NATIONAL INSTITUTE FOR BASIC BIOLOGY

岡崎国立共同研究機構

基 礎 生 物 学 研 究 所

ANNUAL REPORT

1989

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 Ministry of Education, Science and Culture, Japan). The Research Institutes are 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). The NIBB was established in 1977 and celebrated its tenth anniversary in 1987.

The NIBB is an inter-university 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 programs as the Department of Molecular Biomechanics of Graduate University for Advanced Studies and additional 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. The NIBB sponsors "Bioscience Training Course" of about 7–10 days for selected people from all over Japan and organizes national and international conferences on selected topics.

PROFESSOR I. TAKEUCHI

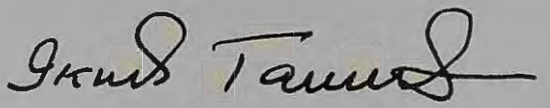
INTRODUCTION

The National Institute for Basic Biology (NIBB) aims to stimulate and promote basic research in the field of biology, by conducting first-rate research on its own, and by cooperating with other universities and research organizations. The Institute concentrates on the studies of cellular functions, growth and development, homeostatic control, and gene expression and regulation in eukaryotic organisms, to elucidate the fundamental mechanisms underlying various living phenomena at the molecular level. To accomplish this goal, the NIBB makes full use of the most modern techniques of biophysics, biochemistry, and cell and molecular biology, including the methods of gene manipulation.

The NIBB contains three departments, Cell Biology, Developmental Biology, and Regulation Biology. The laboratory of Gene Expression and Regulation was inaugurated and newly added to the Institute in 1989. Altogether, the NIBB is divided into 15 divisions, each of which is staffed by a full professor, an associate professor and two research associates. Six of the divisions are adjunct, in that for each division a professor and an associate professor from other universities and institutes are elected for a joint appointment.

The NIBB is an inter-university research institute and plays many roles as a national center for the study of biology. The Institute is responsible for conducting research projects in cooperation with research groups in different universities and institutes. As a part of such cooperative activities, the NIBB carries out Special Programs which are currently directed to "Biomembrane Research" and "Signal Transduction". Based on such programs, the NIBB organizes a series of international conferences which are attended by active scientists in the related fields, both from inside Japan and abroad. In addition, the Institute sponsors symposia and study meetings on current topics at the interdisciplinary level by inviting leading scientists in various related fields, both nationally and internationally. The NIBB also shares its research resources to make them available to biologists both from Japan and around the world. Through such activities, the NIBB is and will continue to be a national and international center to promote basic research in the biological sciences.

This report consists of an outline of activities of the NIBB and a list of papers published by the members during the period of 1988 and 1989.



I. Takeuchi, Ph.D. & 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. T. S. Okada. 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. Toshio Ueno.

Research

The Institute conducts its research programs through three departments and one laboratory organized into 15 divisions. Each division has its own research project and is staffed by a professor, an associate professor and two research associates. A division forms, in principle, an independent project team. Six of the divisions are for adjunct professorship and are under professors who hold joint appointments with other universities. The adjunct division has resident research associates. The arrangement aims to facilitate exchange in research activities in Japan. The Technical Department manages the activities of research techniques and helps to promote research activities of each division and also to maintain the research resources of the Institute. The Department undertakes the technical education of its staffs.

Research Support Facility

The research support facility of the 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

Associate Professor: Toshiyuki Nagata
Research Associate: Kazuo Ogawa

One of the most characteristic features of higher plant cells is their "totipotency": non-dividing somatic cells regain meristematic activity and their offspring redifferentiates to form whole plants under suitable conditions. For the elucidation of the mechanism of totipotency, tobacco mesophyll protoplasts provide ideal material, as dedifferentiation and redifferentiation occur rather synchronously under controlled conditions. This year, the mechanism of dedifferentiation was studied with special attention to the action of auxin, a plant hormone. In order to find genes which could be induced by treatment with auxin, differential screening was conducted of cDNA libraries prepared from the protoplasts cultured for 24 hrs with or without auxin. As the resulting four clones unique to the auxin culture were found to belong to the same gene, the mode of expression of this gene, designated *par*, was examined systematically by Northern blotting. These results showed that the *par* gene was expressed immediately after the addition of auxin and reached maximum expression after 4–12 hrs of culture. However, after 1 day, when DNA synthesis started, the level of its expression gradually decreased. After 2 days, when cell division started, its expression was residual. Thus, the expression of the *par* gene occurs during the transition from G_0 phase to S phase in the cell cycle. The intriguing question is: What is the function of the *par* gene? At the moment we cannot answer this question directly, but the homology search suggests some clues, since the *par* gene has certain homology to the stringent starvation protein of *Escherichia coli*, which binds equimolar to RNA polymerase. Concurrently, the *cis*-acting auxin-regulated elements were identified in the non-coding 5′ region of the genomic clone of the *par* gene using beta-glucuronidase as a reporter gene after delivery into tobacco mesophyll protoplasts by electroporation. Furthermore, the presence of auxin-binding protein (abp) which is immunologically identical to the abp of maize was identified on the surface of tobacco mesophyll protoplasts. Once further *trans*-acting factors have been characterized, it will be possible for the first time to describe a plant hormone signal transduction sequence from auxin supply, *via* the expression of specific genes, to the passage of plant cells from one stage of the cell cycle to another.

Division of Bioenergetics

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

Photosynthetic energy conversion has been investigated at the cellular and molecular levels. Our research is currently focused on (1) regulation of stoichiometry among thylakoid components in response to the light regime for photosynthesis, (2) molecular mechanism of thylakoid protein complexes functioning in the primary process of photosynthetic energy conversion, and (3) nitrogen-fixation in cyanophytes directly coupled with photosynthetic energy conversion.

(1) *Regulation of thylakoid composition*: In response to light regime, stoichiometry between two photosystems is regulated to retain a high efficiency of photosynthesis. We discovered this homoeostatic

regulation in cyanophytes, and are currently studying its mechanism of action. Our previous study revealed that (i) during the formation of thylakoids, assembly of the photosystem I (PS I) complex is regulated so as to balance the electron efflux from electron transport system (ETS) by PS I action with the influx to ETS by photosystem II (PS II) action, (ii) the regulation is induced by a signal elicited from redox state of ETS component(s) between the two photosystems. Current study has indicated that (i) the state of the electron flow within the Cyt b_6-*f* complex, probably the Q or modified Q cycle, forms the signal, and (ii) synthesis or supply of Chl *a* is one of the probable processes for the regulation of PS I assembly.

(2) *Molecular mechanism of electron transport within the PS I complex*: The PS I complex contains two molecules of phylloquinone. The role of this quinone in the electron transport within the PS I complex has been studied using a newly developed method for experimental replacement of the quinone with artificial quinones and herbicides. The effect of replacement has been examined by nanosecond laser spectroscopy and electron spin resonance (ESR) at cryogenic temperatures. Recent results have revealed that (i) protein structure at the quinone-binding site forms a molecular environment which enables the quinone to function at an extremely low redox potential, and (ii) the site accepts herbicides similarly to the Q_B site in the PS II complex.

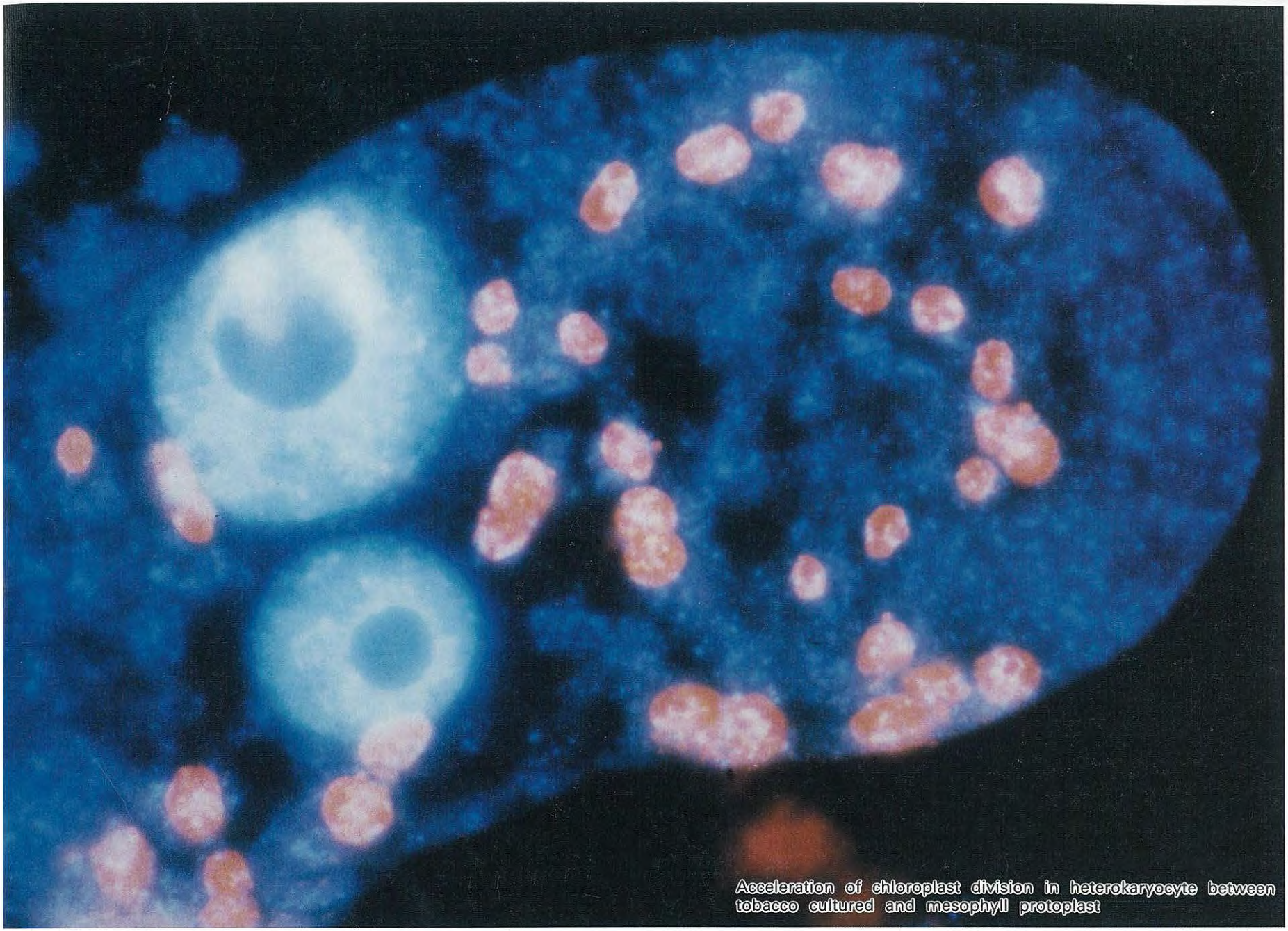
(3) *Molecular mechanism of excitation energy transfer in light-harvesting pigment complexes*: Excitation energy transfer within light-harvesting pigment-protein complexes has been studied in various types of antenna systems: phycobilisome, Chl *a*/*b* and Chl *a*/fucoxanthin/Chl *c* systems, and antenna systems of purple and green bacteria. Analyses of time-resolved fluorescence spectra at the picosecond level and the spectra at steady state have revealed the mechanism of energy transfer characteristic to each type of antenna system.

(4) *Nitrogen-fixation directly coupled with photosynthetic energy conversion*: We have succeeded in culturing a unique strain of N_2-fixation cyanophyte *Trichodesmium* sp. This strain can fix N_2 with a direct coupling to oxygenic photosynthesis. This O_2-tolerancy has been studied in relation to (i) molecular structure of the nitrogenase and (ii) intracellular localization of the enzyme. Current study indicates that: (i) molecular size of the Fe-protein is about 5 kDa larger than that of the nitrogenase sensitive to O_2, and (ii) the enzyme appears to be located around a gas vacuole area, being separated from thylakoids for oxygenic photosynthesis.

Division of Cell Proliferation (Adjunct)

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

Roles of calcium in the regulation of cell proliferation, the mating pheromone response and morphogenesis of the yeast *Saccharomyces cerevisiae* have been investigated using genetic, molecular biological and physiological approaches. The research is currently focused on the following four projects. 1) Our recent studies have established an experimental system suitable for the study of cell cycle regulation by Ca^{2+}. Using this system, we have shown that Ca^{2+} is essential for G_1 and G_2/M events during the cell cycle and regulates cAMP levels. This system will be useful to isolate conditional lethal mutants which can grow only when sufficient concentrations of Ca^{2+} are present in the media. 2) Techniques for measuring the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in individual yeast cells have been established. We have employed fura-2 as a calcium-specific fluorescent probe in conjunction with digital image processing. Images of fura-2 fluorescence under a Nikon Microphot-FX microscope are acquired by a SIT camera and relayed into a TV monitor and a Hamamatsu ARGUS-100 image processor. In *S. cerevisiae*, the mating process of haploid cells is controlled by the mating pheromones, *a*- and α-factors, that induce several



Acceleration of chloroplast division in heterokaryocyte between tobacco cultured and mesophyll protoplast

responses in cells of opposite mating type and are essential for mating. Using this system, we have shown that addition of α-factor to cells of *a* mating type raises $[Ca^{2+}]_i$ to 500-800 nM from a basal level of 100 nM and that this rise is essential for maintaining viability of yeast cells late in the mating pheromone response pathway. 3) To establish another measurement system suitable for detecting a rapid and transient rise in $[Ca^{2+}]_i$ in response to extracellular stimuli, we have employed a luminescent protein, aequorin, as a calcium specific probe. Aequorin is produced by the jellyfish *Aequorea victoria* and consists of apoaequorin and coelenterazine. The binding of Ca^{2+} to aequorin results in an intramolecular reaction, yielding as products light (λ_{max}=465 nm), CO_2 and a blue fluorescent protein. We have constructed plasmids in which the apoaequorin cDNA is joined downstream of either the *GAL1* promoter or the GAP promoter. We successfully regenerated aequorin inside intact yeast cells and detected its luminescent activity when Ca^{2+} was introduced into the cells. 4) A calcium-sensitive *cls4* mutant shows growth arrest in G_1 and seems to be defective in the formation of buds in Ca^{2+}-rich medium. The predicted *CLS4* gene product has been shown to have two putative Ca^{2+}-binding regions. We have begun to purify this gene product from cells bearing a multi-copy plasmid containing the *CLS4* gene, in order to study the role of this gene product and Ca^{2+} in the budding process.

Division of Cell Fusion (Adjunct)

Professor: Yoshio Okada
Research Associate: Masahiro Ishiura

The study of the molecular and cellular biology of mammalian cells, aided by cell engineering and recombinant DNA technology, is the research projects of this division. In relation to the action mechanism of diphtheria toxin in mammalian cells, the structure and function of the elongation factor 2 (EF2) involved in the protein synthesizing system of eucaryotes are currently being extensively studied at the molecular level using cloned EF2 genes. The molecular mechanism for Fabry's disease is being also studied by molecular cloning. Cosmid cloning has been established by constructing simplified cosmid vectors and selecting adequate *Escherichia coli*. Elucidation of the mechanisms of recombination and DNA repair in mammalian cells is also in progress.

Division of Cellular Communication (Adjunct)

The staffs, professor (adjunct), associate professor (adjunct) and research associates, will engage in research problems of cellular communication. The staffs are under consideration.

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
Research Associate: Michiyasu Yoshikuni
Masakane Yamashita
Minoru Tanaka

The germ cell is the most important cell in the body of any species, since it transmits genetic information from one generation to the next. The division of reproductive biology conducts research on the molecular mechanisms of differentiation, growth and maturation of germ cells in multicellular animals, using fish as a primary study model. The sequence of events leading to the production of fertile gametes is controlled by a complex and dynamic interplay of hypothalamic, pituitary, and gonadal hormones. It was in 1985 that we identified, for the first time in any vertebrate, 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) as the maturation-inducing hormone of amago salmon. Along with estradiol-17β (E2), which was identified as the major mediator of oocyte growth, we now have two known biologically important mediators of oocyte growth and maturation in female salmonids. It is established that the granulosa cells are the site of production of these two mediators, but their production by the ovarian follicle depends on the provision of precursor steroids by the thecal cell (two-cell type model). A dramatic switch in the steroidogenic pathway from E2 to 17α,20β-DP occurs in ovarian follicle cells immediately prior to oocyte maturation. This switch is a prerequisite step for the growing oocyte to enter the maturation phase, and requires a complex and integrated network of gene regulation involving cell-specificity, hormonal regulation, and developmental patterning. Our current efforts center on the cloning and sequencing of the genes encoding steroidogenic enzymes responsible for E2 and 17α,20β-DP biosynthesis.

Microinjection experiments have revealed that 17α,20β-DP acts at the oocyte surface; specific 17α,20β-DP binding was found in oocyte cortices. The early steps following 17α,20β-DP action involve the formation of the major cytoplasmic mediator of this steroid, maturation-promoting factor or metaphase-promoting factor (MPF). MPF activity cycles during 17α,20β-DP-induced oocyte maturation; the highest activity occurs at the first and second metaphase. Studies from our laboratories have shown that MPF activity is not species-specific and can be detected in both meiotic and mitotic cells of various organisms, from yeast to mammals. The homolog of the *cdc2* gene product of fission yeast, a protein kinase that prefers histone H1 as a substrate, is a component of MPF in frog, fish and starfish. To characterize fish MPF, we purified histone H1 kinase from mature carp oocytes and confirmed that fractions containing the highest kinase activity contain a *cdc2* kinase homolog, as determined by immunoblotting with a monoclonal antibody against a specific amino acid sequence (PSTAIR) of *cdc2* kinase. The antibody also cross-reacted with cell homogenates in all other species, including a higher plant, the lily. The amount of the *cdc2* kinase homolog was more abundant in germ cells than in somatic cells. The *cdc2* kinase homolog had several forms which differed in their mobility on SDS-PAGE. In all species examined, the form with the fastest mobility appeared or became dominant when cells entered the metaphase.

A steroidogenic switch, from 11-ketotestosterone (11-KT) to 17α,20β-DP, also occurs in salmonid testes around the onset of final maturation. *In vitro* studies using different testicular preparations have revealed that the site of 17α,20β-DP production is in the sperm, but its production depends on the provision of precursor steroids by somatic cells. The site of 11-KT production is in the testicular somatic cell elements. Recent studies have revealed a role of 11-KT in spermatogenesis and 17α,20β-DP in sperm motility. A newly developed organ culture system for eel testes has proved that 11-KT can induce the entire process of spermatogenesis, *in vitro*, from type A spermatogonia to spermatozoa.

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 homeobox genes in *Bombyx mori*. The silk fibroin gene is transcribed specifically in the posterior silk gland cells, starting as soon as silk gland morphogenesis has been completed in the late embryonic stage, and is repeatedly switched on and off in the following larval stages. To analyze the molecular mechanisms of this tissue- and stage-specific regulation of transcription, we have developed cell-free transcription systems from several tissues and cultured cells. Using these systems we have detected tissue-specific as well as ubiquitous factors that *trans*-act on the upstream sequences of the fibroin gene. Some of these factors seem to have the nature of homeodomain proteins. In fact, we have shown that many of homeodomain-containing mRNAs are expressed in the silk gland, and are presumed to be involved in the regulation of the silk genes. Currently we are concerned with how synthesis and specificity of these factors are regulated. In *B. mori*, more than 20 homeotic mutants that mapped to the proximal end of the 6th linkage group had been described as the "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 genes results in embryonic lethality. These genes are probably regulating some structural genes that play important roles in pattern formation. We have cloned certain homeobox 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

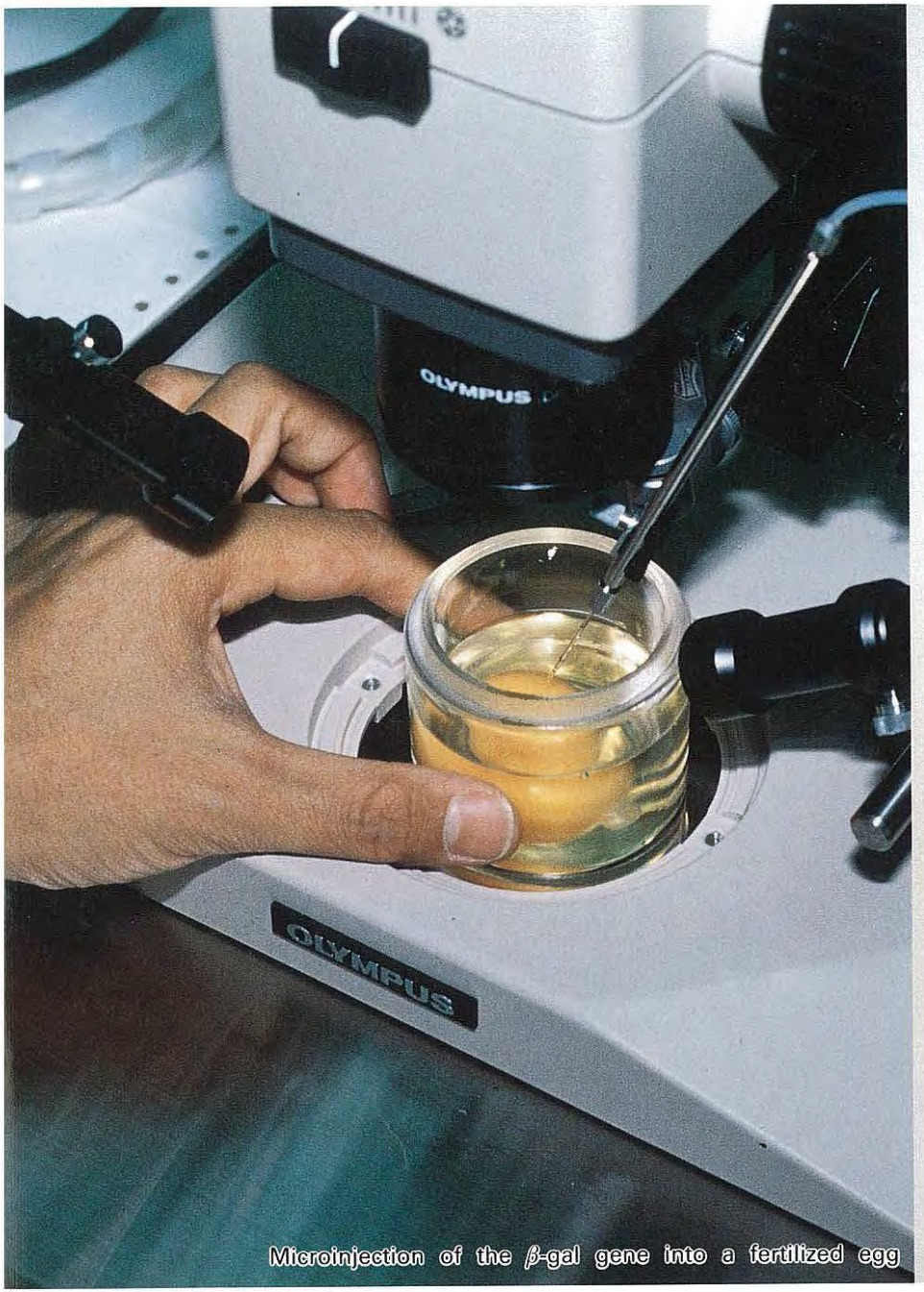
Professor: Goro Eguchi
Associate Professor: Kenji Watanabe
Research Associate: Ryuji Kodama
Kiyokazu Agata

Problems of stability and instability in cell differentiation in the morphogenesis of animal tissues and organs have been studied as current projects in this division.

(1) Utilizing our established cell culture system which allows pigmented epithelial cells (PECs) to transdifferentiate into lens cells, we have demonstrated lens transdifferentiation of PECs isolated from adult human eyes. Fully differentiated PECs from 22- and 80-year-old human eyes, when dissociated and cultured under our established conditions, expressed vigorous growth potential and readily transdifferentiated into lens cells, to organize lentoid structures in the same manner as is shown by PECs of chick embryos.

(2) Phenylthiourea and testicular hyaluronidase are essential substances to provide the permissive culture conditions for PECs to transdifferentiate into lens cells via the multipotent dedifferentiated state. By extensive analysis of the mode of action of these two substances, we have been clarifying actual factors regulating the process of dedifferentiation of PECs and of the switch of differentiation of once dedifferentiated PECs which possess multipotentiality for differentiation. We have also been approaching to cell surface functions of PECs in the process of the transdifferentiation.

(3) We have cloned PEC-specific genes (MMP-115, pP64 and pP344) in addition to lens cell-specific genes (crystallin genes) and described the mode of expression of these genes in the process of transdifferentiation. To search for the regulatory mechanisms governing the switch in expression of these cell type-specific genes during transdifferentiation, we have introduced the highly useful transgenic system of the chick. By collaboration with Dr. Naito



Microinjection of the β-gal gene into a fertilized egg

Expression of the β-gal gene in a chicken embryo

(National Institute for Animal Industry), Dr. Otsuka and Dr. Kino (Aichi-ken Agricultural Research Center), we have established an efficient culture method of fertilized chicken eggs, in which more than 30 % of cultured fertilized eggs can be grown to hatch, and have used this system to improve methods for gene transfer.

Division of Developmental Biology (Adjunct)

Professor: Masaki Iwabuchi
Research Associate: Masao Tasaka

This division is devoted to the study of the regulatory mechanisms of gene expression involved in cell proliferation and differentiation in plants. The research has focused on the following two projects:
(1) Regulation of cell cycle-specific transcription of wheat histone genes.
Almost all the histone genes are expressed in concert with DNA synthesis at the S phase during the cell cycle. The molecular mechanisms of S phase-specific gene expression are unknown. To elucidate the regulatory mechanisms of transcription of plant histone genes, we have investigated the *cis*-acting elements and *trans*-acting factors of wheat (*Triticum aestivum*) histone H3 and H4 genes. Currently we are determining three cis-acting sequences, called the hexameric (ACGTCA), octameric (CGCGGATC) and nonameric (CATCCAACG) motifs, in the proximal promoter region of the H3 gene using the transcription system in sunflower cells transformed by a Ti-plasmid gene transfer technique. These three motifs have been shown to function as positive elements in transcription of the H3 gene. Using techniques of gel shift, methylation interference, and DNase I foot printing assays, we have also identified three nuclear DNA-binding proteins, named HBP-1a, HBP-1b and HBP-2, which interact specifically with the hexameric or nonameric motif. The cDNA clones encoding HBP-1a and HBP-1b specific to the hexameric motif have been isolated from a wheat cDNA library in λ gt11 using the South-western method and their nucleotide sequences determined. The deduced amino acid sequences indicated that both HBP-1a and HBP-1b contain the leucin zipper motif and the basic region as a DNA-binding domain. We have begun to investigate how HBP-1a and HBP-1b are involved in the transcription of the H3 gene using a transient expression system with cultured rice plant cells.
(2) Regulation of cell-type specific gene expression during development of *Dictyostelium discoideum*.
To elucidate the regulatory mechanisms of gene expression during the processes of cell differentiation in the cellular slime mold *Dictyostelium discoideum*, we have isolated several cDNA clones which are specifically expressed in prespore or prestalk cells from cDNA libraries by differential or immunological screening. These cDNA clones were subdivided into three groups on the basis of temporal and cell type-specific accumulation patterns of mRNA. According to the results of *in vitro* 'run on' assays using nuclei isolated from different developmental stage cells and from prespore and prestalk cells, two types of regulation were found to be involved in cell-type enrichment of mRNAs ; one is at the transcriptional level and the other at the post-transcriptional level. The cDNA clones, Dp87 and Sp96 (encoding spore coat protein Sp96) are good cDNA clones to investigate the regulatory mechanisms of the prespore-specific genes, because their mRNAs are synthesized only in prespore cells. Southern analyses of nuclear DNA indicate that the genes for the cDNA exist as unique genes. The genomic clones for Dp87 and Sp96 were isolated and sequenced, and their transcription initiation sites were determined. The structures of Dp87 and Sp96 genes and their deduced amino acid sequences were also determined. A cis-regulatory region for transcription of Dp87 and Sp98 genes was identified, using various 5'deletion mutants in transformed cells. The cis-region in the Sp96 gene coincided well with the DNase-I hypersensitive site that was specifically detected in chromatin isolated at the slug stage. We also identified a presumed *trans*-acting factor which specifically

binds the cis-regulatory region of Dp87 gene and occurs only in the nuclear extract of slug cells.

To understand whether the expression of Dp87 and Sp96 genes is regulated by cAMP, which is known to control prespore cell differentiation, we examined transcription of these genes in cells disaggregated from tissues in the presence or the absence of cAMP. Northern analyses and *in vitro* run-on assays suggest that transcription of the Dp87 and Sp96 gene stopped immediately after the cell disaggregation and resumed upon addition of exogenous cAMP to disaggregated cells. We suppose that the transcription of Dp87 and Sp96 genes is mediated through signal transduction from cell surface cAMP receptors and the transcribed mRNAs are probably stabilized by a protein(s) synthesized after cAMP addition.

DEPARTMENT OF REGULATION BIOLOGY

Chairman: Norio Murata

Two divisions and two adjunct divisions belong to the department and conduct research on regulation mechanisms in biological systems.

Division of Sensory Processing

Professor: Ken-Ichi Naka (moved)
Research Associate: Hiroko Sakai
Eiki Hida (moved)

The major aim of this research division is to elucidate the neural mechanisms of sensory processes. We have focused our effort on the simple retina of channel catfish which has been the principal vehicle of our research since 1968.

The past two years we have studied signal processing in the inner retinal neuron network. Our research has taken advantage of three multidisciplinary means: 1) The intracellular recording and stimulation to show the pathways of signal transmission. 2) Wiener analysis to define dynamics of signal transmission, 3) Cascade analysis which decomposes neuron network into an ordered neuronal connection. By combining these three methods we have produced two sets of major conclusions.

1) Spike analysis. Spike discharge carries information over a long distance. In spite of its importance, little attention has been paid on how to analyze spike discharges, a point process. We have shown that the spike discharge generation can be approximated by a cascade of Wiener structures. The process for spike generation is highly nonlinear, but static. It is, therefore, possible to recover from a spike train the first- and second-order components in a ganglion cell's analog potential. As the cell's response is approximated by these two components, information is transmitted from the retina to the brain with little loss of information.

2) Network analysis. We have injected a test signal into one neuron in the neuron network in the inner retina and recorded the resulting response from near-by neurons. We have discovered that neurons in the network are extensively inter-connected. A current injected into one neuron always produced a response from a nearby neuron. We have discovered two major modes of transmission.

A) Bi-directional fast transmission. This is the common mode of transmission between the amacrine and ganglion cells of the same response polarity. The transmission is linear and likely chemical and mono-synaptic.

B) Complex transmission in which gain of transmission is different for the forward and reverse directions. This mode of transmission is found between neurons of different response polarity.

From these observations we have concluded that neurons in the inner retina form three major clusters, ON-, OFF-, and ON-OFF clusters. In each cluster neurons are tightly inter-connected by a

foot bidirectional pathways. Three clusters, in turn, are interconnected by poly-synaptic and complex pathways. What we have found in the inner retina is new and radical. History of retinal research, however, has repeatedly shown that discoveries can be made. Prof. Tsuneo Tomita's discovery of hyperpolarizing receptor response is one example. Discoveries, from their own nature, are beyond the scope of current research paradigms. We believe one goal of basic science is to discover the unexpected. The vision community may take many years to fully digest the information we have discovered during the last few years.

Division of Cellular Regulation

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

Two important objectives of the current research efforts of this division are to understand (1) the molecular mechanisms of low-temperature sensitivity and adaptation in higher plants and microalgae, and (2) the structure and function of proteins of photosynthetic membranes.

(1) The studies on low-temperature sensitivity and adaptation emphasize the participation of membrane lipids and biological membranes on temperature tolerance: (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. The cDNA and genomic DNA for this enzyme have been cloned from a low-temperature-sensitive plant, squash, and from a low-temperature-resistant plant, *Arabidopsis*, respectively. Studies aiming to transfer, by means of cloned genes, the low-temperature tolerance into low-temperature-sensitive plants are currently being pursued. (ii) A gene, designated as *desA*, required for plant-type fatty acid desaturation, which introduces the second double bond into fatty acids of glycerolipid of biological membranes, has been cloned from a low-temperature-resistant strain of the cyanobacterium, *Synechocystis* PCC6803. A low-temperature-sensitive strain of the cyanobacterium, *Synechococcus* PCC7942, has been transformed with the *desA* gene. This transformation increases the unsaturation of fatty acids of glycerolipids, and makes this cyanobacterium more tolerant to low temperature. These novel results demonstrate that the fatty acid desaturases are also responsible, in part, for regulating the low-temperature sensitivities of plants. (iii) The molecular mechanism of low-temperature adaptation is studied using cyanobacteria, with special emphasis on identifying the temperature sensor and the nature of temperature regulation of gene expression. The promoter region of fatty acid desaturase genes, which sensitively responds to temperature, is being used for cloning mutants and transformants for probing the temperature-sensing mechanism. (iv) Other enzymes related to the biogenesis of plant-membrane lipids are being investigated in relation to the response of plants to temperature; they are: acyl-[acyl-carrier-protein] hydrolase and stearoyl-[acyl-carrier-protein] desaturase.

(2) Photosynthesis research is focused on the machinery of the oxygen-evolving complex of photosystem II. 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 photosynthetic oxygen evolution, the following subjects are presently studied using techniques of biochemistry, physicochemistry and molecular biology: (i) Purification and elucidation of the properties 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 on oxygen evolution. (iv) Identification of low-molecular weight components of the oxygen evolving complex and their genes.

Division of Biological Regulation (Adjunct)

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

Growth and differentiation of higher plants are regulated by both internal and external factors. The internal factors include plant hormones and the external factors include changes in environmental conditions such as light, temperature and water status. This division conducts research to elucidate the biochemical and molecular mechanisms of cellular events affected by plant hormones and environmental stimuli.

The following research projects are being carried out at present:

(1) Development of the sink activity in rapidly expanding tissues. When seeds germinate, young primary leaves and stem present in a seed start to grow and expand rapidly due to cell enlargement. This cell growth is supported by sucrose influx from storage tissues of the seed and accompanies massive accumulation of cell wall materials which are mainly various polysaccharides. We have obtained physiological evidence that a dramatic development of sucrose metabolizing enzymes, invertase and sucrose synthase, in stems and leaves after imbibition supports the continuous influx of sucrose, and thus rapid growth. Invertase and sucrose synthase have been purified from mung bean (*Vigna radiata*) seedlings and their cDNAs are being isolated to analyze regulation of their gene expression triggered by seed imbibition.

(2) Mechanisms of gene expression regulated by plant hormones and their interaction. Auxin induces many physiological processes of plant cells and abscisic acid suppresses the auxin action. Early cellular processes including the synthesis of particular sets of proteins are induced by these plant hormones well before physiological or morphological changes occur. The simultaneous presence of both auxin and abscisic acid, however, induces mutually specific suppression of this protein synthesis. The objective of our study is to elucidate the molecular mechanisms of this specific interaction between auxin and abscisic acid. Currently, cloning of auxin-regulated cDNAs is being carried out with mung bean hypocotyls. Regulation of synthesis of a plant hormone, ethylene, affected by an irregular environmental stimulus, wounding, is also studied.

(3) Mechanisms of acquisition of thermotolerance by non-lethal high temperatures. When plant seedlings are exposed to a lethal high temperature (53°C) for a short period (20 min) and returned to a normal growth temperature (28°C), they are irreversibly damaged and eventually die. However, short (1 hour) exposure to a non-lethal high temperature (40°C) provides plants competence to survive the subsequent exposure to an otherwise lethal high temperature. Physicochemical and biochemical changes of biological membranes induced by those thermal treatments have been analyzed to find specific events related to the acquistion of thermal tolerance. Formation of lysophosphatidyl-ethanolamine was found to correlate with thermal tolerance and its role in physicochemical stability of the biological membranes is being studied.

Extensive studies on the structure and function of phytochrome, which were carried out in this division, have been completed with success.

Division of Behavior and Neurobiology (Adjunct)

Professor: Katsuhiko Mikoshiba
Associate Professor: Masaharu Ogawa
Research Associate: Taka-aki Tamura
Teiichi Furuichi

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) Second messenger signalling in the central nervous system. In the course of a study on cerebellar ataxic mutant mice, a membrane glycoprotein P_{400} was found to be enriched in Purkinje cells from normal mice but reduced in the Purkinje-cell-deficient mutants. cDNA-cloning and expression of functional P_{400} protein show that this protein is a receptor for inositol 1,4,5-trisphosphate ($InsP_3$), a second messenger that mediates the release of intracellular calcium. Our goals in this research are to define how P_{400}/$InsP_3$ receptor molecules recognize the second messenger $InsP_3$ and release Ca^{2+} from the intracellular stores, and determine their function(s) in the central nervous system, especially in the cerebellar Purkinje neurons.
(2) Studies on the mechanism of brain specific gene expression. We are studying this subject using on *in vitro* transcription system derived from animal tissues. We are studying the structure and function of promoters of the myelin basic protein gene. Our aim is to understand the molecular mechanism of tissue-specific gene expression.

LABORATORY OF GENE EXPRESSION AND REGULATION

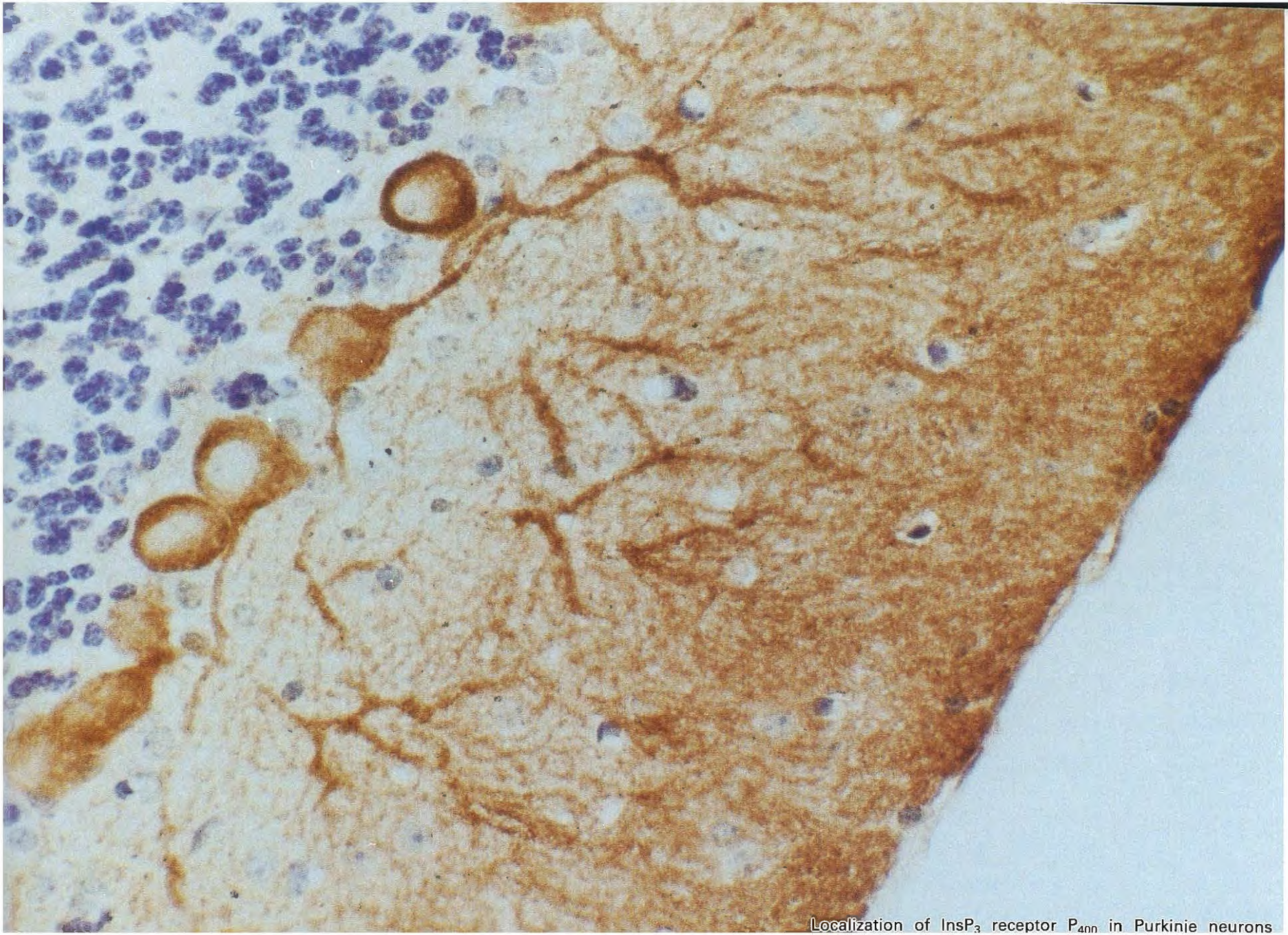
Head (Acting): Goro Eguchi

The laboratory consisting of two research divisions was established in May, 1989 to conduct research into regulatory mechanisms of gene expression in higher plants and animals, particularly using transgenic experimental systems. Special facilities are scheduled to be equipped for experiments with transgenic plants and animals, and also for RI experiments. These facilities will be opened to make collaborations with scientists outside.

Division of Gene Expression and Regulation

Professor: Yoshiro Shimura
Associate Professor: Kiyotaka Okada
Research Associate: Hideaki Shiraishi

The principal interests of this laboratory are the genetic control of higher plant flower development and of root responses to environmental stimuli. Our research is largely based on the small crucifer, *Arabidopsis thaliana*. This plant has some remarkable features which make it ideally suited for genetic and molecular biological studies, including a small genome ($7x10^7$ base pairs per haploid), short life-cycle (5–6 weeks), small size (20–30 cm in hight) and ease of propagation.
Mutational analysis was used to investigate three phenomena: flower development, root gravitropism and a newly described obstacle-evading root response. Mutants in each category were isolated by screening for appropriate abnormalities. Mutants with abnormal floral morphology could be divided into the following six types on the basis of the stages of floral development where the genetic defects were presumed to occur: delayed transition from vegetative to reproductive growth, short inflorescence axis, absence of certain floral organs, asymmetric or aberrant arrangement of floral organ primordia, homeotic transition of floral organs, and abnormal morphogenesis of floral organs. Most of the mutants have been shown to have single, recessive, nuclear mutations. Double mutant analysis revealed mostly additive phenotyes of the parental single mutants, but in some cases flowers with novel and highly complex structures were observed, indicating simultaneous expression and



Localization of $InsP_3$ receptor P_{400} in Purkinie neurons

interaction of two gene products.
Mutants were isolated which show either reduced efficiency or a complete absence of root gravitropism, which fall into two complementation groups. Either alleles include mutants of both types. Analyses of the heterozygous strains indicated that mutants of reduced efficiency have genetic defects lighter than those of no response.
Roots also alter their growth direction when they encounter obstacles. In order to analyze the obstacle-escaping mechanisms of wild-type roots, a simple system which provides a constant obstacle-touching stimulus to root tips was devised. Seedlings growing on vertical agar plates have roots which grow straight downward on the agar surface. If the angle of the agar plate is shifted to 45 degrees, the root-tips bend downward under the influence of gravity and encounter the agar surface. Unable to penetrate the agar, this obstacle-touching stimulus induces root tip rotation which is followed by sideways growth (the obstacle-evading response). The direction of root tip rotation is periodically reversed and the result of this is a wavy growth pattern. Mutants which form abnormal wavy patterns on the inclined agar surface were isolated and genetic analysis has shown that at least 6 genes are involved in this growth response. Two of these six genes are allelic with genes involved in the control of root gravitropism. Attempts are being made to identify and isolate the genes which are mutated in the flower and root mutants by T-DNA tagging.

Division of Gene Expression and Regulation II

The staffs, professor, associate professor, two research associates will engage in research problems of gene expression and regulation in animals. The staffs are under consideration.

TECHNICAL DEPARTMENT

Head: Hachiro Honda
Chief: Hiroyuki Hattori

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.
Technical staffs participate, through the department, in mutual enlightenment and education to increase their capability in technical areas. Of 24 staff members 14 belong to the Commom Facility group (Chief; Hiroyuki Hattori), whereas the other 10 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 the NIBB and the IPS (CENTER for ANALYTICAL INSTRUMENTS, ELECTRON MICROSCOPE CENTER, LABORATORY GLASSWARE FACILITY, MACHINE SHOP and LOW-TEMPERATURE FACILITY).

ag

pi

Flowers of *Arabidopsis thaliana* mutants: *agamous* (top left), *pistillata* (top right), and the double mutant of *agamous* and *pistillata.*

double mutant

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 30 KW-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 fluency 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.

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-videomicrographs of the motile behavior of the cells at the cellular and subcellular levels. Photoreceptive and signal transduction mechanisms of algal gametogenesis are also studied by action-spectroscopy.

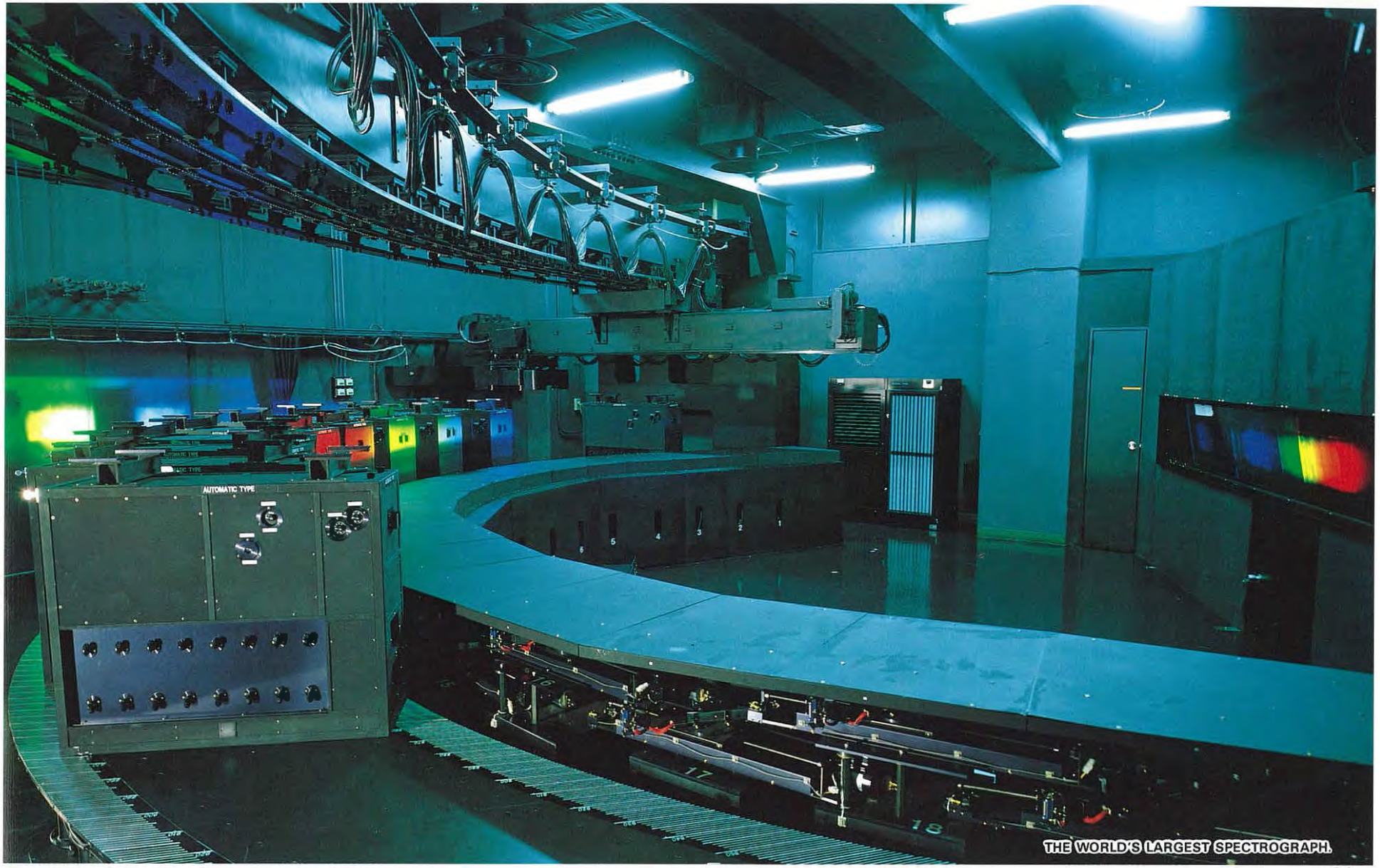
Tissue and Cell Culture Laboratory: This is a facility for tissue and cell culture. This laboratory is equipped with safety rooms which satisfy the P3 physical containment level. This facility is routinely used for DNA recombination experiments. The faculty of the Tissue and Cell Culture Laboratory also 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 mutant cells with altered myosin molecules, though such mutants have not been isolated. The faculty intends 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.

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 greenhouses (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 WORLD'S LARGEST SPECTROGRAPH.

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 analyses 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 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, ^{32}P and ^{35}S in NIBB, ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{45}Ca and ^{125}I in IPS, are processed. The substation in NIBB is equipped with a 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 molecular mechanism of signal transduction in *Neurospora crassa* and *Lemna paucicostata*. Several species of ATP-GTP-binding proteins were detected and ADP-ribosylate by endogenou ADP-ribosyl transferase. The goal of the research is to isolate the genes for ATP-GTP-binding proteins and ADP-ribosyl transferase and to analyze how the external signals such as light and phosphate sources are transduced to cellular responses including gene expression or morphogenesis.

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	SHIMADZU GC-7APTF
HPLC	JASCO TRIROTAR III
	SPECTRA-PHYSICS SP-8700
Ion Chromatograph	DIONEX QIC
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 Spectrophotometric Analysis

Atomic Absorption Spectrophotometer	PERKIN-ELMER 603
Differential Refractometer	CHROMATIX KMX-16
Dual-wavelength Spectrophotometer	HITACHI 557
ICP Spectrometer	SEIKO SPS 1200A
Infrared Spectrophotometer	JASCO A-302
Laser-Raman Spectrophotometer	JASCO R-800

Light Scattering Photometer	CHROMATIX KMX-6DC
Spectrofluorometer	HITACHI 850
	HITACHI MPF-4
	SIMADZU RF-5000
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

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
Two Dimension Microdensitometer	JOYCE LOEBL 3CS

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 one 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, JEOL 100-CX 100KV, JEOL 200-CX 200KV and Philips EM-400HM 120KV.
Transmission microscope, analytical: JEOL 200-CX 200 KV.
Scanning microscope: 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 conferences 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: The NIBB carries out a graduate program as the Department of Molecular Biomechanics of Graduate University for Advanced Studies which was inaugurated in 1989. Besides this program, graduate students from other universities may spend a fixed period of time with members of the NIBB. This allows students to have experience with the very modern facilities of the NIBB.

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 annually with about 12–20 attendants above the graduate student level from other Japanese universities and research organizations (both national and private).

LIBRARY

The three institutes 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 2,299 m^2. The library has 11,275 books in Japanese and 41,430 in foreign languages, and subscribes to 435 Japanese and 551 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 located within a 10-minute-walk distance; Yamate Lodge is within a less than 20-minute-walk distance. 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 the maintenance of 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 that 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 300,000, 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 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 Toyohashi: Two and half hours by the Japan Railway's (JR-Tokai) super-express train (KODAMA). The train runs every 30 min.

From Toyohashi to Okazaki: Twenty five minutes by the Meitetsu (Nagoya Railway) express. Meitetsu's station in Okazaki is named Higashi (or East)-Okazaki. The train runs every 15 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). The train runs every 20 min.

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

From Nagoya to Okazaki: Thirty five minutes by the Meitetsu express which runs every 15 min.

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

BY AIR

International and domestic airlines serve Nagoya Airport (Komaki) which is 1 hour drive from Okazaki. Limousine service is available between Nagoya Airport and Higashi Okazaki Station.

WEATHER

In Okazaki, temperatures reach 30 degrees centigrade in summer and go down to a few degrees above freezing in 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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