RESEARCH SUPPORT

CENTER FOR TRANSGENIC ANIMALS AND PLANTS

Head: Masaharu Noda

Associate Professor: *Eiji Watanabe*
Supporting Staff: *Mie Yasuda*
Ayako Tozaki (June 1, 2000~)

I. Research supporting activity

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

The expected activities of the Center 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 Na_v2 ion channel in collaboration with Division of Molecular Neurobiology. Na_v2 belongs to a group of voltage-gated sodium channels (NaChs) that serve to generate action potentials in electrically excitable cells such as neuronal and muscle cells. Comparing with the other NaChs, Na_v2 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 it must have specific functional properties. To clarify the functional role of Na_v2 *in vivo*, the Na_v2-deficient mice were generated by gene targeting and the physiological phenotypes have been examined. It was suggested that the Na_v2 channel plays an important role in the central sensing of body-fluid sodium level and regulation of salt intake behavior. Details of this study are described in the part of Division of Molecular Neurobiology.

Publication List

Watanabe, E., Fujikawa, A., Matsunaga, H., Yasoshima, Y., Sako, N., Yamamoto, T., Saegusa, C., Noda, M. (2000) Na_v2/NaG channel is involved in control of salt intake behavior in the CNS. *J. Neurosci.* 20, 7743-7751.

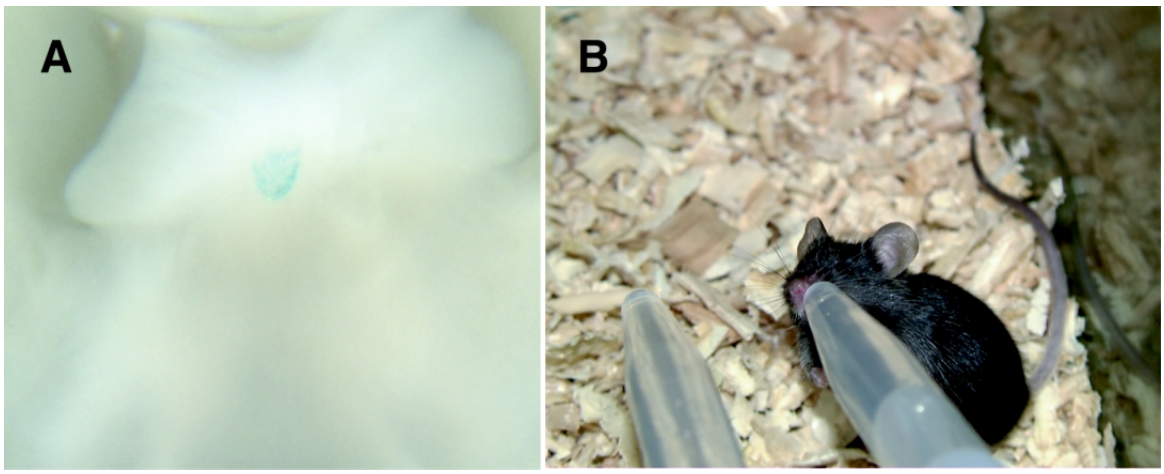


Fig. 1 Na_v2 channel is expressed in specialized neurons and ependymal cells in the adult CNS (A), and the null mutant shows an abnormal ingestion of hypertonic saline (B).
The expression pattern of mouse Na_v2 channel gene was revealed by *lacZ* gene expression in Na_v2+/- mice. The blue signals represent the cells expressing *lacZ* gene. Fig. 1A shows the specific expression of Na_v2 in the subfornical organ. The null mutants showed excessive ingestion of hypertonic saline under both thirst and acute salt appetite conditions. Fig. 1B shows the null mutant ingesting hypertonic saline under the thirst condition.

TECHNOLOGY DEPARTMENT

Head:Hiroyuki Hattori

Common Facility Group

Chief : Kazuhiko Furukawa

Reseach Support Facilities
Shoichi Higashi(Unit Chief)
Chieko Nanba(Subunit Chief)
Hiroyo Nishide
Makiko Itoh(Technical Assistant)
Keiko Suzuki(Technical Assistant)
Yasuyo Kamiya(Technical Assistant)
Yumi Hashimoto (Technical Assistant)
Ayako Tosaki(Technical Assistant)
Nobuko Hattori (Technical Assistant)
Yukiko Tanigawa(Technical Assistant)

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

Center for Analytical Instruments
Sonoko Ohsawa(Unit Chief)
Tomoko Mori(Subunit Chief)
Yumiko Makino
Hatsumi Moribe(Technical Assistant)

Glassware Washing Facility
(Tomoko Mori)
(Kazuhiko Furukawa)

Research Support Group

Chief : Hiroko Kobayashi

Cell Biology Group
Maki Kondo(Unit Chief)
Yukiko Kabeya

Developmental Biology Group
Chiyo Takagi
Sanae Oka
Chiyo Noda

Regulation Biology Group
Hideko Iinuma
Katsushi Yamaguchi
Yasushi Takeuchi
Shigemi Takami
Kaoru Yamada

Gene Expression and Regulation Group
Tomoki Miwa(Unit Chief)
Sachiko Tanaka(Subunit Chief)
Kaoru Sawada(Subunit Chief)
Koji Hayashi
Hideko Utsumi
Naomi Sumikawa

Integrated Bioscience Group
Takeshi Mizutani
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 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 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: *Norio Murata*
Associate Professor: *Masakatsu Watanabe*
Research Associates: *Yoshio Hamada (Tissue and Cell Culture)*
Ikuo Uchiyama (Computer)
Technical Staff: *Sho-ichi Higashi (Large Spectrograph)*
Tomoki Miwa (Computer)
Chieko Nanba (Plant Culture, Farm, Plant Cell)
Hiroyo Nishide (Computer)
Nobuko Hattori (Large Spectrograph)
Makiko Ito (Large Spectrograph)
Yasuyo Kamiya (Tissue and Cell Culture)
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 $\mu m\ s^{-1}$ in tracking speed, down to 2 μm in beam diameter) with an infrared phase-contrast observation system.

2. *Tissue and Cell Culture Laboratory*

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*

Computer laboratory maintains several computers to provide computation resources and means of electronic communication in this Institute. Currently, the main system 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 and color/monochrome printers are also equipped. On this system, we provide various biological databases and data retrieval/analysis programs, and support large-scale data analysis and database construction for the Institute members.

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 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 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 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 clean benches, is also provided.

7. *Laboratory of Stress-Resistant Plants*

This laboratory was founded to study transgenic plants with respect to tolerance toward various enviromental 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-videomiceographs of the motile behavior of the cells at the cellular and subcellular levels. Photo-receptive and signal transduction mechanisms of algal gene expression were also studied by action spectroscopy.

(2) Developmental Biology: Replacement of the ankyrin repeats of mouse Notch2 gene with E.coli b-gactosidase gene induces early embryonic lethality around E10.5. The lethality was suggested due to defects in extraembryonic tissues, because the mutant embryo grew and differentiated further in vitro. Histological examination and in situ hybridization analysis with trophoblast subtype-specific probes revealed that the development of giant and spongiotrophoblast cell layers are normal in the mutant placenta, while vasculogenesis in the labyrinth layer apperaed compromised at E9.5. Since the lethality was circumvented by production o

f chimeric mice with tetraploidy wild type embryos, we concluded that the embryonic lethality is due to defect in growth and/or differentiation of labyrinthine trophoblast cells. The mutant embryo, however, could not be rescued

in the tetraploid chimeras beyond E12.5 because of insurfficient development

of umbilical cord, indicating another role of Notch2 signaling in the mouse development. Chimeric analysis with diploid wild type, however, revealed contribution of mutant cells to these affected tissues by E13.5. Thus, Notch2 are not cell autonomously required for the early cell fate determination of labyrinthine trophoblast cells and allantoic mesodermal cells, but plays an indispensable role in the further formation of functional labyrinth layer andumbilical cord.

(3) Computational Biology: Comparative genomics is a useful approach to find clues to understanding complex and diverse biological systems from rapidly growing genome database. We have constructed a database system for comparative analysis of many of microbial genomes ever sequenced and are developing new computational techniques for large-scale genome sequence comparison. Especially, we are developing a method for orthologous grouping among multiple genomes, which is a crucial step for comparative genomics. Since considerable number of genes consist of multiple domains, we have developed a hierarchical clustering algorithm that can automatically split fusion genes into orthologous domains.

In parallel, we are developing a tool to incorporate various sequence features such as G+C contents, codon usage bias and locations of repetitive elements into the genome comparison. By this approach, we 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 pyroli* strains and two *Pyrococcus* species, 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*

Chinen A., Uchiyama, I., Kobayashi, I. (2000). Comparison between *Pyrococcus horikoshii* and *Pyrococcus abyssi* genome sequences reveals linkage of restriction-modification genes with large genome polymorphisms. *Gene* **259**, 109-121.

Kumano, K ., Saito, T., Kurokawa, M.m Kanda, Y., Hamada, Y. and Hirai, H. (2000) Binding of Delta1, Jagged1, and Jagged2 to Notch2 rapidly induces cleavage, nuclear translocation, and hyperphosphorylation of Notch2. *Mol. Cell. Biol*. **20**, 6913-6922.

Nobusato, A., Uchiyama, I., Kobayashi, I. (2000). Diversity of restriction-modification gene homologues in *Helicobacter pylori*. *Gene* **259**, 89-98

Nobusato, A., Uchiyama, I., Ohashi, S., Kobayashi, I. (2000). Insertion with long target duplication: a mechanism for gene mobility suggested from comparison of two related

bacterial genomes. *Gene* **259**, 99-108.

Uchiyama, I. (2000). Hierarchical clusterng procedure for grouping orthologous domains in multiple genomes. *Currents in Computational Molecular Biology*, 146-147.

Uchiyama, I., Miwa, T., Nishide, H., Suzuki, I., Omata, T., Ikeuchi, M., Murata, N., Kanehisa, M. (2000). Data submission system for cyanobacterial DNA chip consortium, *Genome Informatics* **11**, 235-236.

Uchiyama, I., Higuchi, T., Kobayashi, I. (2000). CGAT: Comparative genome analysis tool for closely related microbial genomes. *Genome Informatics* **11**, 341-342.

Takinami, S., Mochizuki, M., Hayatsu, H., Nikaido, O., Kubota, M., Watanabe, M., Hieda, K. and Negishi, T. (2000) Somatic cell mutation and photoproduct formation in *Drosophila* induced by monochromatic UV light in sunlight, *Environmental Toxicology*, **15**, in press

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

Hada, M., Iida,Y.,and Takeuchi,Y.(2000). Action spectra of DNA photolyases for photorepair of cyclobutane pyrimidine dimers in sorghum and cucumber. *Plant Cell Physiol*. **45***:* 644-648 .

Kawai, H. and Kreimer, G. (2000). Sensory mechanisms: Phototaxes and light perception in algae. In “The Flagellates: Unity, Diversity and Evolution.” (Edited by J. Green and B.S.C. Leadbeater), pp. 124-146. Taylor & Francis Publishing.

Shinomura, T., Uchida, K., and Fruuya, M. (2000). Elementary process of photoperception by phytochorome A for high-irradiance response of hypocotyl elongation in *Arabidopsis*. *Plant Physiol.* **122**, 147-156.

Tada, Y., Wakasugi, T., Nishikawa, A., Furuhashi K. and Yamada K. (2000) Developmental regulation of a gene coding for a low-molecular-weight heat shock protein during haustorium formation in the seedlings of a holoparasitic plant, *Cuscuta japonica*. *Plant Cell Phisiol.* **41**, 1373-1380.

Takeuchi, Y., Iida, Y., Nakajima, N. and Nikaido, O. (2000). Formation of DNA lesions in cucumber cotyledons exposed to solar UV radiation. *Environ. Sci.* **13**, 351-355

Takinami, S., Mochizuki, M., Hayatsu, H., Nikaido, O., Kubota, M., Watanabe, M., Hieda, K. and Negishi, T. (2000) Somatic cell mutation and photoproduct formation in *Drosophila* induced by monochromatic UV light in sunlight, *Environmental Toxicology*, **15**, in press

Torikai, A., (2000), Wavelength sensitivity of photodegradation of polymers. In "Handbook of Polymer Degradation Second Ed.," (Edited by S. Halim Hamid), pp.573-603, Marcel Dekker, Inc. New York.

THE CENTER FOR ANALYTICAL INSTRUMENTS

(managed by NIBB)

Head of Facility: *Tetsuo Yamamori*
Technical Staffs: *Sonoko Ohsawa*
Tomoko Mori
Yumiko Makino
Technical Assistant: *Hatsumi Moribe*

The Center serves for amino acid sequence analysis, and chemical syntheses of peptides and nucleotids to support researchers in NIBB and NIPS. 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 Σ)
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 (BIOIMAGE)
Microscopes (Carl Zeiss Axiophot, Axiovert)
Microscope Photometer (Carl Zeiss MPM 03-FL)

Center for Radioisotope Facilities (CRF)

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

I. Research supporting activity

In this year, the Radioisotope Facility managed by NIBB (National Institute for Basic Biology) was reorganized to the CRF which is included in one of the Common Research Centers belonged to ONRI (Okazaki National Research Institutes).

Technical and supporting staffs of the CRF 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 is also one of our business.

The CRF consists of four controlled areas: Center, NIBB-sub, LGER (Laboratory of Gene Expression and Regulation)-sub, and NIPS (National Institute for Physiological Science)-sub. Users going in and out the controlled areas counted by the monitoring system are 6,273 in 2000. This count is comparable to that (7,912) in 1999. The items in each controlled area is presented in Figure 1.

II. Academic activity

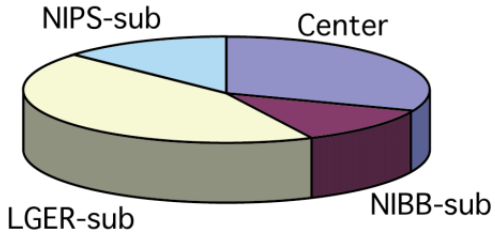
Academic activity by teaching staff is focused on the analysis of the structure and function of a dynein motor protein. Dyneins are a group of microtubule-activated ATPases that serve to convert chemical energy into mechanical energy and divided into axonemal and cytoplasmic dyneins. Figure 2 shows the 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 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.



	Actual count
Center	1,917
NIBB-sub	756
LGER-sub	2,780
NIPS-sub	820
total	6,273

Figure 1. Percentage of users going in and out the controlled areas during April to December, 2000.

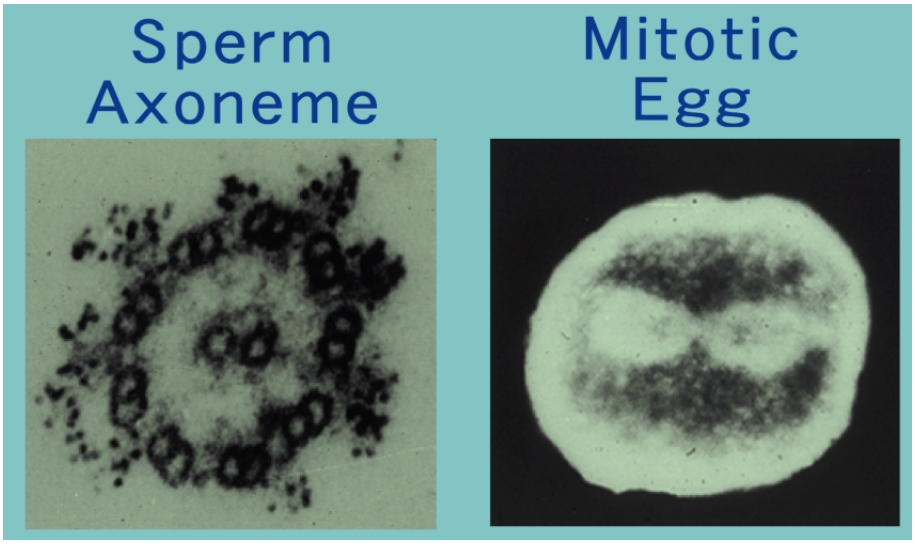


Figure 2. Localization of two dynein isotypes on outer arm of sperm axonemes and mitotic apparatus of cleaving egg.

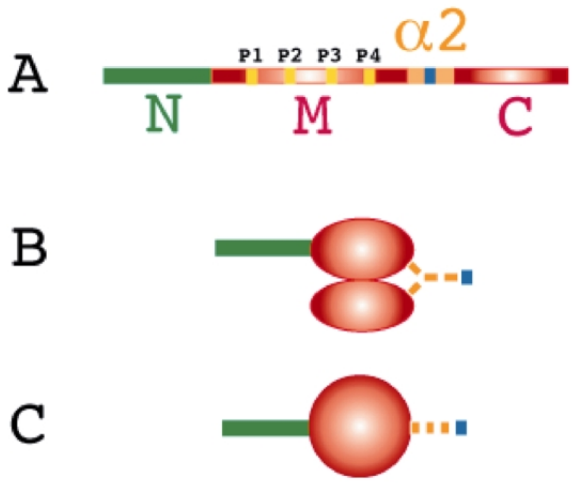


Figure 3. Structure of dynein heavy chain. A; Analysis of amino acid sequence of heavy chain reveals that it consists of three major domains referred to as N, M, and C from the N-terminus. B; M and C domains make larger domain (motor domain) by intramolecular association. C, According to Koonce et al. (1998) showthat recombinant motor domain would be spherical. Vallee et al. (1998) propose that a2 region corresponds to the B-link which is the stalk projected from the globular head structure of dynein, by demonstrating that the recombinant a2 actually binds to microtubules.