pattern of mouse subfamily 2 channel gene (*mNaG*) was revealed by *LacZ* gene expression in *mNaG*^{+/-} mice. The blue signals represent the site expressing *LacZ* gene. (A) E15 whole embryo, (B) tissue section of adult tongue, and (C) adult sympathetic nerve trunk in the thoracic region. In (B), the tissue section was counterstained with cresyl violet.

TECHNOLOGY DEPARTMENT

*Head: Hiroyuki Hattori***Common Facility Group***Chief : Kazuhiko Furukawa**Research Support Facilities*

Shoichi Higashi (Unit Chief)
Tomoki Miwa (Subunit Chief)
Chieko Nanba (Subunit Chief)
Hiroyo Nishide
Makiko Itoh (Technical Assistant)
Keiko Suzuki (Technical Assistant)
Misayo Masuda (Technical Assistant)
Hideyuki Gotoh (Technical Assistant)
Yasuyo Kamiya (Technical Assistant)

Radioisotope Facility

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

Center for Analytical Instruments

Mamoru Kubota (Unit Chief)
Sonoko Ohsawa (Subunit Chief)
Tomoko Mori
Yumiko Makino
Takeshi Mizutani
Hatsumi Moribe (Technical Assistant)

Glassware Washing Facility

Masayo Iwaki
(Kazuhiko Furukawa)

Research Support Group*Chief : Hiroko Kobayashi**Cell Biology Group*

Maki Kondo (Subunit Chief)
Yukiko Kabeya
Katsushi Yamaguchi

Developmental Biology Group

Chiyo Takagi
Sanae Oka
Naomi Sumikawa

Regulation Biology Group

Hideko Inuma
Akiko Oda
Shigemi Takami

Gene Expression and Regulation Group

Sachiko Tanaka (Subunit Chief)
Koji Hayashi
Yasushi Takeuchi
Kaoru Sawada
Hideko Utsumi
Makiko Kondo (Technical Assistant)

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 enlightenment 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 institutes 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
Associate Professor: Masakatsu Watanabe
Research Associates: Yoshio Hamada
 (Tissue and Cell Culture)
 Ikuo Uchiyama
 (Computer) (March 1, 1999-)
Technical Staffs: Sho-ichi Higashi
 (Large Spectrograph)
 Mamoru Kubota (Large Spectrograph) (-March 31, 1999)
 Tomoki Miwa (Computer)
 Chieko Nanba
 (Plant Culture, Farm, Plant Cell)
 Hiroyo Nishide
 (Computer) (June 1, 1999-)
 Toshiki Ohkawa
 (Computer) (-May 31, 1999)
 Kaoru Sawada (Tissue and Cell Culture) (-June 30, 1999)
 Hideyuki Goto (Large Spectrograph) (May 17, 1999-)
 Makiko Ito (Large Spectrograph)
 Yasuyo Kamiya (Tissue and Cell Culture) (May 17, 1999-)
 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 200 $\mu\text{m s}^{-1}$ in tracking speed, down to 2 μm in beam diameter) with an infrared phase-contrast observation system.

2. Tissue and Cell Culture Laboratory

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 four Notch homologues but their developmental significance is not clear. To investigate their role in mammalian development, we have sequenced the murine Notch2 cDNA, determined the primary sequence of its protein, and have produced Notch2 mutant mice by gene targeting procedures. The mutant mice die prior to embryonic day 11.5. Thus, Notch2 plays an essential role in postimplantation development in mice. Chimeric analysis revealed that embryonic lethality of the mutant mice is due to defect in placenta development and Notch2 function is involved in formation of the roof plate of diencephalon and mesencephalon in development later than the lethal stage.

3. Computer Laboratory

Computer laboratory maintains several computers to provide computation resources and means of electronic communication in this Institute. This year, new computer systems were introduced to meet requirement of large scale biological data analysis, and now the system mainly consists of three servers and two terminal workstations: biological information analysis server (SGI Origin 2000), database server (Sun Enterprise 450), file server (Sun Enterprise 3000), data visualization terminal and molecular simulation terminal (both are SGI Octanes). Some personal computers (Macintoshes and Windows PCs) and color/monochrome printers are also equipped. Various biological databases and data retrieval/analysis programs are available on this system.

Computer laboratory also provides network communication services in the Institute. Most of PCs in each laboratory as well as all of the above service machines are connected each other with local area network (LAN), which is linked to the high performance multimedia backbone network of Okazaki National Research Institute (ORION). Many local services including sequence analysis service, file sharing service and printer service are provided for the Institute members through this LAN. We also maintain a public World Wide Web server that contains the NIBB home pages (<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 m² glass-houses with precise temperature and humidity control, three green houses (each 6 m²) at the P1 physical containment level, a small farm, two greenhouses (45 and 88 m²) with automatic sprinklers, two open aquariums (30 and 50 t) 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 (30 km from 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 international 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 unicellular algae are studied action spectroscopically (Watanabe 1995, In CRC Handbook of Organic Photochemistry and Photobiology) by measuring computerized-videomicrographs of the motile behavior of the cells at the cellular and subcellular levels (Horiguchi et al., 1999; Matsunaga et al., 1999; Choi et al., 1999). Photo-receptive and signal transduction mechanisms of algal gene expression were also studied by action spectroscopy (Leblanc et al., 1999).

(2) Computational Biology: Explosively growing biological data produced from various genome projects should contain many clues to understanding complex and diverse biological systems. Comparative genomics is a useful approach to find such clues. We are constructing a database system for comparative analysis of many of microbial genomes ever sequenced and developing new computational techniques for large-scale genome sequence comparison. Currently, our research aim is fo-

cused on the identification of orthologous genes between multiple genomes, which is a crucial step for comparative genomics. Since considerable number of genes consist of multiple domains, we are now developing a hierarchical clustering algorithm that can automatically split fusion genes into orthologous domains using all-against-all homology search results. In parallel, we also make detailed comparison of closely related microbial genomes to investigate the genomic polymorphisms or evolutionary changes in collaboration with Dr. I. Kobayashi's group (Univ. Tokyo). By comparing genomes of two *Helicobacter pylori* strains, we could find interesting insertion/deletion patterns that frequently include restriction-modification genes.

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

- Choi, J-S., Chung-Y-H, Moon, Y-J., Kim, C., Watanabe, M., Song, P-s., Joe, C-O, Bogorad, L., and Park, Y. M. (1999) Photomovement of the gliding cyanobacterium *Synechocystis* sp. PCC 6803. *Photochem. Photobiol.* **70**, 95-102.
- Hamada, Y., Kadokawa, Y., Okabe, M., Ikawa, M., Coleman, J.R. & Tsujimoto, Y (1999). Mutation in ankyrin repeats of the mouse Notch2 gene induces early embryonic lethality. *Development* **126**, 3415-3424.
- Horiguchi, T., Kawai, H., Kubota, M., Takahashi, T. and Watanabe, M. (1999). Phototactic responses of four marine dinoflagellates with different types of eyespot and chloroplast. *Phycol. Res.* **47**, 101-107.
- Kobayashi, I., Nobusato, A., Kobayashi-Takahashi, N., Uchiyama, I. (1999) Shaping the genome – restriction-modification systems as mobile genetic elements. *Curr. Opin. Genet. Dev.* **9**, 649-656.
- Leblanc, C., Falcioratore, A., Watanabe, M. and Bowler, C. (1999). Semi-quantitative RT-PCR analysis of photoregulated gene expression in marine diatoms. *Plant Mol. Biol.* **40** (6), 1031-1044.
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- Shimizu, K., Chiba, S., Kumano, K., Hosoya, N., Takahashi,

- T., Kanda, Y., Hamada, Y., Yazaki, Y. & Hirai, H. (1999). Mouse Jagged1 physically interacts with Notch2 and other Notch receptors. *J. Biol. Chem.* **274**, 32961-32969.
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- II. Cooperative Research Program for the Okazaki Large Spectrograph**
- Andrady, A. L. and Torikai, A. (1999). Photoyellowing of mechanical pulps III. Intensity effects and dose-response relationships. *Polym. Degradn. Stab.*, **66**, 317-322.
- Andrady, A. L., Hamid, S. H., Hu, X. and Torikai, A. (1999). Effects of increased solar ultraviolet radiation on materials. *J. Photochem. Photobiol. B: Biol.* **46**, 96-103.
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- Fujiwara, A., Tazawa, E., Kamata, Y. and Yasumasu, I. (1999). Photo-activation of respiration in degenerated sperm of echiuroid, oyster and sea urchin. *Zool. Sci.* **16**, 237-246.
- Hashimoto, T. and Shichijo, C. (1999). Amplification of phytochrome-induced photomorphogenesis by red light pre-irradiation, and a cryptic red-light signal production. *Trend in Photochem. and photobiol.* vol. **6**, 15-27.
- Horiguchi, T., Kawai, H., Kubota, M., Takahashi, T. and Watanabe, M. (1999). Phototactic responses of four marine dinoflagellates with different types of eyespot and chloroplast. *Phycol. Res.* **47**, 101-107.
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- Shichijo, C., Onda, S., Kawano, R., Nishimura, Y. and Hashimoto, T. (1999). Phytochrome elicits the cryptic red-light signal which results in amplification of anthocyanin biosynthesis in sorghum. *Planta*. **208**, 80-87.
- Torikai, A. and Hasegawa, H. (1999). Accelerated photodegradation of poly(vinyl chloride). *Polym. Degradn. Stab.* **63**, 441-445.
- Torikai, A. and Shibata, H. (1999). Effect of Ultraviolet radiation on photodegradation of collagen. *J. Appl. Polym. Sci.* **73**, 1259-1265.

RADIOISOTOPE FACILITY (managed by NIBB)

Head (Professor, concurrent post): Shigeru Iida
Associate Professor: Kazuo Ogawa
Technical Staffs: Yosuke Kato
(Radiation Protection Supervisor),
Yoshimi Matsuda
(Radiation Protection Supervisor),
Naoki Morooka

Supporting Staff: Takayo Ito
Risako Shirai
Yumiko Iida

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 Regulation)-sub, and NIPS-sub. Users going in and out the controlled areas counted by the monitoring system were 7,912 in 1999. This count was comparable to that (8,483) in 1998. The items in each controlled area are 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 (Fragment 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 present 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 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.

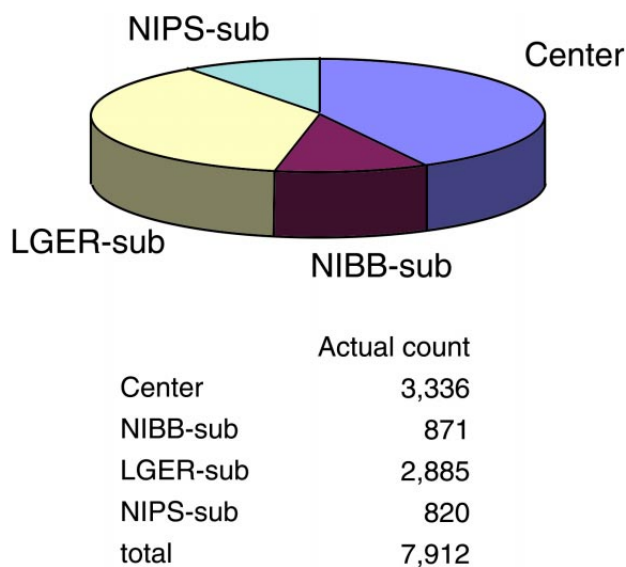


Figure 1. Percentage of users going in and out the controlled areas during April to December, 1999.

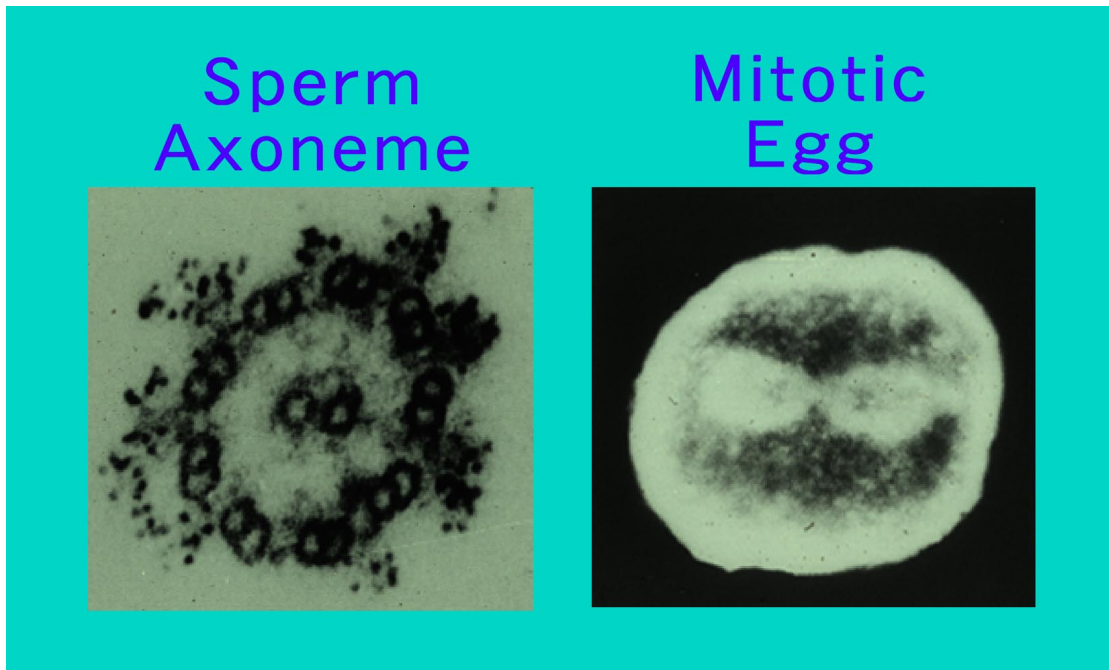


Figure 2. Localization of two dynein isotypes on outer arm of sperm axonemes and mitotic apparatus of cleaving egg.

Publication List:

- S. Watabe, Y. Makino, K. Ogawa, T. Hiroi, Y. Yamamoto, and S. -Y. Takahashi. Mitochondrial thioredoxin reductase In bovine adrenal cortex: Its purification, properties, nucleotide/amino acid sequences, and Identification of selenocysteine. *Eur. J. Biochem.* **204**, 74-84 (1999).
- K. Inaba, O. Kagami, and K. Ogawa. Tctex2-related outer arm dynein light chain Is phosphorylated at activation of sperm motility. *Biochem. Biophys. Res. Commun.* **256**, 177-183 (1999).

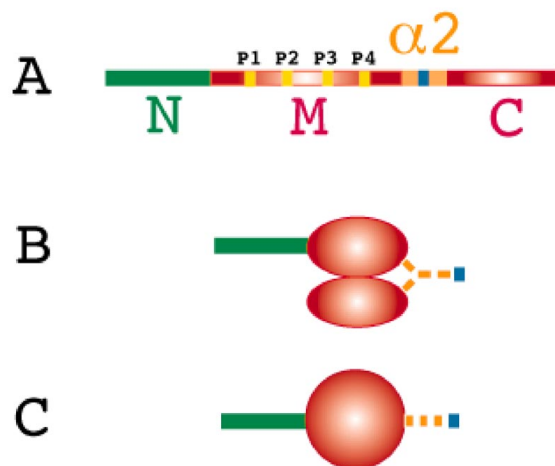


Figure 3. Structure of dynein heavy chain. A; Analysis of amino acid sequence of heavy chain reveals that it consists of three major domains referred to as N, M, and C from the N-terminus. B; M and C domains make larger domain (motor domain) by intramolecular association. C, According to Koonce et al. (1998) show that recombinant motor domain would be spherical. Vallee et al. (1998) propose that $\alpha 2$ region corresponds to the B-link which is the stalk projected from the globular head structure of dynein, by demonstrating that the recombinant $\alpha 2$ actually binds to microtubules.

THE CENTER FOR ANALYTICAL INSTRUMENTS

(managed by NIBB)

Head of Facility: Tetsuo Yamamori
Technical Staffs: Mamoru Kubota
 Sonoko Ohsawa
 Tomoko Mori
 Yumiko Makino
 Takeshi Mizutani (~Feb. 15, 2000)
Technical Assistant: Hatsumi Moribe

The Center serves for amino acid sequence analysis, and chemical syntheses of peptides and nucleotides to support researchers in NIBB and NIPS. Newly installed instruments in 1999 are Biomek 2000 Laboratory Automation System and Automatic Nucleic Acid Isolation System. Instruments of the Center can be used by researchers outside the Institute upon proposal.



Figure 1. Procise 494 Protein Sequencer.



Figure 2. Biomek 2000 Laboratory Automation System.

Representative instruments are listed below.

Protein Sequencers (ABI Procise 494, ABI 473A)
 Amino Acid Analyzer (Hitachi L8500A)
 Peptide Synthesizers (ABI 433A, ABI 432A)
 Plasmid Isolation Systems (Kurabo PI-100)
 Automatic Nucleic Acid Isolation System (Kurabo NA-2000)
 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, Waters 600E)
 Integrated Micropurification System (Pharmacia SMART)
 Flow Cytometer (Coulter EPICS XL)
 Biomolecular Interaction Analysis Systems (Pharmacia BIACORE 2000, Affinity Sensors IAsys)
 Laboratory Automation System (Beckman Coulter Biomek 2000)
 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, Simadzu 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)
 Bio Imaging Analyzers (Fujifilm BAS2000)
 Fluorescence Bio Imaging Analyzer (Takara FMBIO)
 Electrophoresis Imaging Systems (PDI Discovery Series, BIOIMAGE)
 Microscopes (Carl Zeiss Axiophot, Axiovert)
 Microscope Photometer (Carl Zeiss MPM 03-FL)