NATIONAL INSTITUTE FOR BASIC BIOLOGY

基 礎 生 物 学 研 究 所　岡崎国立共同研究機構

ANNUAL REPORT

1988

BRIEF SUMMARY

The National Institute for Basic Biology, NIBB, is a part of the Okazaki National Research Institutes located on a hill overlooking the old town of Okazaki. Its activities are supported by Monbu-sho (The Japan Ministry of Education, Science and Culture). The research institute is composed of three independent organizations, the National Institute for Basic Biology (NIBB), the National Institute for Physiological Sciences (IPS) and the Institute for Molecular Science (IMS). NIBB was established in 1967 and it celebrated its tenth anniversary in 1987.

NIBB is an interuniversity research institute with its own research programs as well as cooperative programs to promote basic biology at the national and international level. The programs are 1) research projects at each division 2) joint research programs in which university scientists are invited to participate in research projects with NIBB members, 3) facility-sharing programs in which university scientists utilize the institute's research resources, 4) graduate student training programs in which graduate students from universities spend fixed periods of time with the NIBB, and 5) international programs in which foreign scientists are invited to NIBB to conduct research projects. NIBB sponsors "Bioscience Training Course" of about 7–10 days for selected people from all over Japan.

PROFESSOR T. S. OKADA

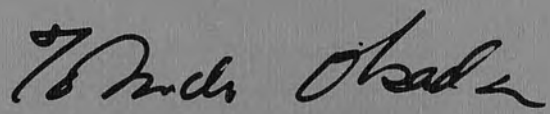
INTRODUCTION

The National Institute for Basic Biology (NIBB) aims to facilitate basic research in the biological sciences in Japan by conducting advanced studies to elucidate various fundamental mechanisms underlying living organisms. Research areas include studies of cell growth, development and differentiation, and the control of various cell functions among others. The ultimate goal is to understand the mechanisms of phenomena in eukaryotic organisms at the molecular level. Analytical approaches include biophysical, cell biological, and molecular techniques, incorporating modern methods of gene manipulation.

The NIBB, is an inter-university research institute, and as such has a two-fold mission: (1) to conduct in-house research and (2) to make the facility available for collaboration with scientists outside the NIBB. Thus, the NIBB is an "open" institute. The Institute contains three departments, Cell Biology, Developmental Biology, and Regulation Biology, which are divided into 13 divisions. Each division is staffed by a full professor, an associate professor and two research associates. Six of the professional appointments are adjunct professorships; these individuals have joint appointments at other institutes in Japan. The NIBB sponsors joint research programs with participating individuals or research groups nationally and internationally.

The NIBB provides an opportunity to share research resources among biologists in Japan and from abroad. It also sponsors symposia on current topics at the interdisciplinary level by inviting participation by leading scientists in various related fields, both from inside Japan and abroad. Thus, the NIBB is becoming and will continue to be an internationally recognized institute for conducting basic research in the biological sciences.

This report consists of an outline of activities of the NIBB and of a list of papers published by the members in the 1986–1987 period.



T.S. Okada, D.Sci.
Director General

ORGANIZATION OF THE INSTITUTE

The Okazaki National Research Institutes, ONRI, are composed of three individual Institutes, NIBB, Institute for Molecular Science (IMS), and National Institute for Physiological Sciences (IPS). The president of ONRI is Prof. Saburo Nagakura. IMS and IPS are headed by Professors Hiroo Inokuchi and Setsuro Ebashi, respectively.

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 and Culture. Currently the chief administrator is Mr. Shousuke Wakisaka.

Research

The institute conducts its research programs through three departments organized into 13 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. Half 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 undertakes the technical education of its staff.

Research Support Facility

The research support facility of NIBB consists of the Large-scale Spectrograph Laboratory, Tissue and Cell Culture Laboratory, Laboratory Computer Facility, Plant Culture Facility, Plant Cell Culture Facility, and Experimental Farm. In addition, seven facilities are operated jointly with the National Institute of Physiological Sciences (IPS); 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 cover 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.

DEPARTMENT OF CELL BIOLOGY

Chairman: Yoshihiko Fujita

Two research divisions and three adjunct research divisions belong to this department.

Division of Cell Mechanisms

Professor: to be appointed
Associate Professor: Toshiyuki Nagata
Research Associate: Kazuo Ogawa

The mechanism of transmission of cellular organelles to the next generation has been pursued in this division for several years. These studies revealed that shortly after zygote formation paternal organelle genomes are digested by Ca^{2+}-dependent nucleases directed by the genetic information from the maternal nucleus in an isogamous organism such as *Chlamydomonas reinhardtii*. This type of organelle trasmission has been found to be widely distributed among lower organisms and should be the explanation of maternal inheritance at the molecular level. On the other hand, although maternal inheritance was first discovered from the study on genetic crosses among various higher plants in 1907 by C. Correns, no appropriate explanation has yet been given. The efforts of this division in the last year, employing detailed observations using fluorescence microscopy after staining with DAPI, a DNA specific fluorochrome, clarified that organelle nucleoids disappeared at a specific stages during the formation of male generative cells in a wide range of higher plants. Such results revealed in heterogamous organism made a more unified explanation of the mechanism of maternal inheritance possible.

The replication mechanism of cytoplasmic genomes thus inherited to the next generation has also been pursued. It has been found from the study of cultured tobacco cells that rapid plastid DNA synthesis started soon after transfer to fresh medium, and this DNA synthesis preceded nuclear DNA synthesis. This separation of the synthesis of plastid DNA from that of nuclear DNA enabled us to analyse the mode of plastid DNA replication, which has not been elucidated yet, while its whole nucleotide sequence data are available.

It has been established from the previous study by this division that plastid nucleoids have a highly organized structure. Biochemical analysis of this plastid nucleoid showed that four proteins separated from the plastid nucleoid are DNA binding proteins, which are supposed to regulate the functional expression as well as the structural integrity of plastid nucleoid.

Thus this division is devoted to the elucidation of the interaction between the cytoplasm and nucleus at the molecular and celluar level in several organisms.

Deparment of Bioenergetics

Professor: Yoshihiko Fujita
Associate Professor: Shigeru Itoh
Research Associate: Mamoru Mimuro
Kaori Ohki

Photsynthetic energy conversion has been investigated at the cellular and molecular levels. Our research is currently focused on (1) regulation of stoichiometry between two phototsystem (PS) complexes in response to the light regime for photosynthesis and (2) reaction mechanism and molecular structure of thylakoid protein complexes functioning in the primary process of photosynthetic energy conversion. Current progress in our research is as follows: (1) *Regulation of thylakoid composition*: Our previous study has revealed that in the cyanobacterial system, stoichiometry between two PS's is regulated responding to the light regime for photosynth-

esis: the ratio of PS I/PS II becomes larger under the light mainly exciting PS II while it is reduced under the light mainly exciting PS I. Determination of the contents of PS I, PS II and cyt b_6/f complexes in a single cell has revealed that the content of PS I complex is regulated. Further study has indicated that the synthesis and/or the assembly, but not the decomposition, of PS I complex is regulated in response to the light regime. These findings have brought about an idea that the synthesis and/or the assembly of PS I complex, the terminal component of thylakoid electron transport system is controlled so as to balance turnovers of two PS's.

(2) *Reaction mechanism and molecular structure of light-harvesting antennae*: The study on the excitation energy transfer within light-harvesting antenna is currently focused on the energy transfer from light-harvesting antennae to the reaction centers in higher plants. Kinetic as well as spectral analyses of time-resolved emission spectra at ps range were made with isolated PS II particles and PS II complexes variously resolved. Analyses have succeeded in the establishment of a model for the energy transfer path from chl's in LHC II to the PS II reaction center chl via those in 43 and 47 kd peptides of PS II complexes. The structural study on phycobilisome (PBS) antenna has been made by electron microscopic analysis of *in vivo* and *in vito* PBS as well as analysis of the pigment composition in a single PBS. The study has brought about a finding of a structurally new type of PBS.

(3) *Reaction mechanism in PS I complex*: The electron transport mechanism in PS I complex has been studied with isolated PS I complexes by analysis of rapid electron flow kinetics induced by laser-flash and of EPR signals. Special attention has been paid to the function of vitamin k-1 in the electron transport after the charge separation in PS I complexes. Based on the finding that diethylether-treatment efficiently removes not only chl *a* but also vitamin k-1 from PS I complexes, the electron flow kinetics were analyzed for the ether-treated PS I complexes before and after adding back vitamin k-1. The analyses have provided a new finding that vitamin k-1 is the secondary electron acceptor mediating the electron flow from the primary to the terminal acceptors in the complex.

Division of Cell Proliferation

Professor: Yasuhiro Anraku
Associate Professor: Souichi Nakamura
Research Associate: Hidetoshi Iida

Versatile roles of calcium signaling in the regulation of cell proliferation of the yeast *Saccharomyces cerevisiae* have been investigated. The research is currently focused on the following two projects. 1) An experimental system suitable for yeast cell culturing under calcium-limiting conditions has been established. This system make it possible to investigate how the initiation of DNA synthesis, budding and mitosis is regulated by Ca^{2+} and to isolate mutants which cannot grow in calcium-deficient medium. 2) Techniques for measuring intracellular free Ca^{2+} in individual yeast cells have been established. We have employed fura-2 as a calcium-specific fluorescent probe in conjunction with digital image processing. We have found that fura-2 can be effectively loaded into yeast cells by electroporation without significant loss of viability. Images of fura-2 fluorescence under the Nikon Microphot-FX microscope are acquired by a SIT camera and relayed into a TV monitor and the Hamamatsu Photonics Argus-100 image processor. Using this system, we have shown that the intracellular free Ca^{2+} concentration in cells of *a* mating type increases 5–8 fold after 1h-incubation with a yeast mating pheromone, α factor that is known to arrest cells in the G_1 phase of the cell cycle.

DATA TO ANALYZE ENHANCER ELEMENT OF THE FIBROIN GENE

Division of Cellular Communication (Adjunct)

Professor: Yoshiro Shimura
Associate Professor: Kenzo Nakamura
Research Associate: Kiyotaka Okada

The division deals with molecular biology of higher plants. Our current interest resides in the genetic control of flower development and morphogenesis, and of gravitropic response in a small crucifer, *Arabidopsis thaliana*. *Arabidopsis thaliana* is well suited for genetic and molecular biological studies, since this plant has some remarkable features such as exceptionally small genome size (7×10^7 base pairs per haploid which is only 20 times that of *E. coli*), short life-cycle (5–6 weeks), small size of plant body (20–30 cm in height) and easy cultivation in laboratories under continuous illumination at a constant temperature (22°C).

We have isolated a number of mutants which show aberrant phenotypes in flower morphology and/or development and in root gravitropism. These mutants have been analyzed genetically, so that the number of mutational loci, dominance and allelism have been clarified in at least some of the mutants. In addition to the search and characterization of the mutants described above, we have also isolated clones carrying the calmodulin genes of the plant. These studies will be detailed in the following section:

The developmental and morphological processes of flowers consist of at least several stages: (1) generation of floral primordia, (2) arrangement of primordia of floral organs (sepal, petal, stamen and pistil), (3) development and differentiation of the organ primordia, (4) maturation of each floral organ, (5) blooming and (6) fertilization. Of some 150 independent flower mutants of *Arabidospsis* isolated, we have found some mutant strains whose genetic defects seem to correspond to each of the stages described above. For example, a mutant which generates no flowers on inflorescences, mutants which lack petals and/or stamens, and mutants which have abnormal structure in pistils could be interpreted to have abnormalities in stages (1), (2), and (3), respectively. Of particular concern is a class of mutants which have homeotic conversions between floral organs. For example, sepals are converted to pistils in one mutant strain, and petals to stamens and sepals to leaves in another mutant. These mutants could be caused by a genetic defect in a developmental process which determines the fate of an organ primordium. Genetic analyses of these mutants clearly show that most of these flower mutants carry single, recessive, nuclear mutations.

We have also isolated at least 10 mutants which show an abnormal gravitropic response in root elongation. The mutants are classified into at least three groups phenotypically: a group showing delayed responce, a group which does not respond to a change of gravity direction, and a third group which does not grow straightly downward but fluctuates mincingly. Genetic analyses of the gravitropic mutants are in progress.

Calmodulin is known to be a ubiquitous regulatory protein in higher and lower eukaryotes. Cloning of calmodulin genes from an *A. thaliana* genomic library has revealed that there are at least a few non-allelic calmodulin genes in the plant. The nucleotide sequences of the two indicate that they are highly homologous to each other and are also quite similar to the calmodulin genes of other eukaryotes. The exon-intron organization is almost identical in the two non-allelic genes of the plant but is considerably different from that of other eukaryotes including yeast, trypanosome and mammals.

Attempts are being made to identify and isolate the genes responsible for the flower mutants as well as the gravitropic mutants using transformation systems mediated by the Ti-plasmid and Ri-plasmid vectors or by a direct gene-tagging system. For such experiments, it is absolutely necessary to develop a good, efficient transformation system and also to construct a good genomic library of the Ti-derived cosmid vector.

Division of Cell Fusion (Adjunct)

Professor: Tsuyoshi Uchida
Associate Professor: Masaru Yamaizumi
Research Associate: Masahiro Ishiura
Kenji Kohno

Molecular and cellular biology of mammalian cells, aided by cell engineering and recombinant DNA technology, are the research projects of this division. In relation to the action mechanism for diphtheria toxin in mammalian cells, the structure and function of the elongation factor-2 (EF-2) involved in the protein synthesizing system of mammalian cells are currently being extensively studied at the molecular level using cloned EF-2 genes. Cosmid cloning has been established by constructing simplified cosmid vectors and selecting adequate *E. coli*. Elucidation of the mechanism for DNA repair in mammalian cells is also in progress.

DEPARTMENT OF DEVELOPMENTAL BIOLOGY

Chairman: Goro Eguchi

The department has three research divisions and one adjunct research division which conduct research into the cellular and molecular mechanisms of various processes involved in developmental phenomena.

Division of Reproductive Biology

Professor: Yoshitaka Nagahama
Associate Professor: to be appointed
Research Associate: Michiyasu Yoshikuni

The division conducts research on the molecular mechanisms of differentiation, growth and maturation of gonads (ovary and testis) in multicellular animals, particularly the hormonal control of oocyte growth, oocyte maturation, spermatogenesis, and spermiation. These studies combine biochemical, ultrastructural and physiological approaches. Our current investigations are designed to identify and characterize the molecules which regulate ovarian and testicular functions. This information will provide the basis for a study at the molecular level of their precise mode of action.

Research centers around oocyte maturation in teleosts and starfish. Our previous studies have established that three major mediators are involved in the regulation of oocyte maturation in these animals: gonadotropins (GTH) or gonad-stimulating substances (GSS), maturation-inducing substances (MIS) and maturation-promoting factor, or M-phase promoting factor (MPF). These three mediators function sequentially at the levels of the follicle layer, the oocyte surface and the oocyte cytoplasm, respectively. In 1985, we identified for the first time in any vertebrate the MIS of salmonid fishes as 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DHP). Production of this steroid occurs via the interaction of two distinct cell types in the follicle and is controlled by GTH. We are now focusing on the molecular mechanisms involved in the synthesis and action of 17α,20β-DHP.

Our current research concerns (1) the characterization and/or synthesis of teleost GTHs and starfish GSS, (2) the molecular mechanisms of biosynthesis of salmonid MIS, 17α,20β-DHP, and starfish MIS, 1-methyladenine, in ovarian follicle cells, (3) the purification and characterization of GTH receptors located on salmonid ovarian follicle cells, (4) the characterization of the MIS

receptors located on the oocyte surface, and (5) the purification, characterization and function of fish MPF. Other ongoing investigations concern the genetic control of sex differentiation, and defining the molecules (proteins and steroids) involved in the initiation of meiosis, spermatogenesis and spermiation.

Division of Cell Differentiation

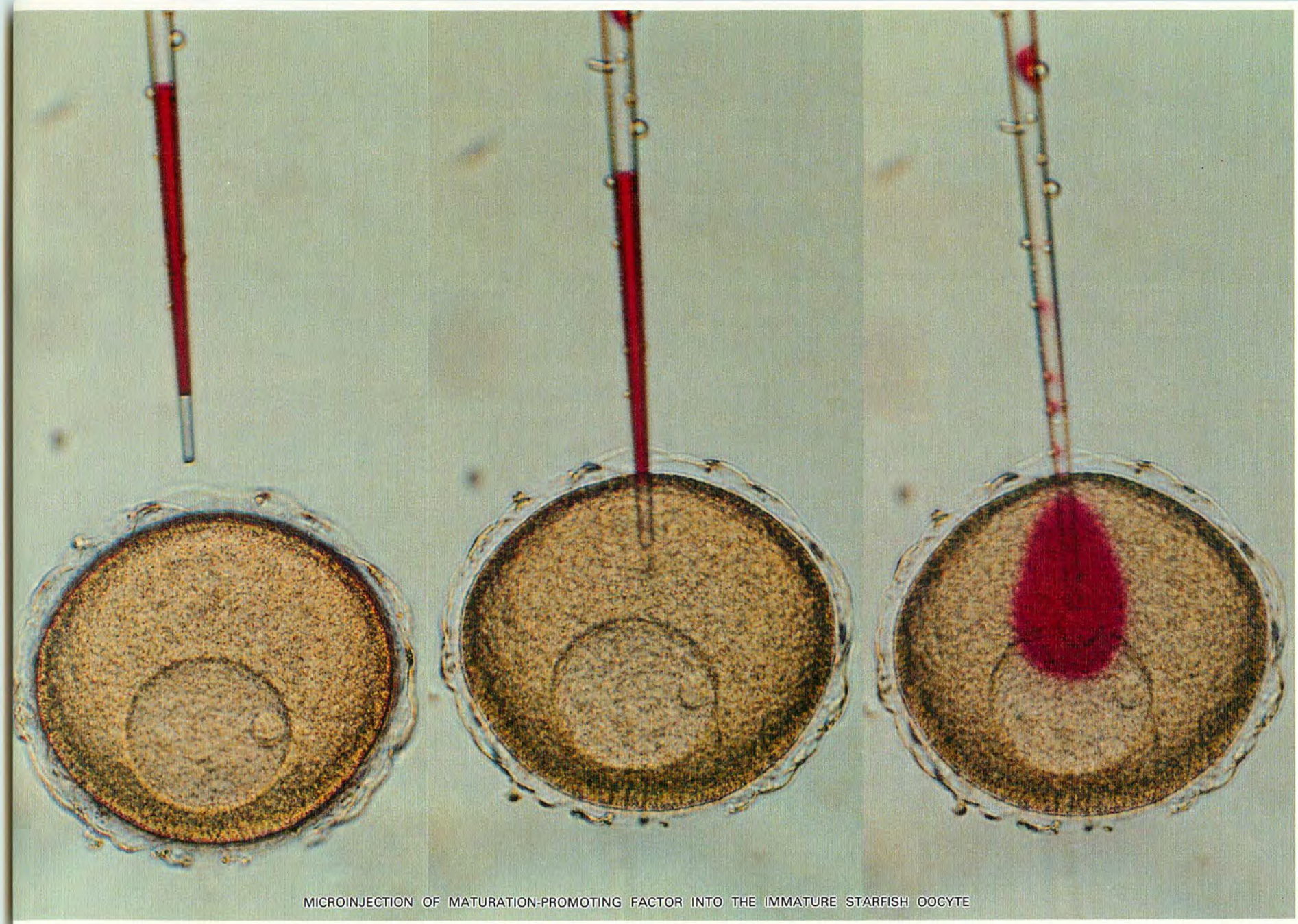
Professor: Yoshiaki Suzuki
Associate Professor: Kohji Ueno
Research Associate: Shigeharu Takiya
Toshiharu Suzuki

Members of the division have been analyzing developmental regulation of the tissue-specific genes and the homeotic genes in *Bombyx mori*. Recently we have described that the transcription of the silk fibroin gene in the posterior silk gland cells is initiated at around E25 stage when silk gland morphogenesis has been completed in the embryos. The fibroin gene is repeatedly switched on and off in the following stages of larval development. To analyze the molecular mechanisms of the tissue- and stage-specific regulation of transcription, we have developed 18 cell-free transcription systems from embryos and several tissues from various developmental stages and cultured cells. These cell-free extracts reveal transcription in a stage- and tissue-specific manner, and differential transcription of two genes can be demonstrated. Using these systems and techniques of gel shift, footprint, and biochemical fractionation of the extracts, we have detected tissue-specific factor(s) as well as ubiquitous factors that interact with the fibroin DNA and sericin DNA. Currently we are concerned with how synthesis and specificity of the factors are regulated. In *Bombyx mori*, more than 20 homeotic mutants that mapped to the proximal end of the 6th linkage group were described as E-group during the period of 1930 and 1960 in Japan. They exhibit a variety of developmental abnormalities in organogenesis in various body segments, and in most cases homozygosity of the mutant gene results in embryo death. These genes probably regulate some structural genes that play important roles in pattern formation. We have begun to clone some of these homeotic genes in order to analyze the molecular events during embryonic development and hopefully to clarify the relationship between these regulatory genes and those structural genes such as the fibroin gene that are expressed tissue-specifically.

Division of Morphogenesis

Professor: Goro Eguchi
Associate Professor: Kenji Watanabe
Research Associate: Ryuji Kodama
Kiyokazu Agata

Problems of stability and instability in cell differentiation have been studied as current projects in Division of Morphogenesis.
(1) Remarkable progress has been attained in understanding the transdifferentiaton of pigmented epithelial cells(PECs) into lens cells. Utilizing our established culture system of chick embryonic PECs, we have cloned genes coding for several molecules characteristic of PECs and lens cells. Melanosomal matrix protein (MMP-115) is detected with a monoclonal antibody, and its cDNA is cloned and fully sequenced. MMP-115 gene is expressed specifically in PECs and skin melanocytes. MMP-115 will be a cue to dissect the formation of melanosomes with gene engineering. Two other genes (pP344 and pP64) specifically expressed in PECs are cloned and sequenced and their functions are now being studied. Expression of pigment cell-specific genes (MMP-115, pP344 and pP64) and lens cell-specific crystallin genes (α A and β) are entirely repressed in the multipotent (at least bipotent) dedifferentiated state of PECs. Gap junctions are responsible for intercellular communication in the epithelial structure organized by PECs. They diminish in the dedifferentiated state of PECs. In normal lens cells and transdif-



MICROINJECTION OF MATURATION-PROMOTING FACTOR INTO THE IMMATURE STARFISH OOCYTE

ferentiated lens cells, another type of gap junction is expressed. We are purifying both types of gap junction proteins to clarify regulatory mechanisms of their switching expression in the transdifferentiation of PECs.

Ability of PECs to transdifferentiate was first found in the newt. Now it is possible to assume that such an ability to transdifferentiate is conserved widely in vertebrates including humans. PECs of humans have high growth ability and are able to transdifferentiate into lens cells under similar conditions used in chicken PECs culture. Dedifferentiated PECs of humans have been maintained in culture for more than 6 months, expressing lens phenotype continuously and are expected to become a cell line.

(2) Although lens regeneration from PECs *in situ* is possible only in a limited number of species, PECs of various animal species can transdifferentiate into lens when dissociated and cultured.

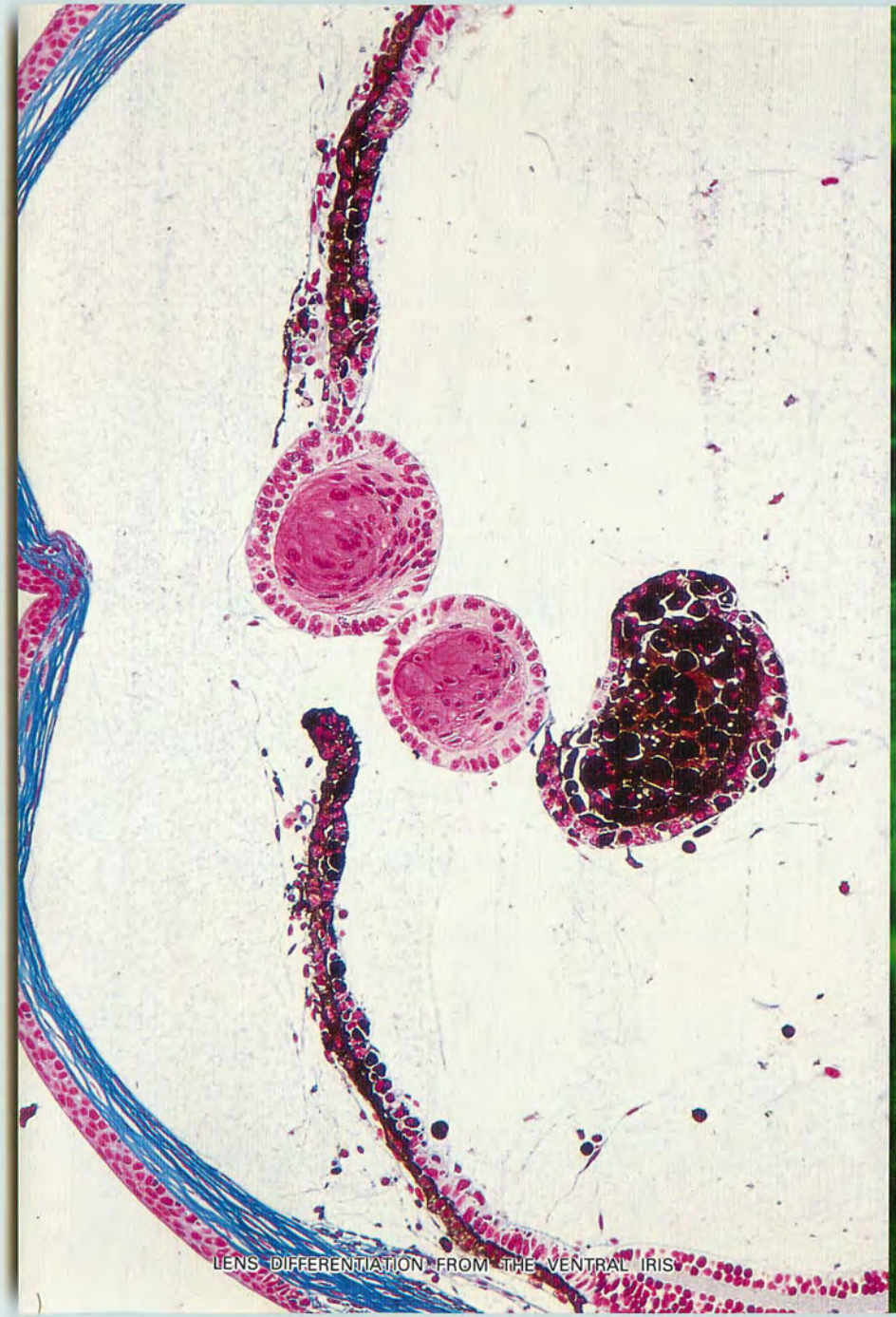
Transdifferentiation of PECs *in vitro* was achieved by manipulation of cellular environments, suggesting the presence of some key molecules responsible for stabilization of the differentiated state of PECs *in situ*. We have obtained an exciting monoclonal antibody by immunizing mice with the homogenate of adult newt irises. The monoclonal antibody stained the cell surface or intercellular materials of various newt tissues including the iris. Soon after lentectomy, however, antigen molecules began to decay only in the dorsal marginal iris pigmented epithelium, the site of lens regeneration, and became temporarilly undetectable prior to dedifferentiation of PECs. Fragments dissected from the ventral iris, from which lens regeneration never occurs in the eye *in situ*, became capable of lens formation, when pretreated with the antibody. The antigen molecules reacting with the monoclonal antibody must be required for stabilization of the differentiated state of the newt iris PEC, and the loss of these molecules might be closely related to the initiation of dedifferentiation of dorsal iris PECs prior to transdifferentiation.

(3) Senile cataracts are one of the important problems associated with prolonged life in humans. What mechanism underlies in the progress of senile cataracts?. The lens is highly susceptable to environmental disorders such as high glucose in the blood, leading to cataracts. This property of the lens might be due to the characteristic structure of this organ. The lens is a large avascular tissue composed of a sheet of lens epithelium and a mass of lens fibers. Lens epithelium is responsible for maintaining the inner environment of the lens fiber mass. Aging of lens epithelial cells must lead to deterioration of their function and cause cataracts. To investigate the aging of lens epithelial cells, we are studying the growth and differentiation potential of lens cells from humans of various ages and also from chickens of various developmental stages.

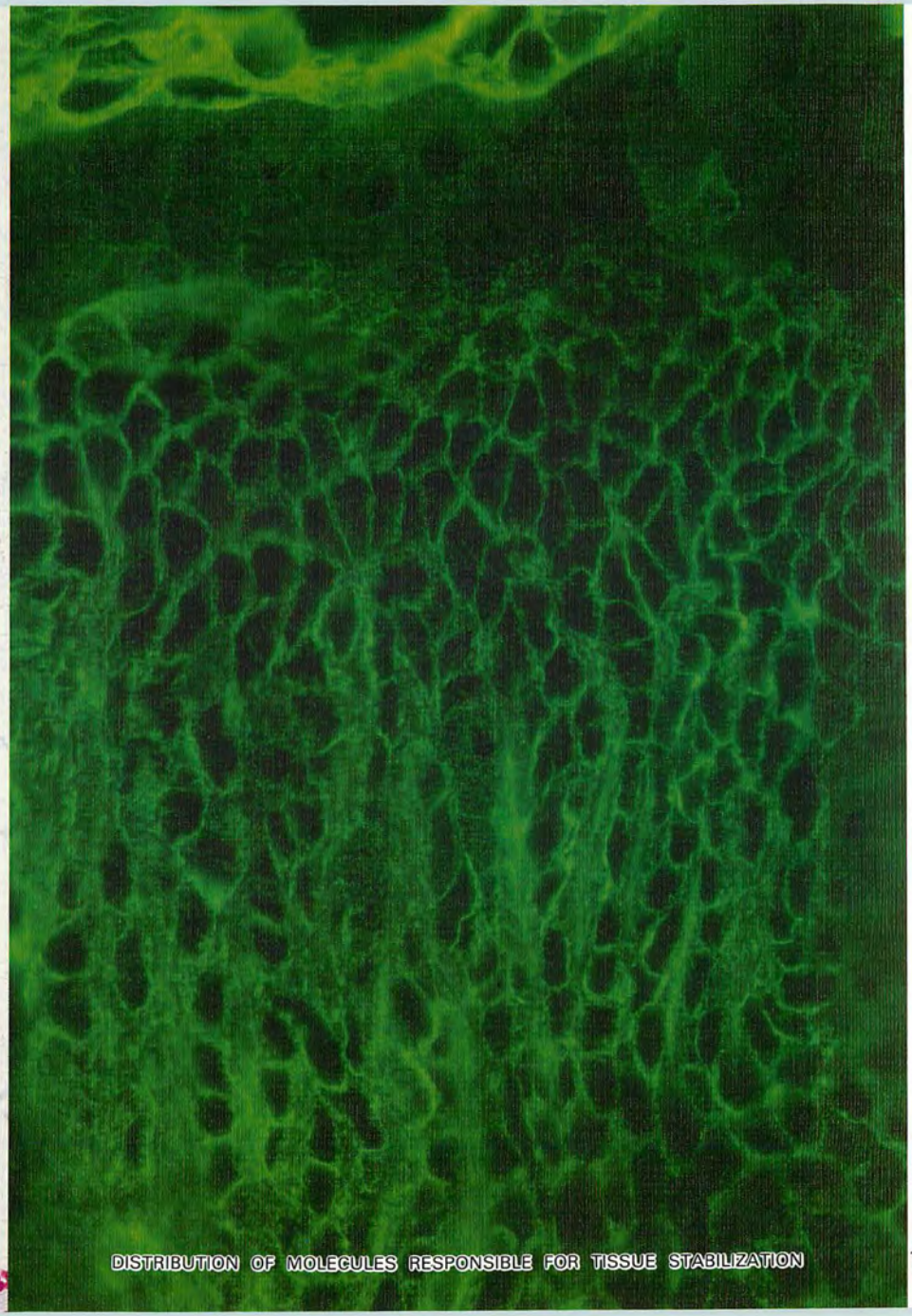
Division of Developmental Biology

Professor: Ikuo Takeuchi
Associate Professor: Masaki Iwabuchi
Research Associate: Masao Tasaka

This division is devoted to the study of regulation in development, using the developmental system of cellular slime molds. The development of these organisms is characteristic in that after tissue formation, two types of cells, called prestalk and prespore cells, differentiate in a fixed proportion at the anterior and posterior parts of the tissue respectively and that removal of a portion of the tissue brings about cell-type conversion between the two presumptive cells and results in regeneration of the normal differentiation pattern without accompanying cell division. To elucidate the regulatory mechanisms of gene expression during the processes of cell differentiation, we have isolated 2 and 6 cDNA clones for mRNA which specifically accumulate in prestalk and prespore cells, respectively. Analyses using cDNA clones indicated that both cell types begin to differentiate around the same stage in development, accompanying changes in transcription of a set of cell-type specific genes. Further analyses along this line were pursued during 1987,



LENS DIFFERENTIATION FROM THE VENTRAL IRIS



DISTRIBUTION OF MOLECULES RESPONSIBLE FOR TISSUE STABILIZATION

as follows:

I. Prespore specific gene expression

a) Gene encoding a spore coat protein

By screening the cDNA libraries in λ gt11 with a polyclonal antibody against *Dictyostelium discoideum* spores, we have obtained a cDNA clone encoding a spore coat protein. The Northern blotting analyses showed that the mRNA of *ca.* 2.2kb first appeared at the tipped aggregate stage and accumulated in prespore and spore cells at the later stages of development. The Southern blotting analyses indicated that the gene is unique. The genomic fragments covering the coding, 5'upstream and 3'downstream regions were isolated. The gene includes one open reading frame separated by one intron and encodes the peptide composed of 595 amino acids (Mr.59158.27). This peptide has a leader sequence at the N-terminal, one possible N-glycosylation site in the amino-half and three independent repeated amino acids sequences. We detected a few DNase-I hypersensitive sites in the 5'upstream region characteristic of the expressed gene.

b) Gene encoding elongation factor 2

Full length cDNA clones hybridized with the hamster elongation factor 2 (EF2) cDNA were isolated from the libraries. The complete amino acid sequence of *D. discoideum* EF2 deduced from the nucleotide sequence of cDNAs indicated more than 60% overall homology with the hamster EF2, the homology being as high as *ca.* 80% in its GTP- and ribosome-binding regions. Its molecular weight was estimated 91,699 and the site of ADP-ribosylation by diphtheria toxin was identified. The Southern blotting indicated that the gene encoding the EF2 is unique. We have isolated the genomic DNA clones including the coding, 5'upstream, and 3'downstream regions. The Northern blotting analyses indicated that the expression of the mRNA was highest during the vegetative stage and gradually decreased through development. As prestalk cells differentiated, the mRNA disappeared in these cells, thus becoming highly enriched in prespore cells compared to prestalk cells. Whether this was due to specific turning-off of transcription of this gene or degradation of the mRNA in prestalk cells is now being examined.

II. Prestalk specific gene expression

By the use of a prestalk specific cDNA clone, the genomic DNA fragment, including the coding 5'upstream and 3'downstream regions, was isolated and sequenced. We have obtained an antibody produced against the fusion protein encoded by this gene. The antibody recognized a protein of Mr45,000. Expression of this gene in cells and tissues as well as the nucleotide sequences involved in regulation of its expression are now being examined.

DEPARTMENT OF REGULATION BIOLOGY

Chairman: Goro Eguchi (adjunct)

Two divisions and two adjunct divisions belong to the department, and conduct research on information processing and control mechanisms in biological systems.

Division of Sensory Processing

Professor: Ken-Ichi Naka
Associate Professor: Shozo Yasui
Research Associate: Hiroko Sakai
Eiki Hida

NEURON NETWORK IN THE VERTEBRATE RETINA

The goal of our study is to define how signals are processed in a neuron network. The network we have chosen is that in the vertebrate retina because: 1) the retina is essentially a two-dimensional structure of a few hundred microns in thickness, 2) it

FLOWERS OF *ARABIDOPSIS THALIANA*: WILD TYPE (LEFT) AND A HOMEOTIC MUTANT, FL-40 (RIGHT)

is distinctly layered and constituent neurons have a characteristic morphology and physiology, 3) the input-output relationships are well-defined, and 4) neurons within can be reached from outside without much difficulty. These factors make the vertebrate retina an 'ideal model' to unravel the working of a neuron network. In essence the retina is an approachable part of the brain. From the nature of our subject our research is interdisciplinary and is distinctly different from the other types of 'biological' research which are mostly matter oriented. To achieve our goal we have made two original choices in methodology and preparation.

One of the most basic approaches in science and engineering is to stimulate (or to excite) a system and to observe its response. This is to identify a system by observing how it responds to a certain input: a black-box approach. Norbert Wiener suggested the use of Gaussian white-noise as test signal and to identify a system through a series of orthogonal functionals known as the Wiener G-functionals or kernels. Gaussian white-noise is a formal derivative of the fundamental chaos, Brownian motion, a concept examined rigorously in mathematics.

Twenty years ago, we began our pioneering effort to apply the theory to identify a practical system, the neuron network in the retina, and our past effort has made it a legitimate means in neurophysiology. This is testified by the fact that Dr H. M. Sakai has lately been invited to contribute a chapter, "White-noise Analysis in Neurophysiology", to the prestigious Annual Review of Physiology. In addition to the esthetic appeal of the theory, Wiener's methodology has many practical advantages over the traditional testing of systems by sinusoidal or pulsatile inputs. It is a tribute to the great mathematician that he formalized the theory in his late fifties. During the course of our application effort, we have developed a comprehensive software system to execute analysis efficiently and effectively as prescribed by Wiener's theoretical framework.

In biology, the choice of preparation is often as important as the choice of methodology. The preparation must be original and should offer the best chance of achieving the research objective. We have chosen the retina of channel catfish, *Ictalurus punctatus*, as the subject of research. The retina is now the most thoroughly studied vertebrate retina, and our choice made twenty years ago has been vindicated. N. Osborne and J. Chader wrote on catfish research: "This is not only due to improvements in technology but to the brilliance and dedication of workers in the field who have been able to put together the functional circuitry of the retina in relation to its morphology".

Our research has produced several salient results. In morphology, we have discovered three new synapses: the synapse made by the horizontal cells back to the receptors, the synapse made by the horizontal cells onto the amacrine cells and the synapse made by the ganglion-cell dendrites. Functional evidence shows that the synapse is functional. In view of the fact that retinal ganglion cells are the most extensively examined neurons in the central nervous system, this discovery is noteworthy.

In the functional domain, we have identified the dynamics of retinal neurons to show that there are two principal transformations in the retina: the piecewise linearization in the outer retina and the generation of static (second-order) nonlinearity in the amacrine cells. These linear and non-linear components are encoded into spike trains, a point process, to be sent to the brain. Cross-correlation between white-noise inputs and resulting spike discharges recovers the linear and nonlinear components. Thus the neuron network in the retina can be approximated by the Wiener/Korenberg cascade structure.

We have also discovered that neurons in the inner retina are extensively coupled by fast and bi-directional pathways and such interactions are linear. Groups of tightly inter-connected neurons are loosely coupled to each other through a band-pass filter. Thus, the notion of central interaction through either excitatory or inhibitory synapses needs to be modified in the retinal neuron network.

Division of Cellular Regulation

Professor: Norio Murata
Associate Professor: Hideaki Nakashima
Research Associate: Takao Kondo
Ikuo Nishida

This division studies two important phenomena in higher plants and microalgae: (1) The molecular mechanisms of low-temperature sensitivity and adaptation to temperature stress. (2) The structure and function of chloroplast membrane proteins in photosynthesis. The temperature work emphasizes the participation of biological membranes and membrane lipids: (i) An enzyme (glycerol-3-phosphate acyltransferase) has been isolated which produces special lipid molecules that are responsible for the low-temperature sensitivity of higher plants, and its cDNA has been cloned. Studies aiming at the plant transformation in the low-temperature sensitivity by the cloned DNA are taking place. (ii) Adaptation of plants to temperature stress is studied using tobacco culture cells and cyanobacteria. These organisms increase the degree of unsaturation of membrane lipids when exposed to low temperature. Enzymes which catalyze the desaturation of fatty acids are under investigation.

Photosynthesis research is focused on the machinery of the oxygen-evolving complex. This complex is embedded in chloroplast thylakoid membranes and consists of about 20 kinds of protein components. In order to elucidate the molecular mechanism of the oxygen evolution, the following subjects are presently studied by techniques of biochemistry, physicochemistry and molecular biology: (i) Purification and property of the structural components of the complex. (ii) Three-dimensional interaction of the protein components within the complex. (iii) The functional role of each component in oxygen evolution.

Division of Control of Gene Regulation

This division was newly opened in 1988. Its chief intention is to study the control mechanisms of gene expression in multicellular organization, with particular attention to the introduction of technology of transgenesis of animals and plants. Research activities are expected to start from the later half of the fiscal year of 1988.

Division of Biological Regulation (Adjunct)

Professor: Hidemasa Imaseki
Associate Professor: Mikio Nishimura
Research Associate: Kotaro Yamamoto
Satoru Tokutomi
Wataru Mitsuhashi

Higher plants always regulate their growth rate and differentiation processes according to their environmental conditions such as light, temperature, water availability and so on. Under environmental stimuli, changes in cellular functions take place in plant cells long before any changes are detected morphologically. The Division of Biological Regulation conducts research at the biochemical and molecular biological level on the controlling process of higher plants triggered by changes in environmental condition.

The research programs being carried out at present are (1) study of the rapid growth and opening of plumules during germination of mung bean (*Vigna radiata*) seeds, (2) study of the acquisition of thermotolerance in mung bean seedlings, and (3) study of the structure-function relationship of phytochrome, a photoreceptor which senses light as environmental stimuli.

(1) Biochemical and molecular biological study on rapid growth of plumules: During germination of a mung bean seed, the plumule starts to grow and open after imbibition. The growth and opening is mainly due to the enlargement of cells. Since the process requires synthesis and accumulation of large amounts of carbon and nitrogen compounds, sucrose and amide metabolism must be activated prior

to it. The aim of this study is to clarify the molecular mechanism of activation of the metabolism, which is triggered by imbibition.
At present, we are studying the role of invertase and sucrose synthase in carbon metabolism. Activities of these enzymes increase dramatically two days after imbibition. As for nitrogen metabolism, we are investigating the role of asparaginase since asparagine comprises 40 % of nitrogen compounds supplied to a plumule from the other parts of the seedling. We are using immunochemical methods to test whether the increase of enzyme activity results from the activation of inactivated enzymes or from the *de novo* expression of their genes.
(2) Mechanism of the aquisition of thermotolerance: When plant seedlings are exposed to high temperature (*ca.* 50°C) for about 20 min, they are irreversibly damaged and die, even if they are returned to a normal growth temperature (*ca.* 28°C) again. However, pretreatment at medium temperature (*ca.* 40°C) provides protection (thermal tolerance) to a subsequent exposure to the high temperature. The acquisition of thermotolerance has been observed universally in organisms. Some changes in the structure and function of biological membranes may be involved in thermotolerance, as is observed in thermal adaptation. To discover the mechanisms of aquisition of thermotolerance, investigations on the involvement of biological membranes in the phenomena have been started. The effect of these thermal treatments on the physicochemical properties, such as fluidity, permeability and surface potential has been studied in total lipid membranes.
(3) Structure-function study of phytochrome: Phytochrome is a major photoreceptor of photomorphogenic responses in plants. It absorbs light and transmits a signal to other cellular components in an unknown manner. In order to uncover the primary reaction of phytochrome, this study aims to identify functional domains of phytochrome with respect to light absorption and conformational changes which subsequently take place. At the moment, we are investigating the macromolecular structure of phytochrome by small angle X-ray scattering, and characterizing a functinal domain for light absorption by the methods of protein chemistry and spectroscopy.

Division of Behavior and Neurobiology (Adjunct)

Professor: Katsuhiko Mikoshiba
Associate Professor: Masaharu Ogawa
Research Associate: Takaaki Tamura

In our research division, we are studying the mechanism of development, growth, and differentiation of the mammalian nervous system at the molecular level. The main themes of our division are:
(1) Studies on myelin-deficient mutant animals. We have produced several mutants with abnormalities at different steps of myelinogenesis. We are studying myelination as a typical morphogenetic phenomenon in the central nervous system, and also as a neuron-glia inter-relationship.
(2) Studies on the mechanism of brain specific gene expression. We are studying these subjects with both *in vitro* and *in vivo* systems. In the *in vivo* system, we are studying promoter activity of myelin basic protein (MBP), proteolipid protein (PLP) and JC virus genes by brain-specific *in vitro* transcription system.
(3) Studies on the structure and function of brain-specific proteins. Brain-specific proteins such as P_{400}, which is specifically expressed in Purkinje cells in the cerebellum. Antisera and cDNA clones of the P_{400} have been obtained. We are studying the structure of the mRNA and the genomic DNA of the P_{400} as well as protein structure and its functions.

TECHNICAL DEPARTMENT

Chief: Hachiro Honda

The Technical Department is a supporting organization for researchers and research organizations 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.
Technicians participate, through the department, in mutual enlightenment and education to increase their capability in technical areas. Of 26 staff members 14 belong to the Common facility group (Chief; Hiroyuki Hattori), whereas the other 12 are supporting research at each division.

RESEARCH RESOURCE

There are four categories of research support facilities available to research members and visiting biological scientists: 1) Campus-wide facilities (CENTRAL COMPUTING CENTER and LIBRARY), 2) NIBB's own research support facilities (RESEARCH SUPPORT FACILITY, intramural), 3) facilities jointly maintained by the NIBB and the IPS but managed either by the NIBB (RADIOISOTOPE FACILITY), or by the IPS (ANIMAL-CARE FACILITY), 4) facilities jointly run by NIBB and IPS (CENTER for ANALYTICAL INSTRUMENTS, ELECTRON MICROSCOPE CENTER, LABORATORY GLASSWARE FACILITY, MACHINE SHOP AND LOW-TEMPERATURE FACILITY).

RESEARCH SUPPORT FACILITY

Head of Facility: Yoshitaka Nagahama
Faculty: Masakatsu Watanabe (Spectrograph)
Yoshio Hamada (Tissue and Cell Culture)

The facility maintains large-scale experimental equipment and facilities for growing and maintaining biological specimens. The facility is shared among the research members, and has five laboratories.
The Large Spectrograph Laboratory: This laboratory has the largest spectrograph in the world dedicated mainly to action spectroscopical studies of various light-controlled biological processes (the Okazaki Large Spectrograph, OLS). The spectrograph runs on a 30KW-Xenon arc lamp and has a compound grating-surface composed of 36 smaller individual gratings. It projects a spectrum of a wavelength range from 250 nm to 1,000 nm onto its focal curve of 10 m in length. The fluence rate (intensity) of the monochromatic light is more than twice as much as that of the tropical sunlight at noon at each wavelength. More than 30 projects every year are conducted by visiting scientists including foreign scientists as well as those in the Institute under the NIBB Cooperative Research Program for the use of the OLS.
Tissue and Cell Culture Laboratory: This is a facility for tissue and cell culture. This laboratory is equipped with safety rooms which satisfy the P3 physical containment level. This facility is routinely used for DNA recombination experiments.
Laboratory Computer Facility: The NIBB's computing is handled by a network of Digital Equipment Corporation's VAX 11/780 and VAX 2,000 with Floating Point System's AP120B and 5310 array processors. An Ethernet interfaces the network with a number of laboratory PC's. An extensive software system for time-series analysis developed in-house has been operating for the last 8 years.

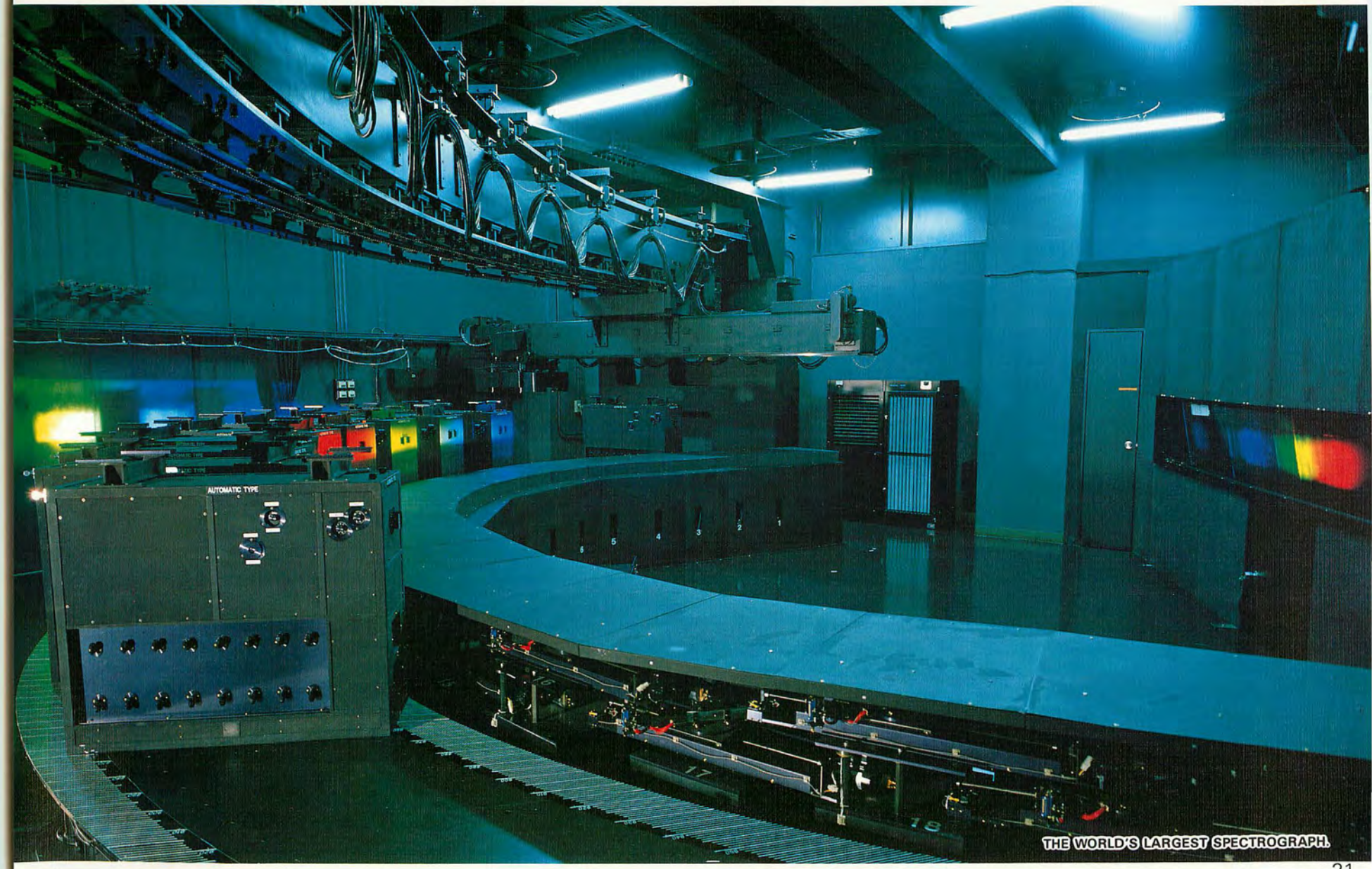
A limited number of image processing routines are also available.
Plant Culture Facility: There are a large number of culture boxes, cubicles, and a limited number of rooms with environmental control for plant culture.
Experimental Farm: This facility consists of two 20 m^2 glass-houses with precision temperature and humidity control, a limited farm, two green houses (45 m^2, 88 m^2) with automatic sprinklers and window control, two open aquariums (30 t., 50 t.) and several smaller tanks. The facility also includes a building with office, storage and work-space.
Plant Cell Culture Facility: Autotrophic and heterotrophic culture devices are equipped for experimental cultures of plant and microbial cells.
The faculty of the Large Spectrograph Laboratory conducts its own work. Photoreceptive and signal-transduction mechanisms of phototaxis of single-celled, flagellate algae are studied action-spectroscopically by measuring computerized-videomicrographically the motile behaviors of the cells at the individual cell level and at the subcellular level. Photoreceptive and signal transduction mechanisms of algal gametogenesis are also studied action-spectroscopically.
The faculty of the Tissue and Cell Culture Laboratory also conducts its own work. Myosin is a ubiquitous protein in eukaryotes. It is presumed that this molecule plays a key role in cell motility as well as other functions. The role of myosin in non-muscle cells might be better understood if wild-type cells could be compared with mutants cells with altered myosin molecules, though such mutants have not been isolated. We intend to examine the function of the myosin heavy chain in vertebrate non-muscle cells by interrupting its synthesis with the introduction of antisense RNA molecules.

RESEARCH FACILITIES RUN JOINTLY WITH IPS

RADIOISOTOPE FACILITIES (managed by NIBB)

Head of Facility: Yoshihiko Fujita
Faculty: Kohji Hasunuma

The facility is composed of a center and two subcenters, one in NIBB and the other in IPS. The facility is being used for molecular anaylses of eukaryotes. At the center, a variety of radioisotopes such as ^{3}H, ^{14}C, ^{22}Na, ^{32}P, ^{35}S, ^{45}Ca and ^{125}I are handled, as well as various species of beta and gamma-ray emitting nucleides. A P3-level laboratory for recombinant DNA research is included in the center facilities. At the substations, only a limited variety of radioisotopes such as ^{3}H, ^{14}C and ^{32}P are processed. The substation in NIBB is equipped with a P2-level recombinant DNA research laboratory. The members of the Radioisotope Facility maintain and control the center, and give users appropriate guidance for radioisotope handling.
The faculty of the facility conducts its own research on the signal transduction chain of light to cyclc nucleotides in *Neurospora crassa* and *Lemna paucicostata*. Several species of ATP-GTP-binding proteins were ADP-ribosylated by endogenous ADP-ribosyltransferase. The goal of the research is to isolate the genes for ATP-GTP-binding proteins and ADP-ribosyltransferase and to analyze how the external singals such as light and hormones were transduced to the intracellular second messengers.



THE WORLD'S LARGEST SPECTROGRAPH.

CENTER FOR ANALYTICAL INSTRUMENTS
(managed by NIBB)

Head of Facility: Norio Murata

The Center for Analytical Instruments consists of the following five sections. (1) Chemical analysis, (2) Preparation of Biological Materials, (3) Spectroscopic analysis, (4) Physical analysis, and (5) Microscopic analysis. Each section is equipped with instruments for general use as listed below.

1. Section for Chemical Analysis

Amino Acid Analyzer	HITACHI 835
Gas Chromatograph	SHIMAZU GC-7APTF
HPLC	JASCO TRIROTAR III
Protein Sequence Analyzer	JEOL JAS-47K
Peptide Synthesizer	BECKMAN 990C

2. Section for Preparation of Biological Materials

Coulter Counter	COULTER ZB
Isotachophoresis System	LKB 2127 TACHOPHOR
Preparative Ultracentrifuge	BECKMAN L8-80
Two Parameter Cell Sorter	BECTON-DICKINSON FACS-II

3. Section for Spectroscopic Analysis

Atomic Absorption Spectrophotometer	PERKIN-ELMER 603
Differential Refractometer	CHROMATIX KMX-16
Dual-wavelength Spectrophotometer	HITACHI 557
Infrared Spectrophotometer	JASCO A-302
Laser-Raman Spectrophotometer	JASCO R-800
Light Scattering Photometer	CHROMATIX KMX-6DC
Spectrofluorometer	HITACHI MPF-4
Spectrophotometer	GILFORD 250 HITACHI 330
Spectropolarimeter	JASCO J-40S

4. Section for Physical Analysis

Analytical Ultracentrifuge	HITACHI 282
Differential Scanning Calorimeter	PERKIN-ELMER DSC-2
EPR Spectrometer	BRUKER ER 200D
GC Mass Spectrometer	HITACHI M-80
GC/LC Mass Spectrometer	JEOL DX-300
Superconductive FT-NMR Spectrometer	BRUKER WM 360wb
Viscometer	CONTRAVES RM-30

5. Section for Microscopic Analysis

2-Dimension Microdensitometer	JOYCE LOEBL 3CS
Film Data Analysis System	NAC MOVIAS GP-2000
Image Analyzer	KONTRON MOP-AM03
Interactive Image Analyzer	KONTRON IBAS-I & II
Microscope Photometer	CARL ZEISS MPM 03-FL

ANIMAL-CARE FACILITIES
(managed by IPS)

Vivarium: This is a 2,100 m^2 building for maintaining land animals, including insects. Operations and experiments of a limited scope may be performed in the vivarium.

Aquarium: This is a 600 m^2 facility for both fresh- and sea-water animals. There are twelve 1-ton and fourty 0.5-ton tanks in addition to one 7-ton and one 2-ton circular tanks. All tanks are individually temperature controlled and are supplied either with deionized water or seawater. There is a lorry with a 2.2-ton temperature-controlled tank to transport aquatic animals and plants.

ELECTRONMICROSCOPE CENTER
(managed by IPS)

This facility maintains the following microscopes for the use by the institute's members as well as researchers from other universities and research institutions.
Transmission microscope: Hitachi H-500 125KV, JOEL 100-CX and 200-CX 100 and 200KV and Philips EM-400HM 120KV.
Transmission scope, analytical: JOEL 200-CX, 200KV.
Scanning scope: Hitachi S-450 25KV.

COOPERATIVE RESEARCH ACTIVITIES

The NIBB sponsors four cooperative research activities.

Individual and group cooperative research program: Scientists from other Japanese universities and research institutes are invited to undertake joint research projects with the institute's members. Limited funds are available for travel and expenditures related to the projects.

Research conferences: The NIBB sponsors research conference on important subjects in biology. Ten to twenty scientists are invited and intense discussion is held for two to three days. The NIBB provides financial support for the participants. Conferences may be initiated by the institute's members or scientists from other institutes.

Facility-sharing program: Scientists with other universities are permitted to use specified instruments maintained by the NIBB. No financial support is available except for the Large Scale Spectrograph whose user is provided with limited financial support.

Graduate student programs: Graduate students with other universities may spend a fixed period of time with members of NIBB. This allows students to have experience with the very modern facilities of NIBB. The NIBB is expected to have its own graduate program in the near future.

Bioscience training course: Since 1986, the NIBB has organized an advanced course with practical exercises on a special subject on the fore-front of the biological sciences. The course is held annualy with about 12–20 attendants above the graduate student level from other Japanese universities and research organization (both national and private).

LIBRARY

The three institues of the Okazaki National Research Institutes share a common library facility. The library is a part of the main administration building and has a floor space of 2,500 m^2.

The library has 9,350 books in Japanese and 30,050 in foreign languages, and subscribes to 237 Japanese and 446 foreign journals. Lending records, inventory and literature searches are computerized. The library is open 24 hours a day.

LODGING FACILITY

The Okazaki National Research Institutes maintain two lodging facilities, the Mishima Lodge and Yamate Lodge.

Mishima Lodge is a few minute walk away; Yamate Lodge is less than 20 minutes. The lodges are for scientists and their families staying for fixed periods of time with the institutes. Some suites and bungalows are provided with kitchenette facilities. There is a modest charge to help maintain the facilities.

Mishima Lodge has 54 single rooms, 4 twin rooms, 6 suites, and 10 bungalows for large families. Yamate Lodge has 11 single rooms, 4 suites and 2 family complexes.

On the campus there is a dining facility which is open Monday through Saturday.

THE CITY

The city of Okazaki, incorporated in 1916, is located 30 kilometers southwest of Nagoya, the fourth largest city in Japan. A high-speed urban train connects the two cities. Okazaki, with a population of 275,000, is a typical medium-size city in Japan and offers the convenience of urban life while avoiding the disadvantages of a large city. Okazaki is the commercial as well as cultural center of the Mikawa (Three River) district with its rich historical heritage. Iyeyasu Tokugawa, the first Tokugawa Shogun, was born here in 1542 and built a castle here. The original parapets and moats and the rebuilt castle still dominate the city as they did 500 years ago. When he established the Shogunate in Edo (the former name of Tokyo) in 1603, Iyeyasu took a large contingent of Mikawa Bushi (Mikawa Samurai or professional warriors) with him. Those Mikawa Bushi formed the nucleus of Iyeyasu's new administration.

Within a radius of 10 kilometers from the city center are located Mitsubishi Motor's Okazaki Plant, Toyota Motor's main production facilities in Toyota city, and Sony's ultramodern Koda plant which produces video tape/tape recorders.

TRANSPORTATION

BY TRAIN

From Tokyo to Toyo-hashi: Two and half hours by the Japan Railway's (JR-Tokai) super-express train (KODAMA or ECHO). The train runs every 30 min.

From Toyo-hashi to Okazaki: Twenty five minutes by the Mei-tetsu (Nagoya Railway) express. Mei-tetsu's station in Okazaki is Higashi (or East)-Okazaki. The train runs every 20 to 30 min. This is the most convenient route to come to Okazaki from Tokyo.

From Tokyo to Nagoya: Two hrs by the JR's super-express train (HIKARI or LIGHTENING). The train runs every 30 min.

From Kyoto/Osaka to Nagoya: One to two hrs by the JR's super-express train (HIKARI/KODAMA). Kin-tetsu (Kinki Nippon Railway) also serves Osaka/Nara and Nagoya.

From Nagoya to Okazaki: Thirty five minutes by the Mei-tetsu express which runs every 20 min.

In March 1988, New Station, Mikawa-Anjyo, was opened for the super-express train (KODAMA). About 45 minutes from there to Okazaki by taxi.

BY AIR

Domestic airlines serve Komaki, Nagoya, airport which is 1 hour drive from Okazaki. Limosine service began in July 1988 between Nagoya airport and Okazaki.

WEATHER

In Okazaki, temperatures reach 30 degrees centigrade in the summer and go down to a few degrees above freezing in the winter. May to June is the rainy season and the spring (March to May) and fall (September to November) are the most pleasant.

THE OKAZAKI CASTLE,
THE HOME OF THE FIRST
TOKUGAWA SHOGUN, IYEYASU.

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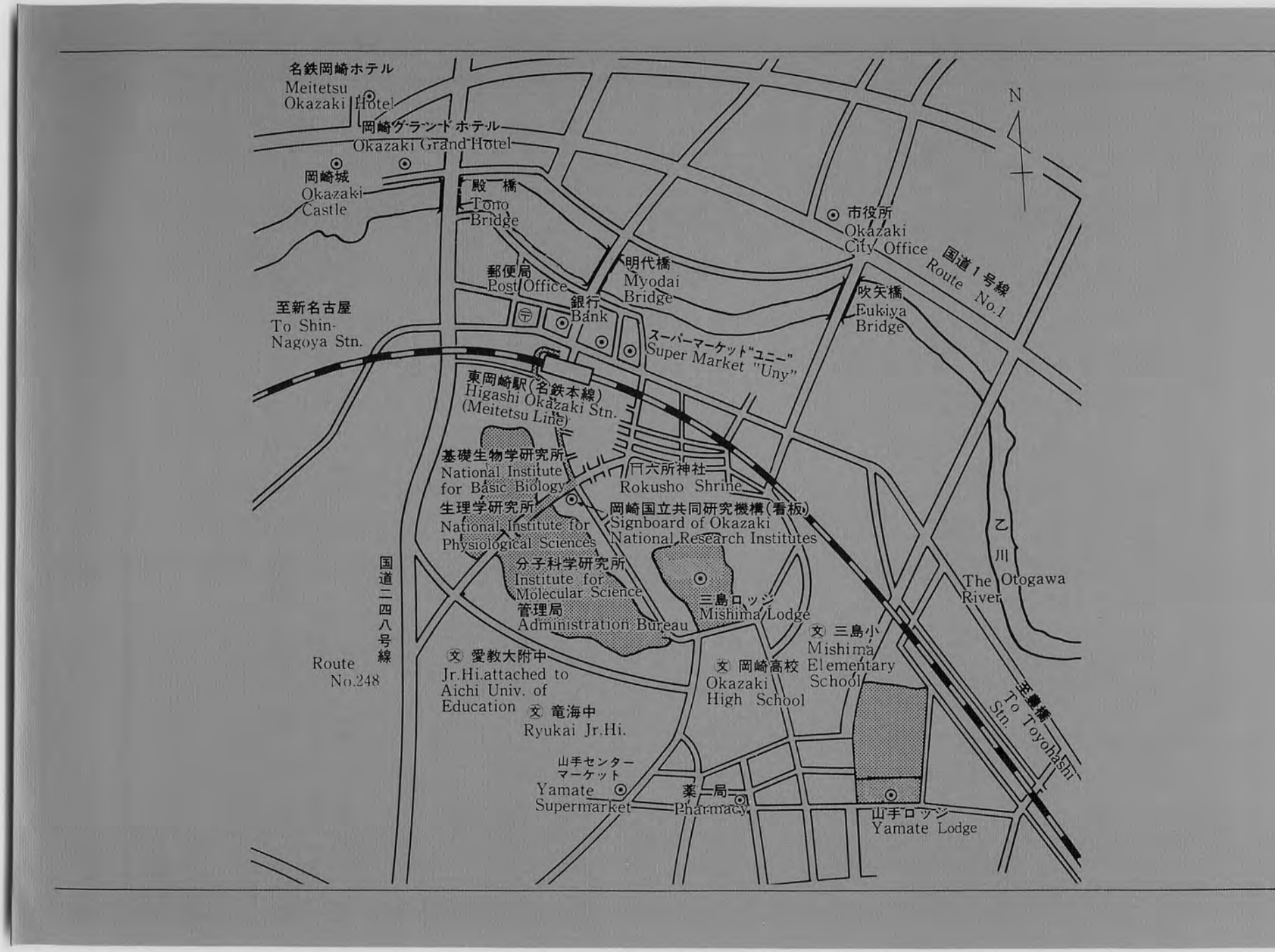
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