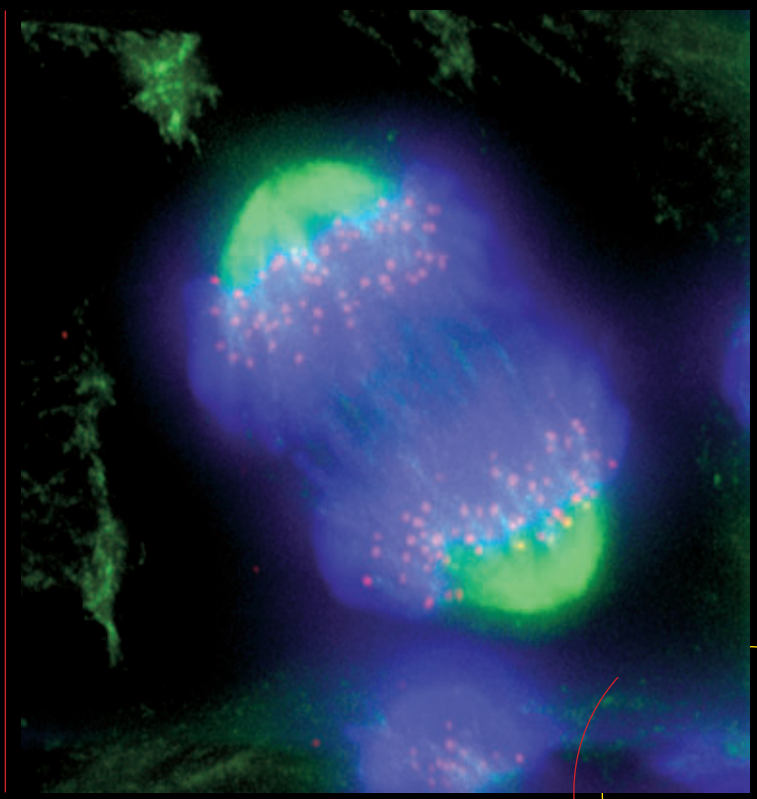
GRADUATE SCHOOL OF BIOSTUDIES, KYOTO UNIVERSITY

THE BEGINNING OF A NEW ERA OF LIFE SCIENCES



2003 PROSPECTUS / ANNUAL REPORT 2002

Preface

The Graduate School of Biostudies, Kyoto University was founded in April, 1999. Heads of about twenty laboratories scattered in the campus decided with enthusiasm to unite and make a graduate school of biosciences. Although their backgrounds are diverse previously belonging to different schools (Agriculture, Medicine, Pharmacology, Science), their researches share a common language of "genes", "molecules" and "cells" thanks to very fast developments in the whole life science research fields. For last three years, the school has experienced very favorable interactions among people and gratifying outcome. It has emerged as a center of excellence in research and education, not only in this country but also in the world. In this fall the school was awarded a grant of the '21st Century COE Program' from the Monkasyo.

The mission of Graduate School for education is summarized below. (1) Training of graduate students to acquire the ability to pursue new biostudies at the worlds' top level. (2) Training students to apply the new life sciences and technologies for human welfare and protection of the global environment. (3) Nurturing individuals who can understand the various vital phenomena of the living organisms as a systemic function, and pursue these systemic functions.

The Graduate School of Biostudies has annual acceptance of about 85 students in the two-year master course and subsequently 40 to 50 graduate students (the number dependent on the year) enter the three year doctor course. Although the first graduate students will obtain the doctoral degree in the spring of 2004, the school has already 253 graduate students (157 in the master course and 96 in the doctor course) and predicts to increase the number of graduate student to about 300 in the spring of 2003. It is a most active and flourishing life science graduate school in Japan. The ratio of students who want to continue from the master to the doctor course was the highest among the science and technology graduate schools in Kyoto University. The ratio of female students is currently 28%.

The Graduate School of Biostudies has two divisions, the Division of Integrated Life Science (ILS) and the Division of Systemic Life Science (SLS). Laboratories in ILS investigate fundamental life mechanisms at the levels of gene, chromosome, cell, muticellular organization and surrounding environment. Research topics include gene mechanisms, chromosome segregation, cell cycle control, cell and developmental biology, plant gene and totipotency, applied molecular biology,

environmental signals and stresses. Education and research in SLS are focused on the elucidation of the fundamentals of molecular and systemic biology, cell biology and immunology, and immunobiology for the recognition of self-and non-self, in response to various factors. Research areas include molecular and system biology, animal development and physiology, signal transduction, functional biology, mammalian regulatory network.

ILS and SLS consist of 12 and 7 core laboratories, respectively. In addition, ILS cooperate with two groups in the Institute of Virus Research and one in the Center for Radiation Biology of Kyoto University. SLS cooperate with two laboratories in the Institute of Virus Research, one in the Experimental Unit of Genetic information, one in the Osaka Bioscience Institute and one in the Center of Developmental Biology, RIKEN.

The governing body of the Graduate School of Biostudies is the monthly-held meeting of laboratory heads (full professors) of the two divisions. Almost all the issues are discussed and decided in this meeting chaired by the dean. The division meetings are held only for discussions and primary decisions for appointing junior members or selecting graduate students.

It took us nearly ten years of preparations to make the Graduate School of Biostudies. In most of these years, our effort was concentrated on mutual understandings among people in the core laboratories. For several years, regular joint seminars attracted many graduate students by excellent talks and beer with sandwich. We understood the necessity of acquaintance at the level of sciences but also of personality, before making any concrete plan for the new graduate school. Enthusiasm was mounted year by year. Gladly, when we finally submitted our plan of the new Graduate School to the University headquarter and also to the Monbusyo (now Monkasyo, the Ministry of Education, Culture, Sports, Science and Technology) in the early summer of 1998, it was immediately accepted. Each five labs from the Schools of Agriculture and Science, each two labs from the Schools of Medicine and Pharmacology, each one lab from Institute of Virus Research and Integrated Human Studies and three new labs were united and opened the Graduate School of Biostudies as the first merging attempt in the country's national universities.

Research activities of Graduate School of Biostudies may be judged by the number of very large research grants obtained (10 are more than hundred million yen) and highly-cited publications (seven are over 1,000 citations and 300 papers more than 100 citation) and patents (over 100). Many laboratory heads play leading roles in their research areas, and work as the board members for a number of academic journals. Visibility in the international scene might be ex-

ampled by the foreign membership election of National Academy of Science, USA and Royal Society of London for two professors. Only within three years after its foundation, four junior faculty members were promoted to full professors in other universities or other institute of Kyoto University, and one full professor to the director of a large research center of RIKEN. Even one full professor was 'rented' to be the dean of other graduate school in Kyoto University. Our graduate school has ample interactions with industries as two full professors and nine junior faculty members have had experiences to work in the company's research institutes. Every year average fifty grants are given by the private sectors. The ratio of female faculty is not high (one full professor and two junior faculty members). The ratio of foreign faculty is low (currently only one junior faculty). We will be obliged to improve these two points.

For improving the research and educational environment for graduate students and young investigators, we decided to use the grant of 21st Century COE program for giving an opportunity to improve their speaking and writing abilities in English. Our plan is to hire native foreign teachers who will come to the University campus so that students can have personal or group lessons. We also decided to use the grant for hiring students as research and education assistants for easing their financial difficulty. The grant will be also used for hiring post-doc fellows in certain research labs that really need active post-docs for promoting their own researches.

To sum up, the Graduate School of Biostudies has made an excellent start since 1999. For further development, we need to examine ourselves and seek for outside opinions. For this end, we have annual two-day symposium where every lab head presents their recent research activities. This symposium has been extremely useful and stimulating for students and members of the graduate school. In next December, we will have the first conference, which is intended for the external examination by outside researchers. Eminent researchers (three foreign and four Japanese) will attend the conference and give us valuable comments. In this year, approximately half of the lab heads will speak about their study, and the remaining half will be scheduled for the next year conference. We believe that this kind of activity together with publication of this booklet introducing our graduate school in English will serve for the further development in research and education and also for showing our aim and reality to the outside people.

Mitsuhiro Yanagida
Dean, Professor

October 30th, 2002

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Laboratory of Chromosome Transmission

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Mitsuhiro Yanagida

Chromosome dynamics in the cell cycle and mechanisms of eukaryotic chromosome segregation

Chromosomes are correctly segregated to daughter cells in each cell division. This laboratory's long range goal is full understanding of chromosome behaviour during mitosis, using an excellent model organism, the fission yeast Schizosaccharomyces pombe. We have tackled problems of chromosome condensation and sister chromatid separation, and try to understand the mechanisms at the molecular level. The genes required for condensation and segregation are now known to be the same between fission yeast and human. Molecular functions of essential proteins discovered in this laboratory are investigated. Research topics include kinetochore formation and its interaction with the spindle, the roles of condensin, cohesin, securin-separase and APC/cyclosome, and genome stability. As a post-genome project, the whole gene interaction network is under construction.

Kinetochore structure and function

Kinetochore, DNA-protein complex crucial for equal segregation, consists of a large number of proteins. Two functional domains, outer repetitive and central, exist and are bound to different proteins. We intend to identify molecular activities of kinetochore proteins Mis6, Mis12, Mis12-Mis22, identified in this lab. Our focused interests are on how kintochore is formed and activated, and how it interacts with spindle microtubules. Dis1, 2 and 3 proteins are studied as they relate to dephosphorylation, acetylation and exoribonuclytic activity. We also employ HeLa with the RNAi method for making the eukaryotic model for the kinetochore.

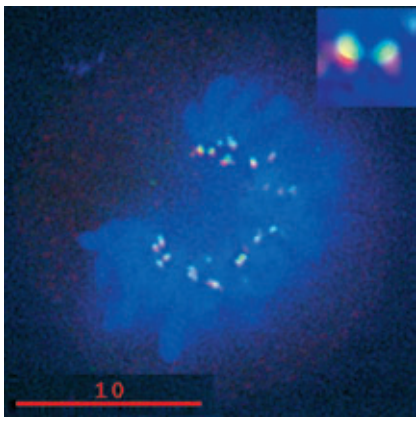

Figure 1
Human hMis12-GFP (green) locates at kinetochore of mitotic chromosomes. Checkpoint protein Mad2 (red) also situates at kinetochore but slightly different positions. DNA, blue. HeLa prometaphase cell (Tomomi Kiyomitsu)

Condensin, cohesin and securin-separase complexes, and regulation of anaphase promoting proteolysis

Condensin, cohesin and securin-separase are crucial for understanding chromosome dynamics that lead to condensation, cohesion and sister chromatid separation, respectively. In this laboratory, the genes encoding the essential components of these complexes were identified through analyses of mutants that displayed characteristic pheno-

types. Our current research plans are to elucidate the role of mitotic and interphase roles of condensin and cohesin, the cell cycle regulated interaction between cohesin and separase, and the upstream regulators and the down stream target of securin-separase. Specific mitotic roles of individual APC/cyclosome subunits are also investigated.

Genome stability and checkpoints

Crb2 is a major player in the fission yeast DNA checkpoint. Upon damage, Crb2 and Rad3/ATR activate Chk1 kinase. Crb2 is the target of Cdc2, and is regulated in damage-induced cell but also in normal cell cycle. Since Crb2 is implicated in repair *per se* and replication, proteins that interact with Crb2 are investigated. Cnd2, a non-SMC condensin subunit, is required for both repair and activation of checkpoint kinase Cds1/Chk2. A question is being addressed how Cnd2 becomes essential for the genome stability during interphase.

Post-genomic approach toward the whole gene network

Over 1000 temperature-sensitive mutants individually characterised are used for gene cloning. The mutant genes as well as high-copy suppressers are obtained. Combined with other post-genomic approaches, the functional gene network, which cover a significant portion of the whole genome, are being constructed.

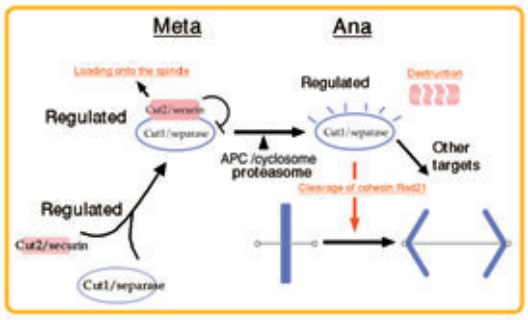

Figure 2
Three regulatory steps for securin-separase, and separase targets other than Rad21 are the subjects of investigation (Koji Nagao).

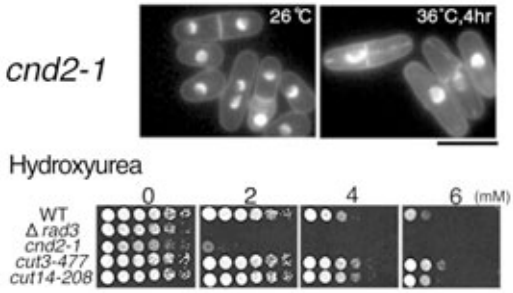

Figure 3
Mutant *cnd2-1* defective in condensin subunit fails in mitotic condensation at 36ºC and is hypersensitive to hydroxyurea, an inhibitor for replication, at 26ºC (Nobuki Aono).

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Esashi, F. et al (1999). Cdc2 phosphorylation of Crb2 is required for reestablishing cell cycle progression after the damage checkpoint. *Mol Cell 4*, 167-74.

Laboratory of Gene Biodynamics

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Tan Inoue

Nanobio RNA/RNP architecture

The catalytic RNAs called ribozymes exist as single molecular RNAs, small RNA-protein complexes (RNPs), and central components of ribosomes and spliceosomes. We showed that a self-splicing ribozyme is composed of physically separable modular units which are commonly identified in other ribozymes. On the basis of this finding, we have developed a new method for the molecular design and construction of RNAs and RNPs with difined 3-D structures (ca. 10 nm scale). Our current goal is to establish the method as a new tool for the field of nanobio science and engineering, and to fully elucidate the architecture of natural RNAs and RNPs by employing the method. Further research areas include the molecular mechanisms of gene expression, development and senesence of one of the simplest multicellular organisms called Volvox.

Minimal catalytic domain of a self-splicing intron RNA

The self-splicing intron ribozymes have been regarded as primitive forms of splicing machinery for eukaryotic pre-mRNAs. The splicing activity of group I self-splicing intron is dependent on its absolutely conserved and exceptionally dense-packed core region composed of two characteristic modular units. We found that only one unit is responsible for the RNA catalysis. This establishes that two physically and functionally separable components compose the core of the RNA, and presents a model for the architecture of a prototype of this class of intron (Ikawa et al., 2000).

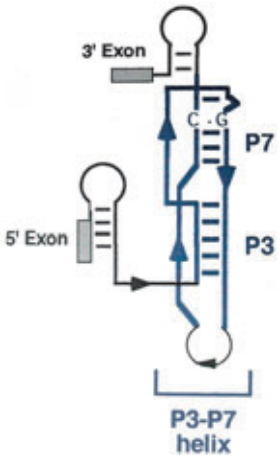

Figure 1

Secondary structure of minimal catalytic domain in the self-splicing group I intron RNAs.

Designed self-splicing RNP

Ribonucleoproteins consisting of a derivative of the Tetrahymena group I self-splicing RNA, and an RNA binding protein were designed and constructed based upon high-resolution structures of the corresponding prototype molecules: the protein contains two RNA-binding

motifs from bacteriophage λ N and HIV Rev. The splicing reaction of the RNP proceeds efficiently only when the RNA associates with the protein. The fact that the conversion of a ribozyme to a ribonucleoprotein can be accomplished by a simple molecular design suggests that a natural catalytic RNP might have evolved readily from a ribozyme (Atsumi et al., 2001).

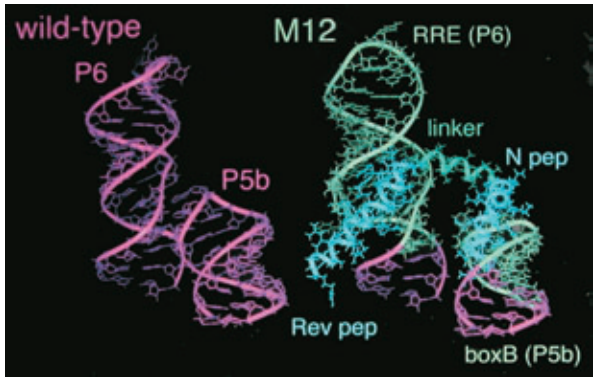

Figure 2

Designed conversion of an RNA-RNA interaction with an RNA-protein -RNA interaction (from left to right panel).

Gene expression, development and evolution of volvocaceans

Volvox is known as one of the simplest multicellular organism consisting of only two cell-types, that are, somatic cells and reproductive cells. Phylogenetic analysis suggests that *Volvox* may have shared a common ancestor with *Chlamydomonas* as recently as 35 million years ago. To investigate the evolutional pathways and mechanisms from single-cellular to multi-cellular organisms, we have isolated stage-specific cDNAs from *V. carteri* that may be acquired by this organism concomitant with the evolution of multicellularity. Initial characterization of the cDNAs that are specifically expressed during late somatic cell phase has shown that extensin-family proteins fused to metalloproteinase domains or a PR (pathogenesis-related) domain are specifically expressed in this stage, suggesting that the shuffling of functional modules of proteins has occured during the evolution of this organism (Shimizu et al., 2002). (Figure 3)

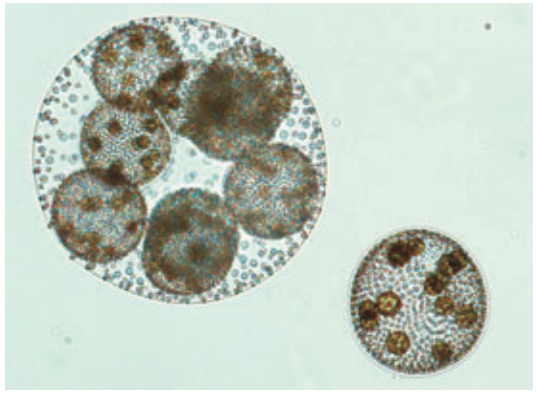

Figure 3

Spheroids of *Volvox carteri*.

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Laboratory of Cell Cycle Regulation

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Fuyuki Ishikawa

Chromosome behavior in cell cycle, differentiation, aging and cancer development

The continuity of life relies on the faithful transmission of genetic information from one cell to its daughter cells and from one generation to the next. In most organisms, DNA encodes genetic information, but DNA alone cannot accomplish the functions required for the continuity of life. Together with numerous protein and RNA components, DNA forms a huge multi-functional complex called chromosomes. It is our goal to understand how chromosomes behave faithfully and adaptively, as if they embody the will of life to transmit DNA. Because the basic mechanistics of chromosome biology are highly conserved among eukaryotes ranging from yeast to human, we attempt to reach this goal by exploiting such model systems as fission yeast, *Xenopus*, mouse and human cells.

Sex and Death, a link elucidated by the surprisingly adaptive behaviors of chromosomes

Eukaryotes are characterized by linear chromosomes, whereas most prokaryotes, by circular chromosomes. Accordingly, eukaryotes possess specialized structures called telomeres at both ends of a single DNA molecule. Telomeres have been implicated in diverse pathologies, such as aging and cancer. Then, why is it that eukaryotes do not maintain chromosomes in circular forms as prokaryotes do? Fission yeast possesses only three linear chromosomes. Upon introducing mutations that led to telomere dysfunction, most fission yeast cells died due to chromosome instability. However, we found that some fission yeast cells survived this crisis by self-circularizing the three chromosomes. This indicates that circular chromosomes *per se* are not lethal to eukaryotes. However, we also found that fission yeast possessing circular chromosomes does not undergo meiosis, a process necessary for sexual reproduction, thereby indicating that eukaryotes have evolved to maintain linear chromosomes due to the necessity to perform sexual reproduction. This hypothesis suggests that death (caused by telomere-based aging) and sex (meiosis) are two opposite faces of a single coin.

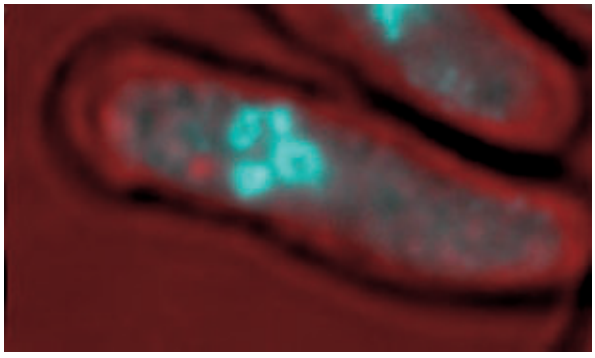

Figure 1
Circular chromosomes in fission yeast. Three condensed circular chromosomes are visualized by arresting the telomere-defective cell in metaphase.

A look into sister chromatid cohesion in higher eukaryotes

Chromosomes are replicated during the S phase to produce identical sets of chromosomes called sister chromatids. The two sister chromatids are associated with each other until the cells are ready to segregate each of the chromatids into two daughter cells in the M phase. The sister chromatid association, cohesion, is maintained by cohesin, a molecular glue. Cohesin has been extensively studied in yeast, but its functional role in higher eukaryotes is largely unknown. We have identified a dominant-negative allele of the human cohesin subunit Scc1. When we introduced a dominant-negative Scc1 into cells, we found that cohesion does not take place, as expected. However, we also observed additional phenotypes not straightforwardly expected with a simple glue molecule: the kinetochores in the disjoined sister chromatids did not interact with the spindle microtubules. This indicates that cohesin is not simply a molecular glue, but is responsible for multiple functions in chromosome dynamics.

Current and Future Goals

In addition to chromosome dynamics during the cell cycle, we are currently investigating how chromosomes change their functions either reversibly or irreversibly, during differentiation, aging and cancer development. These efforts will lead us to understand how aging and cancer occur, and to the discovery of new therapeutic strategies for these conditions.

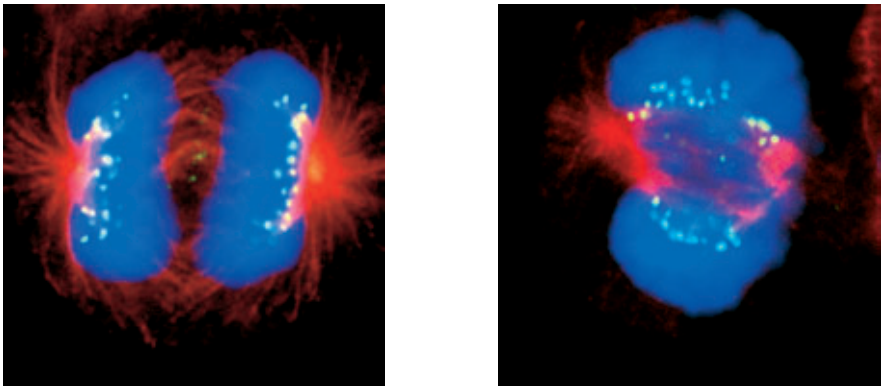

Figure 2

The kinetochore-microtubule association in the M phase is not established in cells defective in cohesin. In the normal M phase (left), sister chromatids (blue) are segregated in anaphase by the pulling force of the spindle microtubules (red) applied to the kinetochores (green). In cohesin-defective cells (right), microtubules do not interact with kinetochores, leaving DNA masses separated in a direction perpendicular, instead of parallel in wild-type cells, to the orientation of spindles.

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Laboratory of Signal Transduction

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Yoshihiko Miyata (Instructor, D.Sc.)
Makoto Fukuda (Instructor, D.Sc.)

Eisuke Nishida

Molecular mechanisms of signal transduction

We are interested in identifying and elucidating molecular mechanisms that regulate cell proliferation, cell differentiation, cell cycle and developmental processes. The current topics include 1) regulatory mechanisms and functions of the MAP kinase pathways, 2) control mechanisms of nucleocytoplasmic transport of signaling molecules, 3) roles of protein kinases in cell cycle progression and regulation, 4) growth factor signaling mechanisms in developmental processes, 5) regulatory mechanisms for cell polarity, and 6) molecular mechanisms of aging.

Regulatory mechanisms and functions of the MAP kinase pathways

We have been analyzing function and regulatory mechanisms of the MAPK pathways since we described classical MAP kinase (ERK MAPK) as serine/threonine kinase that is commonly activated by various growth factors and tumor promoters. We have identified vertebrate MAPK activator (MAPKK, MEK) and shown that the MEK/ERK pathway lies downstream of Ras and plays a crucial role in various biological processes. The receptor tyrosine kinase (RTK)/Ras/ERK MAPK pathway has now been shown to be one of the central signaling pathways that regulate cell proliferation, cell differentiation and various developmental processes. We have identified several upstream kinases that activate other members of the MAPK family (JNK and p38), such as MKK6, MKK7, TAK1, ASK1 and MLTK. Our recent studies revealed spatiotemporal control mechanisms of ERK and its activator MEK, and established the importance of docking interactions in enzymatic reactions in the MAPK cascade pathways, such as interactions of MAPK with their activators MAPKKs, their inactivators MKP (MAPK phosphatase) s, and their substrates including MAPKAPK (MAPK-activated protein kinase) s. Most recently, we have shown that Sprouty, a signaling inhibitor originally found in *Drosophila*, is a novel type of a negative feedback inhibitor in the RTK/Ras/ERK pathway and plays a key role in regulation of cell differentiation, cell proliferation and various developmental processes.

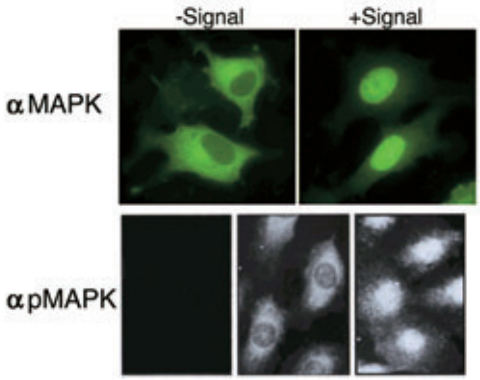

Figure 1

Activation and nuclear translocation of ERK MAPK

Mechanisms of nucleocytoplasmic transport of signaling molecules

We identified a leucine-rich nuclear export signal (NES) sequence in MEK that regulates cytoplasmic localization of MEK. We have then identified NES in a number of key molecules, including cyclin B1, Cdc25C, and Smad4. We have shown that CRM1 is a receptor or transporter for NES-containing cargoes. We are studying molecular mechanisms that regulate nucleocytoplasmic trafficking of key signaling molecules.

Identification and functional analyses of other signaling pathways

We are interested in signaling pathways that regulate longevity and aging. The best characterized pathway is an insulin/IGF-1-like endocrine system that regulates the life span of *C.elegans*. We are searching for crucial genes whose expression extends the life span of this animal. We are also analyzing signaling pathways that regulate vertebrate developmental processes by using both *Xenopus* and mouse embryos.

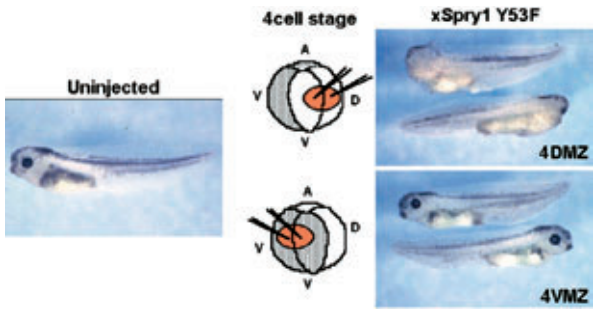

figure 2

Sprouty regulates brain patterning

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Hanafusa, H. et al (2002). Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nature Cell Biol.* 4, 850-858.

Laboratory of Plant Molecular Biology

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Hideya Fukuzawa (Associate Professor, D.Agr.)
Katsuyuki Yamato (Instructor, D.Agr.)

Kanji Ohyama

Sexual development and carbon concentrating mechanism in plants

In contrast to most animals, most plant species are bisexual. Only a few plants are unisexual ("dioecious") and have sex chromosomes. Structure and molecular function of the sex chromosomes in plants are largely unknown. We are investigating the structure of plant sex chromosomes and their function in sex determination and differentiation, using the dioecious liverwort, *Marchantia polymorpha* L. This plant can reproduce asexually from a single cell as well. We are also interested in how plants acclimate to the environmental stresses, especially shortage of CO_2 and high light. In order to elucidate the "carbon concentrating mechanisms" or "molecular acclimation process" in plants, we are studying genes for the CO_2-signal transduction pathway using model photosynthetic organisms, *Chlamydomonas* and cyanobacteria.

Structure of sex chromosomes and sexual development in liverwort

Since liverwort is haploid, a Y chromosome is present only in the male, and an X chromosome is found only in the female plants. This X-Y exclusiveness in liverwort makes it possible to investigate the genetic content of each sex chromosome separately. In addition, the sizes of the Y and X chromosomes of liverwort are estimated to be approximately 10 Mb and 20 Mb, respectively, and thus liverwort is an excellent model for molecular investigation of the sex chromosome system in plants. We have shown that unique repeat sequences are accumulated in a portion of the Y chromosome (Okada et al., 2001) and that novel multicopy genes are embedded among the repeat sequences (Okada et al., 2001; Ishizaki et al., 2002) We have isolated DNA markers for the sex chromosomes (Fujisawa et al., 2001). Sex chromosome-specific genes and their functions are under investigation using transformation technique.

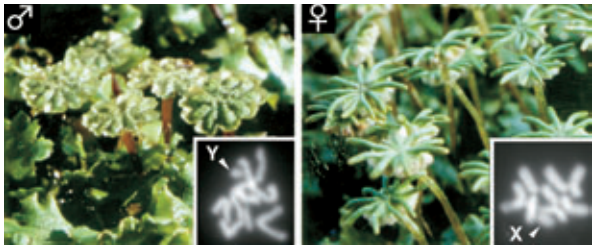

Figure 1

M. polymorpha is dioecious; male (left) and female (right) plants. Structures which look like "a plate on a stick" and "a broken umbrella" are male and female sex organs, respectively. Chromosomes are shown in the insets with sex chromosomes indicated by arrow heads.

Lipid metabolism in liverwort

Another interesting feature of liverwort is that it contains high levels of polyunsaturated fatty acids (PUFA), such as arachidonic and eicosapentaenoic acids. The accumulation of these PUFAs indicates respective high activities of the PUFA-synthesizing enzymes in liverwort. We have isolated genes encoding fatty acid elongase and desaturase from liverwort.

Molecular mechanisms of acclimation to CO_2-limiting conditions and high light

Aquatic photosynthetic organisms can adapt to CO_2-limiting conditions by inducing a set of genes for carbon concentrating mechanisms (CCM). High-CO_2 requiring mutants of a unicellular green alga, *Chlamydomonas reinhardtii*, were isolated by gene-tagging. One of the mutants is aberrant in most of the normal responses to CO_2 limitation and does not accumulate inorganic carbon into the cell. The gene impaired in this mutant appeared to be a regulatory gene, which responds to the decrease of environmental CO_2 level. We have isolated the regulatory gene, *Ccm1*, and found that the *Ccm1* gene codes for a protein with a novel zinc-finger motif (Fukuzawa et al., 2001). By exploiting cDNA arrays, we have identified a number of genes involved in CCM, including those regulated by the *Ccm1* gene. Genome-wide analyses provide us excellent opportunities to understand how cells acclimate to the change of environmental CO_2 concentration to attain efficient photosynthesis. Random transposon-knockout library was generated and used to isolate a gene encoding a new type of sodium-dependent bicarbonate transporter from a cyanobacterium (Shibata et al. 2002).

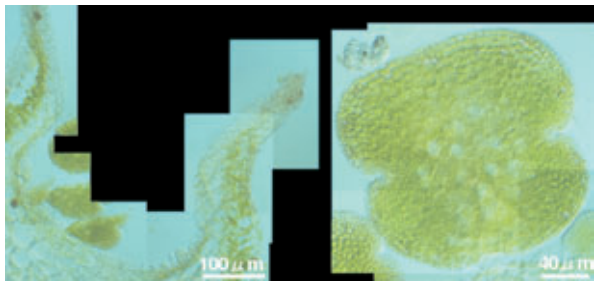

Figure 2

M. polymorpha can proliferate asexulally. Cross section of a gemma cup with immature gemmae at various stages (left), and an immature gemma still attatched to a basal cell at the bottom of the gemma cup (right).

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Laboratory of Molecular and Cellular Biology of Totipotency

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Fumihiko Sato

Functional differentiation and totipotency in plant cells

Plant cells have high potentials of totipotency, which enables the multiple functional differentiation from a single cell. To understand the molecular and cellular basis of totipotency in plant cells, we have been studying the molecular mechanism of functional differentiation in plant cells using in vitro cultured cells and transgenic plants. Several unique cell lines with photoautotrophic potential or secondary metabolite productivity etc have been established and used for further molecular characterization. Using genes identified, molecular engineering of plant productivity and stress tolerance has been conducted to provide novel genetic resources for the sustainable agriculture and green industry.

Chloroplast DNA-binding protease (CND41), chloroplast development and plant growth

CND41, a 41-kD DNA-binding protease in chloroplasts, has been isolated from cultured tobacco cells (Nakano et al., 1997). The physiological role of CND41 was investigated using transgenic tobacco with reduced CND41 (low CND41 tobacco). Since low CND41 tobacco showed retarded senescence, we examined the function of CND41 as a protease during the senescence (Murakami et al., 2000). Further analyses indicated that CND41 plays an important role in the *in vivo* degradation of Rubisco in senescent leaves and the induction of senescence. On the other hand, low CND41 tobacco also showed a dwarf phenotype, whereas plastid development in shoot apex cells was more accelerated in low CND41 tobacco. Analysis of gibberellin (GA) content confirmed the reduction of active GA in transformant. The characterization of molecular mechanism of multiple functions of CND41 in plant growth and chloroplast development is undergoing.

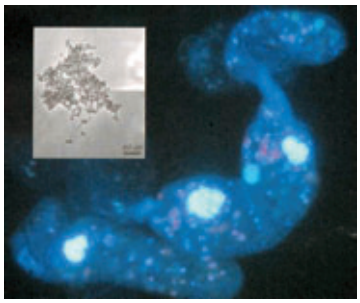

Figure 1
Different forms of DNA packaging in plastid nucleoids are found in cultured tobacco cells. Undifferentiated plastids showed more condensed blue fluorescence due to DAPI-staining of DNA, whereas chloroplasts with red fluorescence have rather diffused blue signals.

Figure 2
Control (L) and low CND41 tobacco (R) under nitrogen depletion condition.

Secondary metabolism in plant cells

Functional differentiation of secondary metabolism in isoquinoline-alkaloid-producing plants has been investigated. First, characterization of whole biosynthetic pathway of berberine alkaloid in high berberine-producing *Coptis japonica* cells has been conducted. Several biosynthetic enzymes have been purified and those cDNAs were isolated (e.g., Choi et al, 2002). Further characterization of gene expression using these cDNAs and ESTs suggested that selected *C. japonica* cells are useful materials to understand the whole regulatory system of isoquinoline alkaloid biosynthesis. Molecular engineering of secondary metabolism also provided new insights of metabolic regulation of secondary metabolism and evolution of new biosynthetic pathway (Sato et al., 2001).

Molecular characterization of gene functions using mutants and transgenic plants

Molecular mechanisms for the regulation of photosynthetic functions are investigated using mutants and transgenic plants. The cyclic electron transport around PSI by NAD(P)H dehydrogenase complex (NDH) in tobacco chloroplast has been demonstrated using chloroplastic transformants in which *ndh* genes were insertionally inactivated. On the other hand, several mutants with high level of the minimum yield of chlorophyll fluorescence, possibly including mutation in oxidizing side of PSII, photoihibition machinery or plastid gene expression were also isolated and characterized (Murakami et al. 2002).

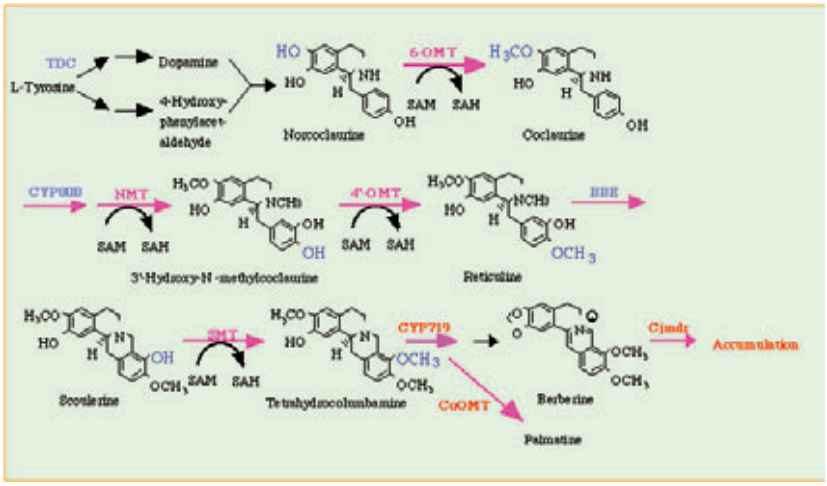

Figure 3
Isoquinoline alkaloid biosynthetic pathway in cultured *Coptis japonica* cells.
cDNAs of biosynthetic enzymes and a transporter in red have been isolated and characterized. 6-OMT, norcoclaurine 6-O-methyltransferase; NMT, coclaurine N-methyltransferase; 4'-OMT, 3'-hydroxy N-methylcoclaurine 4'-O-methyltransferase; SMT, scoulerine 9-O-methyltransferase; CYP719, canadine (tetrahydroberberine) synthase; CoOMT, columbamine O-methyltransferase; Cjmdr, berberine-import ABC transporter

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Laboratory of Biosignals and Response

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Masaya Nagao

Erythropoietin and heavy metals

Research in this laboratory focuses on multiple physiological functions of erythropoietin (Epo). Epo is a major physiological regulator of erythropoiesis. This laboratory has found that Epo is also produced in the brain, uterus, oviduct and epididymis. Epo protects neurons from ischemia-induced cell death and is involved in the uterine angiogenesis. To understand the mechanisms that Epo exerts, the study of tripartite (erythropoietic, neurotrophic and angiogenic) functions is of great interest.

Another area of research is the studies on the mechanism of heavy metal metabolism. Trace heavy metals such as iron and zinc are indispensable for life but toxic when they exist in excess. Uptake and excretion of them, therefore, must be tightly regulated. We employ a variety of biochemical, molecular biological and genetic approaches to clarify the heavy metal metabolism.

Neurotrophic function of erythropoietin

We demonstrated that cerebroventricular infusion of Epo prevents the ischemia-induced learning disability and rescues hippocampal CA1 neurons from lethal ischemic damage. Molecular basis of therapies for ischemic damage or neuronal degeneration is under investigation.

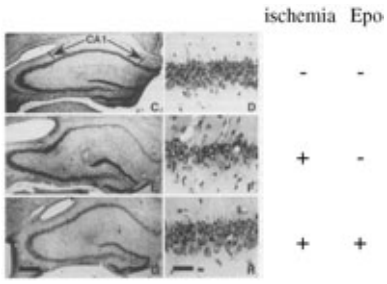

Figure 1

Erythropoiein protects hippocampal CA1 neurons from ischemic damage.

Regulation of iron metabolism

Iron is an essential nutrient for virtually all organisms yet toxic when exists in excess. The uptake of iron must therefore be tightly regulated. In *Saccharomyces cerevisiae*, the Aft1 transcriptional activator controls iron homeostasis. Aft1 activates a battery of genes required for iron uptake under iron-starved conditions, whereas Aft1 function is inactivated under iron-replete conditions. Our main focus is to understand the mechanism underling this change in gene expression responding to iron status. We

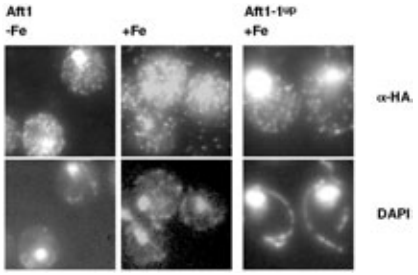

Figure 2

Subcellular loalization of Aft1 and Aft1-1^{up} proteins in the cells grown in iron-starved (-Fe) and iron-replete (+Fe) medium.

have shown that the regulation is mainly governed by the iron-regulated nuclear localization of Aft1. (Yamaguchi-Iwai et. al., 2002). We are currently studying the mechanism how iron status is sensed and regulates subcellular localization of Aft1 in the cells by using genetic, biochemical and chemical approaches. We also plan to examine the involvement of mitochondrial iron homeostasis on this iron-dependent nuclear localization of Aft1.

Zinc transport into secretory granules in pancreatic β cells

Zinc is an essential trace element required for the key structural components of a large number of proteins such as metalloenzymes and transcriptional factors. In addition, zinc plays unique roles in some specialized cells. In pancreatic β cells, zinc is required for insulin storage in secretory granules for formation of zinc-insulin crystals. We have identified a candidate for transporting zinc into secretory granules and named zinc transporter 5 (ZnT-5). ZnT-5 is a vesicular zinc transporter abundantly expressed in pancreatic β cells and localized in association with insulin crystals. We are studying the mechanism of transporting zinc, regulation of gene expression and another function of ZnT-5 by the use of biochemical approaches.

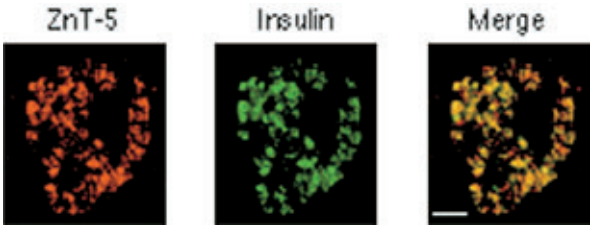

Figure 3

ZnT-5 protein is specifically detected in insulin-containing β cells, but not in other endocrine cell types or acinar cells. Scale bar = 50 mm.

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Laboratory of Applied Molecular Microbiology

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Hidehiko Kumagai

Basic and applied studies on microbial enzymes

The Laboratory of Applied Molecular Microbiology gives courses in (1) fundamental aspects of microorganisms and the application of microbiology to industrial fermentation, food production, and processing; (2) microbial technology including gene engineering and its application; (3) microbial physiology; (4) laboratory work on applied molecular microbiology.

Research projects include (1) Studies on microbial enzymes and their genes concerned with metabolism of amines, amino acids, and peptides; (2) Studies on microbial enzymes and their genes concerned with metabolism of oligosaccharides and organic acids; (3) Studies on signal transduction systems in yeast.

Analysis and construction of L-DOPA production system with bacterial cells

L-DOPA is used in the treatment of Parkinson's disease and about 100 tons of L-DOPA is now produced by a method involving the enzyme tyrosine phenol-lyase. This method was developed by Kumagai et al., employing *Erwinia herbicola* cells exhibiting high activity of tyrosine phenol-lyase. The molecular mechanism of functional proteins, transcription regulating factor and a tyrosine transporter were investigated to analyze the cell system and to produce L-DOPA much more effectively (Katayama et al. 2000 and 2002).

Cloning and expression of thermo-tolerant cellulases in a thermophilic yeast

Thermo-tolerant cellulases are useful in biomass utilization. The expression of the enzymes in a thermophilic yeast will be also useful to produce alcohol from biomass.

Characteristics of γ-glutamyltranspeptidase as an N-terminal nucleophile hydrolase: Elucidation of the mechanisms of the enzyme reaction and autocatalytic processing

The reaction catalyzed by γ-glutamyltranspeptidase (GGT, EC 2.3.2.2) has been speculated to proceed *via* a γ-glutamyl enzyme intermediate. Using a novel mechanism-based affinity labeling agent, we showed that the oxygen atom of the side-chain of Thr-391, which is the N-terminal residue of the small subunit, is a catalytic nucleophile and that GGT is a new member of the N-terminal nucleophile hydrolase superfamily. Enzymatically active GGT, which consists of one large subunit and one small subunit, is generated from an inactive common precursor through post-translational proteolytic processing. The processing mechanism for GGT has been analyzed by means of *in vitro* studies using purified precursors. We showed that the processing of its precursor is an intramolecular autocatalytic event and that the catalytic nucleophile for

the processing reaction is the same oxygen atom as that for the enzymatic reaction (Suzuki and Kumagai, 2002). Further study is being performed to elucidate the molecular mechanism of the processing reaction by crystallographical study.

Application of bacterial γ-glutamyltranspeptidase to improve the taste of food

We found that the taste of bitter amino acids was improved dramatically by γ-glutamylization and developed an enzymatic method for the synthesis of γ-glutamyl amino acids involving GGT (Suzuki et al., 2002). Theanine (γ-glutamylethylamide) is not only the major umami component of tea, but also has favorable physiological effects on mammals. An enzymatic method for synthesizing theanine involving GGT was also developed. A salt-tolerant GGT was found in *Bacillus subtilis* and we are studying its application for food fermentation as glutaminase in industry.

Nutrient sensing and signal transduction in yeast

How cells monitor the availability of nutrition and transduce signals is a fundamental, unanswered question. We have found that Gpr1p, a recently identified G-protein coupled receptor in yeast *Saccharomyces cerevisiae*, regulates the cellular cAMP level in response to glucose (Yun et al., 1998). We are studying the role and the mechanism of nutrient signal transduction.

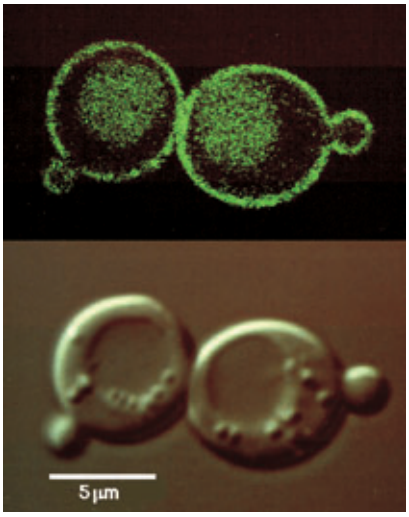

Figure 1

Localization of Gpr1-GFP

Katayama, T. et al (2000). Cloning and random mutagenesis of the *Erwinia herbicola tyrR* gene for high-level expression of tyrosine phenol-lyase. *Applied and Environmental Microbiology* 66, 4764-4771.

Katayama, T. et al (2002). Functional analysis of the *Erwinia herbicola tutB* gene and its product. *Journal of Bacteriology* 184, 3135-3141.

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Laboratory of Molecular Biology of Bioresponse

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Kenji Yamamoto

Elucidation of the role of sugar chain

The sugar chains of some glycoconjugates have been shown to play important roles in biological phenomena such as cellular recognition, immune response, and lectin binding, among others. Recently, glycoconjugates are gathering much attention, and the studies in this field of "glycobiology" or "glycotechnology", are increasing rapidly. We study about microbial enzymes acting on sugar chains of glycoconjugates and their applications. We apply such microbial glycosidases to synthesize bioactive compounds and to develop the enzymatic means for research of cell biology. We also study on interaction between lactic acid bacteria and cell membrane of host intestine through sugar chains, and on investigation of factor that promoted adhesion of useful microorganisms to intestine.

Analysis of specific activity of microbial endoglycosidase and its application

Endo-β-*N*-acetylglucosaminidase is a unique endoglycosidase that hydrolytically cleaves the di-acetylchitobiose linkages in oligosaccharides bound to the asparaginyl residue of various glycoproteins. We found a specific endo-β-*N*-acetylglucosaminidase in the culture fluid of a fungus, *Mucor hiemalis*, isolated from soil. This enzyme, Endo-M, acted on the complex type of sugar chains as well as on high-mannose and hybrid types of sugar chains, and had transglycosylation activity. We analyzed this specific activity and applied it for adding the oligosaccharide to various compounds.

1. Synthesis of bioactive glycopeptide

We chemo-enzymatically synthesized novel glycopeptides using the transglycosylation activity of Endo-M. The strategy of the synthesis of bioactive glycopeptide involves the following three steps: the synthesis of an *N*-acetylglucosaminyl asparagine derivative which is the building block of *N*-acetylglucosaminyl peptide, the preparation of an *N*-acetylglucosaminyl peptide, and the transglycosylation of N-linked oligosacchareides of natural origin to the *N*-acetylglucosaminyl moiety of the peptide, which is catalyzed by Endo-M. Using this chemo-enzymatic

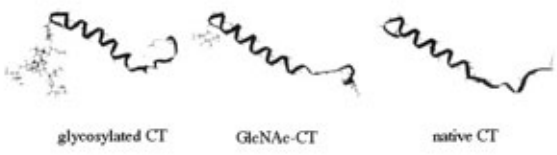

Figure 1
Tertiary Structures of Glycosylated Calcitonin and Native Calcitonin
These are the tertiary structures of glycosylated calcitonin which was chemo-enzymatically synthesized using the transglycosylation activity of Endo-M, N-acetylglucosaminyl calcitonin and native calcitonin, determined by NMR. All of them were found to have an identical conformation in micelles characterized by an amphipathic α-helix followed by an unstructured region at the C-terminus.

method, we artifically added oligosaccharides to the asparagine or glutamine residue of various bioactive peptides such as Peptide T, calcitonin, Substance P and yeast α-mating factor, originally having no sugar chain. These glycosylated peptides were more stable against protease digestion than the original molecule.

2. Remodeling of oligosaccharides of glycoproteins

The oligosaccharide-tranferring reaction by Endo-M could be used for the remodeling of oligosaccharides of glycoproteins. Using the transglycosylation activity, we exchanged the high mannose type of oligosaccharides in glycoproteins that are produced by yeasts or molds to a complex type of oligosaccharides which are human compatible oligosaccharide.

3. Effective preparation of monoclonal antibody against sugar chain of glycoprotein

Various monoclonal antibodies directed to sugar chains of glycolipids have been established using the glycolipids itself as the immunogen. However, those directed to sugar chains of glycoproteins have not been established because the protein moiety has high antigenicity. We synthesized novel glycolipids having a sugar chain of glycoprotein using the transglycosylation activity of Endo-M, and prepared a monoclonal antibody against the N-linked oligosaccharide using the novel glycolipid as immunogen.

Adhesion mechanism of lactic acid bacteria bound to host intestine through sugar chain

Many viruses, pathogenic bacteria, and bacterial toxins specifically bind to sugar chains of eukaryotic cell surface. For intestinal bacteria, adherence to cell surfaces for colonizing epithelial tissues is an important event. *Lactobacillus*, a representative useful bacterium, in the intestinal tract was found to bind to some specific glycosphingolipids, like the pathogenic intestinal bacteria. We found that *Lactobacillus casei* bound to specific glycosphingolipids. The bacteria generally bound to non-acid glycosphingolipids having short sugar chains and galactosyl moiety in the non-reducing terminal, but not to any acid-glycosphingolipids.

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Laboratory of Plant Physiology

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Katsura Izui

Regulatory mechanisms for carbon and nitrogen metabolism in plants

Our research is mainly focused on molecular mechanisms for the regulation of C4 photosynthesis and N_2-fixation. We are identifying and characterizing novel components of these metabolic processes using a combined biochemical, molecular and transgenic approach. Our studies will hopefully provide basic knowledge for the improvement of crop productivity.

Structure and function of phosphoenolpyruvate carboxylase (PEPC)

PEPC plays a key role in the photosynthetic CO_2 assimilation in C4 plants such as maize and sugarcane. We have long been studying on PEPC, and cloned the gene in 1984 and recently elucidated the 3-D structure for the first time (Kai et al., 1999). The reaction mechanism, and non-covalent (allosteric) and covalent (phosphorylation) regulatory mechanisms are being studied, and genetically engineered enzymes are produced.

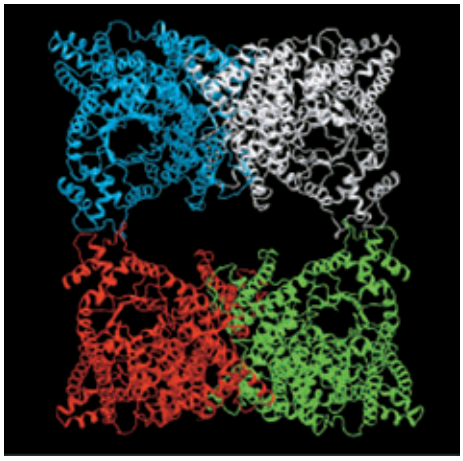

Figure 1

Tetrameric structure of PEPC from maize.

Signal transduction for the regulatory phosphorylation of PEPC

PEPC for C4 photosynthesis is activated by phosphorylation under light. A cDNA for protein kinase (PK) specific to PEPC was cloned from a model C4 plant *Flaveria trinervia* and characterized (Tsuchida et al., 2001). The enzyme was purified to homogeneity from maize and possible redox regulation of its activity via thioredoxin was proposed (Saze et al., 2001). On the other hand, sense and antisence expression of the PEPC-PK gene in *F. trinervia* is also under progress to evaluate its physiological significance in C4 photosynthesis.

Search of novel proteins indispensable for the C4 photosynthesis

There are still many unknown genes indispensable for C4 photosynthesis. By differential screening of cDNA libraries between mesophyll cells and bundle-sheath cells of maize (Furumoto et al., 2000), and between the leaves of C3- and C4 *Flaveria* species, many novel candidate genes have been obtained and now being characterized.

Nitrogen-fixing nodule formation of leguminous plants

Promoter analysis of a nodule-enhanced PEPC gene of soybean was carried out and the process of molecular evolution of the gene was investigated. Plant-microbe interaction was also investigated using *Lotus japonicus*, a model legume. We found that *Rhisobium etli*, which is a microsymbiont of *Phaseolus*, forms early senescent nodules on *L. japonicus* roots (Figure 2). The mechanism of programmed cell death in the artificial nodules is under investigation with the aid of a cDNA array of *L. japonicus*. (This project is led by S.H.)

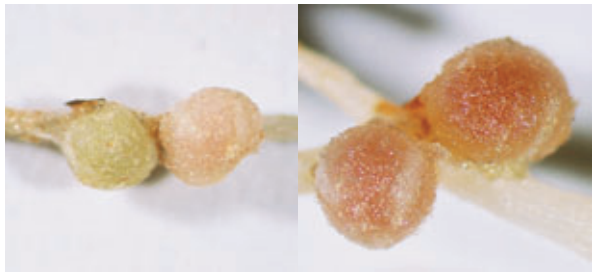

Figure 2

Early-senescent nodules (left) and regular nodules (right).

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Laboratory of Plasma Membrane and Nuclear Signaling

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Kunio Takeyasu

Plasma membrane and nuclear signaling

We are interested in how the cell signaling is regulated by specific molecular elements. We have been investigating the molecular structure and function relationships between the signaling elements (e.g., membrane receptors and transcription factors) and their targets (e.g., specific enzymes and DNA) by using a variety of techniques in biochemistry, molecular and cell biology, and structural biology. It is particularly exciting to elucidate the higher-order structures of cellular machinery (e.g., chromosomes in nucleus) by scanning probe microscopy. Molecular evolutionary aspects of the P-type ATPase superfamily proteins are also investigated. A comparative phylogenetic analysis demonstrates the relationship between the molecular evolution of these subfamilies and the establishment of the kingdoms of living things.

Nuclear dynamics

A single molecule imaging technique (Atomic Force Microscopy: AFM) is applied to the nano-strucutural biology of DNA, nucleosome, chromatin, chromosome and nucleus. We have reconstituted chromatin fibers and observed them by AFM (Figure 1). With this technique, a loop formation of DNA with multiple enhancer proteins (Yoshimura et al., 2000a) and a relaxation of supercoiled plasmid induced by a replication initiator protein (Yoshimura et al., 2000b) were observed. A “beads on a string” structure can now be obtained routinely by using centromeric histone octomer in which CENP-A replaces histone H3 (Yoda et al., 2000). When histone H1 was added the chromatin, the nucleosomes were compacted. The structure and function of the condensin molecule have also been elucidated (Yoshimura et al., 2002). We now focus upon the nuclear structures of eukaryotes (Figure 2) and prokaryotes (Figure 3). A series of basic structures common to human and *Escherichia coli* has been identi-

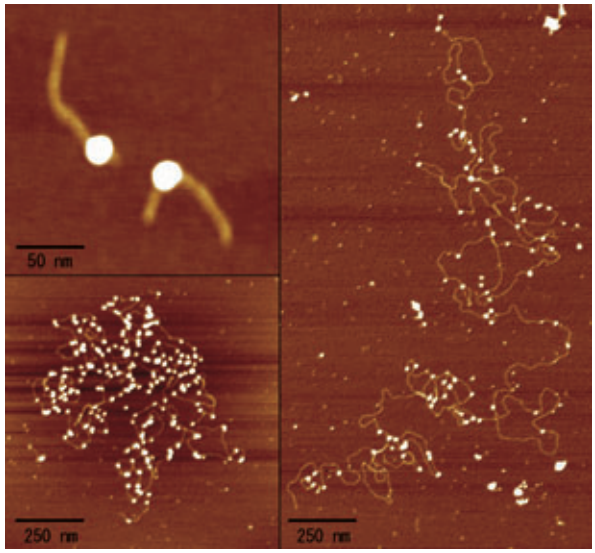

Figure 1
AFM images of the nucleosomes reconstituted by a salt dialysis method
Mono-nucleosomes were formed on 5S RNA gene (437 bp) that has two nucleosome positioning signals (left-upper panel). Right and left-bottom panels were reconstituted chromatin fiber on a linear (right) or a closed circular (left-bottom) 56 kb DNA. Nucleosomes were formed much more efficiently on the closed circular DNA than the linear DNA.

fied. Monoclonal antibodies against nuclear proteins have now become powerful tools to study the 4-dimensional dynamics of nucleus.

Molecular evolution and function of membrane proteins

Membrane transportors are essential for the cellular ion homeostasis. Some of them play an important role in sensing and transducing the extracellular information into the nucleus. We study how the biogenesis and turnover of the Na/K-ATPase, a multi-subunit membrane protein, is regulated at the ER, Golgi, and plasma membrane levels. How they assemble, how they are transported to the plasma membrane, and how they function at the plasma membrane, and how they are degraded, are the major focuses of the questions. Phylogenetic analyses of the primary sequences among the membrane transportors, specifically the P-type superfamily, have postulated a new evolutionary process of the 5 kingdoms (Takeyasu et al., 2001).

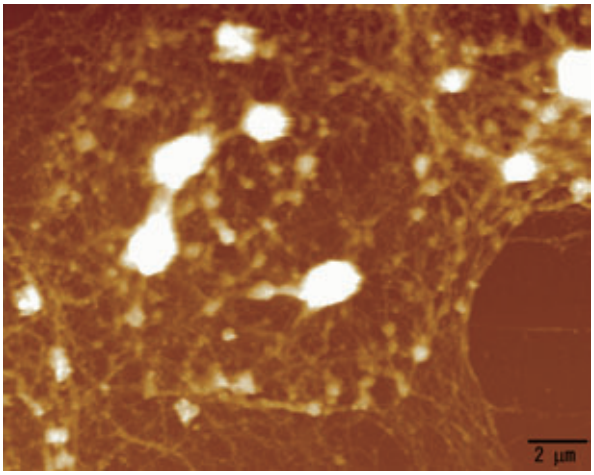

Figure 2
Fibrous structures in nuclear matrix revealed by AFM.
HeLa cells growing on a caver glass were treated with detergent to remove membrane and cytoplasm and then with DNase to remove genome DNA. The remained structures were observed under AFM.

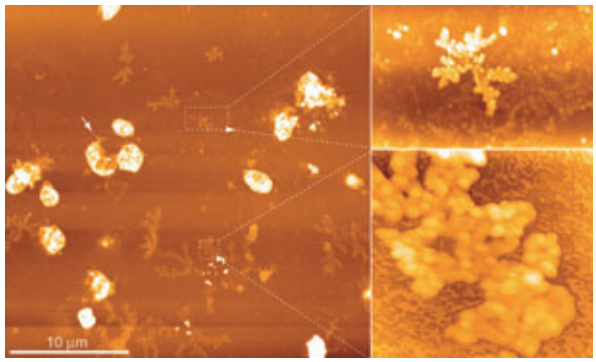

Figure 3
Escherichia coli nucleoids revealed by AFM
E. coli cells in the late stationary phase were lysed mildly on a cover glass. Left panel shows the incompletely broken cells (allow), which still retain the cell shape. The spread-out nucleoids (magnified in the right-bottom panel) and F-plasmids (right-top panel) are clearly identifed. The nucleoids and plasmids show the same coral leaf structures. A detail image (right-bottom panel) depicts nodes on the coils that resemble the plectonemic supercoiled structure.

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Takeyasu, K., et al (2001). P-type ATPase diversity and evolution: the origins of ouabain sensitivity and subunit assembly. *Cell Mol. Biol.* (Noisy-le-grand) 47, 325-333.

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Laboratory of Genome Stability

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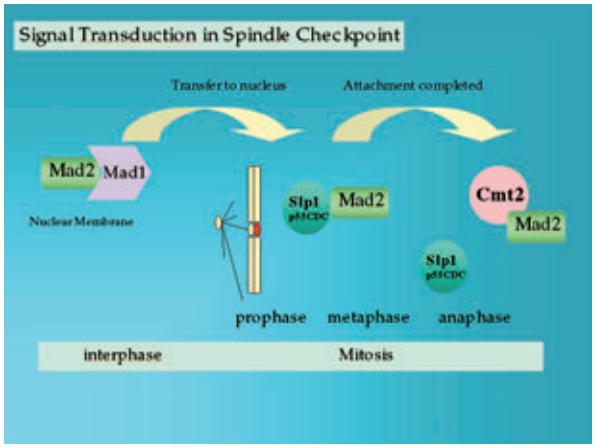
Tomohiro Matsumoto

Checkpoints in the cell cycle

The cell cycle is a series of biochemical reactions required for faithful duplication of cells. Most of these reactions are initiated in a step-wise manner and have to be completed once and only once in the cell cycle. Radiation, chemical compounds and other stress prevent these reactions from their completion and thus bring the cell cycle to a crisis. Depending on the stage of the cell cycle, eukaryotic cells employ various strategies to cope with the crisis. At the first step of the crisis control, eukaryotic cells make a decision that is “to survive or to die”. The signal cascades of checkpoints are activated if they chose to survive. The checkpoints delay progression of the cell cycle, and allow time for repair and removal of lesion. On the other hand, if they chose to die, the cascades for the programmed cell death (apoptosis) are activated. Most of the anti-cancer drugs target a step-wise reaction in the cell cycle and activate a cascade for checkpoint or apoptosis. Our research focus is on the molecular mechanisms of checkpoints.

Mad2 and its target in the spindle checkpoint

The spindle checkpoint delays the onset of anaphase when a kinetochore is not attached to the spindle. The delay induced by the spindle checkpoint requires that Mad2 binds to its target, fission yeast Slp1, which normally promotes proteolysis required for sister chromatid separation, an action occurs at anaphase. *slp1-mr63*, a mutation on the *slp1*$^{+}$ gene, abolishes the binding between Slp1 and Mad2, however, restores activities essential for cell growth. In the *slp1-mr63* mutant, a defect in the spindle attachment does not induce a delay in cell cycle progression. The mutant exits from mitosis and initiates the next round of DNA replication even when the spindle attachment in incomplete (Kim et al., 1998). A similar result has been reported for budding yeast Cdc20 that is homologous to Slp1. In higher eukaryotes such as human and mouse, p55CDC, a structural homolog of Slp1/Cdc20,



binds to Mad2 and is believed to be a target of the spindle checkpoint.

Silencing the spindle checkpoint

It has been well documented that sister chromatids separate, with a lag-time of 20-30 minutes, after the last kinetochore is attached to the spindle. The separation is highly synchronous, suggesting that the attachment of the spindle to the last kinetochore triggers a process that promotes separation of all the sister chromatids at once. Perhaps, a diffusible element is responsible for triggering such a process. Prior to sister chromatid separation, the spindle checkpoint must be silenced so that Cdc20/Slp1/p55CDC can promote the proteolysis. We have recently identified a novel Mad2-binding protein, CMT2, through yeast-two hybrid screen and shown that 1) Formation of the CMT2-MAD2 complex coincides with or immediately follows dissociation of the p55CDC-MAD2 complex in mid-mitosis, 2) Overexpression of CMT2 abolishes the function of the checkpoint by interfering with stability of the p55CDC-MAD2 complex, 3) Inactivation of CMT2 results in a transient metaphase arrest followed by cell death. These results would suggest that CMT2 may play a central role in silencing the spindle checkpoint for transition from metaphase to anaphase (Habu et al., in press).

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Laboratory of Cell Patterning

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Tadashi Uemura

Neuronal and epithelial single-cell patterning

During development, individual cells decode multiple polarity cues, reorganize cytoskeleton, and eventually adopt a vast range of asymmetrical patterns. We have tackled mysteries of "single-cell patterning" of two cell types: neurons and epithelial cells. Epithelial cells construct actin-based extensions on their apical surfaces; and neurons develop processes such as dendrites that are specialized for signal reception. How do the cells select specific intracellular sites for groundbreaking? How is the growth of the extensions limited? How are branching complexities of dendritic arbors controlled? We are investigating those molecular mechanisms by using Drosophila and mammals as model systems. Our long-term goal is to shed light on pathogenesis of human diseases that are caused by defects in the single-cell patterning.

Multiple roles of a seven-pass transmembrane cadherin Flamingo

A seven-pass transmembrane cadherin, designated Flamingo, plays multiple roles in planar cell polarization (PCP) and limiting outgrowth of neuronal processes in Drosophila. In epithelial cells, subcellular distribution of Flamingo becomes highly asymmetric and this biased redistribution appears to be prerequisite for normal cell polarization. We are investigating how this asymmetric pattern generated by developing in vivo time-lapse analysis of GFP-tagged molecules. In neurons, Flamingo appears to function as a receptor for an as yet unidentified ligand, and we are performing structure-function analysis to isolate the ligand and downstream components. In addition, we are challenging functional analysis of seven-pass transmembrane cadherins in mouse development.

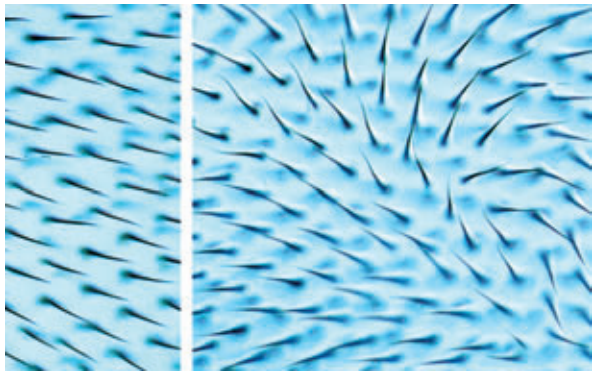

Figure 1

In the normal Drosophila wing, each epithelial cell produces a single hair that points distally (left). Mutations of polarity genes including *flamingo* disrupt the oriented pattern (right).

Control of actin reorganization by Slingshot that dephosphorylates actin depolymerizing factor (ADF)/Cofilin

ADF (actin depolymerizing factor)/cofilin is a stimulus-responsive mediator of actin dynamics. In contrast to mechanisms of inactivation of ADF/cofilin by phosphorylation, much less was known about its reactivation through dephosphorylation. We isolated *slingshot* (*ssh*) mutations that dramatically increased levels of both F-actin and phospho-cofilin, and disorganized epidermal cell extensions. ssh encodes an evolutionary conserved phosphatase and we showed that SSH family members dephosphorylate phospho-cofilin. We are studying whether SSH contributes to stimuli-driven cell shape change including neurons, and if so, how the SSH activity is regulated.

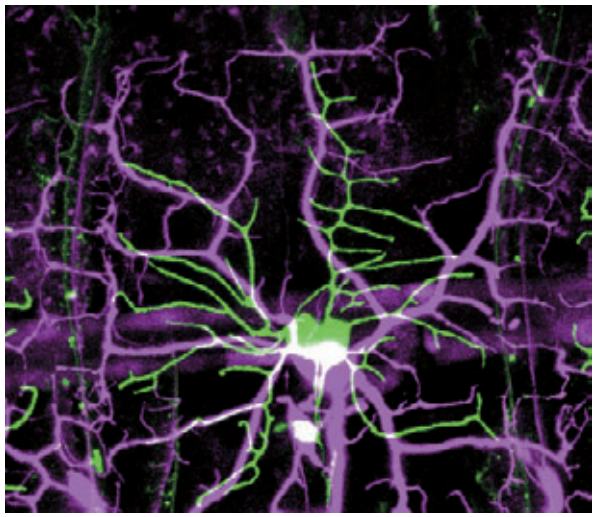

Figure 2

Dendritic arborization neurons (da neurons) in the Drosophila larva are classified into four categories, class I- IV, in order of increasing arbor complexity. Two class I and a class IV neurons are labeled green and purple, respectively.

Formation of dendritic arbors in vivo

Neurons extend many dendrites, specialized for signal reception, as well as a single axon, specialized for signal transmission. In contrast to a large body of knowledge about axon guidance, a number of questions remain about mechanisms underlying dendritic outgrowth and branching in vivo. We have developed GFP-markers for a subset of Drosophila sensory cells, dendritic arborization neurons (da neurons), and taken both cell biological and genetic approaches to full understanding of dendritic patterning.

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Sugimura, et al. Distinct developmental modes and lesion-induced reactions of dendrites of two classes of Drosophila sensory neurons. (submitted)

Laboratory of Mammalian Molecular Biology

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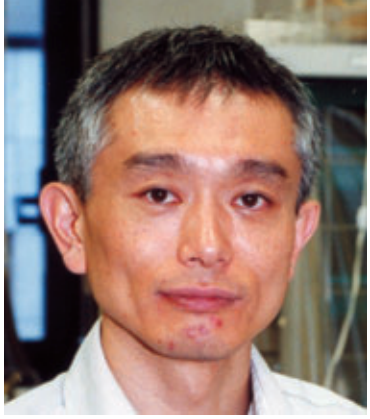

Yoichi Shinkai

Chromatin higher order structure and its function

Our principal objective is to understand the molecular mechanisms which control chromatin function and genome diversity & stability in mammals. Current our interest centers on two chromosomal-associated molecules, telomere binding proteins and SET (Suppressor of variegation, Enhancer of zeste and Trithorax)-domain containing histone methyltransferases (HMTases). Both molecules were shown to be critical for chromatin higher order structure and its functions. However, it is still poorly understood how they function at a molecular level.

Function of mammalian telomere binding proteins

Telomeres are special structures at the end of eukaryotic chromosomes and protect the chromosome end from degradation and fusion to other chromosomes (Niida et al., 1998, Niida et al., 2000). It has been described that telomere binding proteins play important roles for these telomere functions. To elucidate functional telomere structure, we are studying how mammalian telomere binding proteins contribute to chromosomal stability and cellular proliferation.

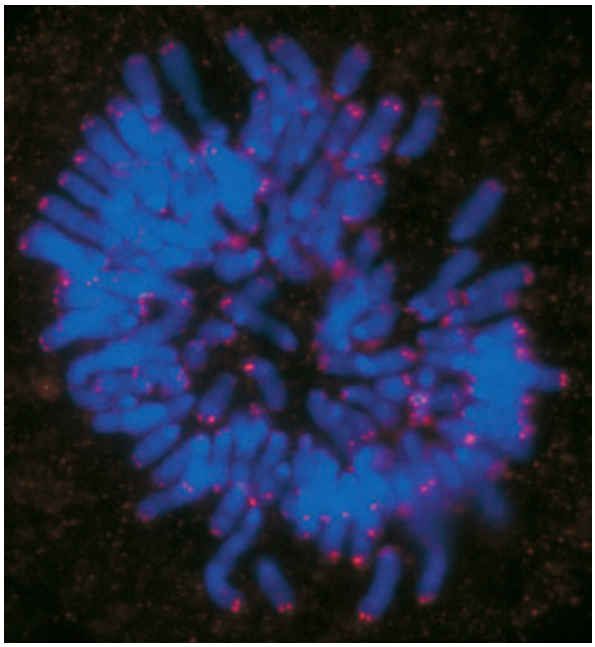

Figure 1

Localization of telomere binding protein, TRF1

Histone methylation and chromosomal function

The covalent modification of histone tails has regulatory roles in various nuclear processes, such as control of transcription and mitotic chromosome condensation. The different groups of enzymes are known to catalyze the covalent modification, including acetylation, phosphorylation, ubiquitination, and methylation. Recent studies revealed that lysine or arginine methylation is important for transcriptional control, mitosis, and meiosis. Among HMTases, G9a is a mammalian histone H3 lysine 9 (H3-K9)-preferring HMTase (Tachibana et al., 2001). We have established G9a KO mice and ES cells and shown that G9a is a dominant euchromatic H3-K9 HMTase and plays an essential role on murine early embryogenesis (Tachibana et al., 2002). G9a as a model system, we are studying the molecular basis of transcriptional control and other chromosomal functions regulated by HMTases.

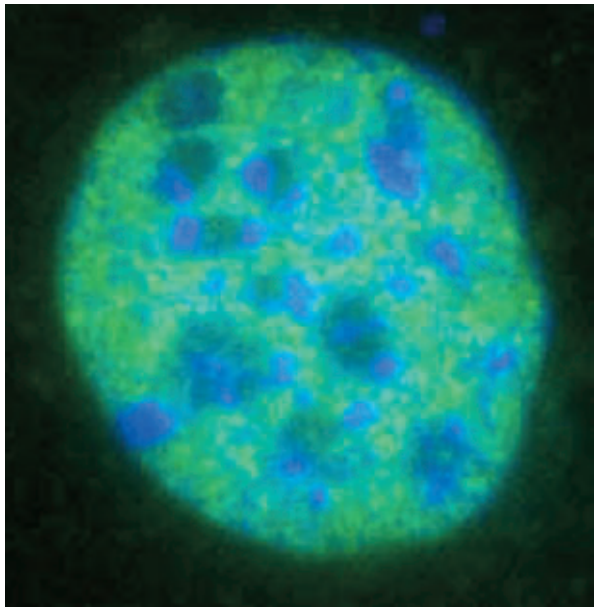

Figure 2

Euchromatic accumulation of histone methyltransferase, G9a

DAPI (Blue) and EGFP-G9a (Green)

Niida H. et al. (1998) Severe growth defect in mouse cells lacking a telomerase RNA component. *Nature Genetics.* 19, 203-206.

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Laboratory of Neuroscience

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Jun Kitano (Instructor, M.D., D.Med.Sc.)
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Shigetada Nakanishi

Investigations of synaptic operation and regulation are important for a better understanding of mechanisms responsible for brain function and dysfunction. The research projects in this laboratory are directed toward: 1) the structure, function and regulation of diverse members of glutamate receptors; 2) signal transduction and functional organization of glutamate receptors; 3) synaptic integration of neurotransmission in the neural network and 4) neural disorders derived from synaptic dysfunction.

Molecular diversity and function of glutamate receptors

Glutamate receptors are important in neural plasticity, neural development and neurodegeneration. Glutamate-gated ionotropic receptors are subdivided into NMDA receptors and AMPA/kainate receptors, whereas metabotropic glutamate receptors (mGluRs) are coupled to intracellular signal transduction via G proteins. We cloned NMDA receptors and mGluRs by developing a novel cloning strategy that combined electrophysiology and a Xenopus oocyte expression system. Our studies indicated that diverse members of receptors exist in both families (Fig. 1) and exhibit specialized functions in various brain regions.

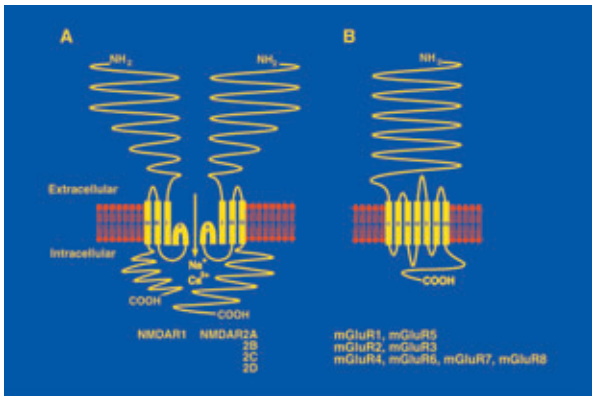

Figure 1
A family of NMDA receptors (A) and mGluRs (B)

Segregation and integration of visual information in the retinal network

In the retina, light and dark signals generate ON and OFF responses in distinct bipolar cells (Fig. 2A). Using molecular cloning, immunohistochemistry and gene targeting, we showed that mGluR6 in ON bipolar cells is a key receptor responsible for ON responses. In contrast, ionotropic receptor is used as a postsynaptic receptor in OFF bipolar cells. Two distinct types of glutamate receptors thus effectively operate for segregating visual information in response to the common glutamate transmitter. Direction-selective (DS) responses of retinal ganglion cells to a moving stimulus represent a primitive pattern recognition. The role of starburst cells (a retinal amacrine cell) in DS responses was investigated using the immunotoxin-mediated cell targeting (IMCT) technology. In this technology, we generated transgenic mice that expressed human IL-2 receptor (hIL-2R)/GFP in starburst cells and ablated these cells by intravitreally injecting the

immunotoxin composed of the hIL-2R antibody fused to bacterial toxin. Selective elimination of starburst cells abolished not only direction selectivity of ganglion cell responses but also an optokinetic eye reflex derived by stimulus movement. The synaptic integrity of neurotransmittions at the level of starburst cells underlies as a mechanism for a pattern recognition of visual information.

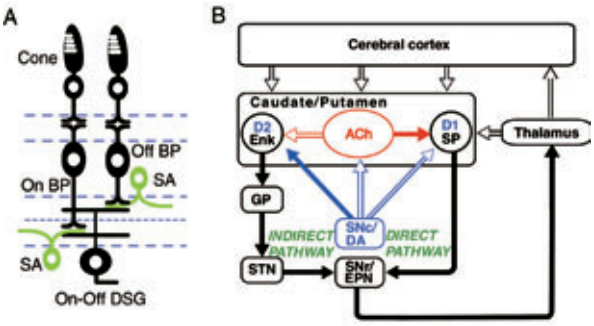

Figure 2

(A) A retinal neural network for direction-selective responses of ganglion cells. BP, bipolar cell; SA, starburst amacrine cell; ON-OFF DSG, ON-OFF direction-selective ganglion cell.

(B) A basal ganglia neural circuit. Arrow-headed open and closed lines indicate excitatory and inhibitory pathways, respectively. DA, dopamine; Ach, acetylcoline; Enk, enkephalin; SP, substance P; D1 and D2, D1 and D2 receptors; GP, globus pallidus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; EPN, entopeduncular nucleus; STN, subthalamic nucleus.

Synaptic integration in motor balance and abuse of drugs

The basal ganglia circuit is modulated by dopamine neurotransmitter (Fig. 2B). To investigate the role of acetylcholine (ACh) in the basal ganglia circuit, we selectively ablated striatal cholinergic neurons by IMCT techniques. Our studies indicated that the basal ganglia function is concertedly and adaptively regulated by synaptic integration of cholinergic and dopaminergic transmissions. Futhermore, ACh in the nucleus accumbens plays a pivotal role in the sensitivity to addition of cocaine and morphine, indicating the involvement of ACh in synaptic plasticity in the basal ganglia circuit.

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Kunishima N. et al (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 407: 971-977

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Laboratory of Immunology and Cell Biology

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Yoshimasa Tanaka (Instructor, D.Agr.)

Nagahiro Minato

Recognition and functions of immune system

T cells in the immune system play a central role for specifically recognizing invading pathogens or endogenous abnormal cells and eliciting diverse responses to protect the body. While recognition of antigens *per se* is mediated by antigen receptors, it is a functional site formed by the cognate interaction between T cells and antigen-presenting cells (APC) that determines whether the T cells respond to the antigen or not, and if they do how. One of our major research foci is directed to the understanding of molecular basis for such cognate cellular interactions to control the modes of immune responses. We are also studying possible modes for the immunological recognition of endogenously arising neoplasmic cells to explore a long-standing yet poorly corroborated concept of immune surveillance against cancer.

Integrin-mediated cognate cellular interactions in immune and hematopoietic systems

Integrins are the major cell adhesion proteins involved in the formation of immune synapse between T cells and APC for immune responses and hematopoietic niche for generation of blood cells. We have reported that a Ras-family GTPase Rap1 plays a crucial role for integrin activation and thereby controls these cognate interactions. Activation levels of Rap1 in T cells, for instance, regulate the integrity of immune synapse and affect the fates of antigen-recognized T cells, namely controlled activation and proliferation, forced unresponsiveness (anergy), or exhaustive activation followed by death. Those in hematopoietic progenitors also regulate the cognate interaction with stroma cells and control the extent of hematopoiesis. We are currently investigating the molecular mechanisms involved in the cell fate decision of T cells and hematopoietic cells by the cognate cell-cell interactions, which ultimately affect the modes of immune responses and hematopoietic homeostasis, respectively.

Unique mode of antigen recognition by γδ T cells

While the mode of protein antigen recognition by αβT cells in the context of peptides -associated major histocompatibility (MHC) molecules has been well established, we have shown that human γδ T cells exhibit a distinct mode of antigen recognition, namely recognition of a

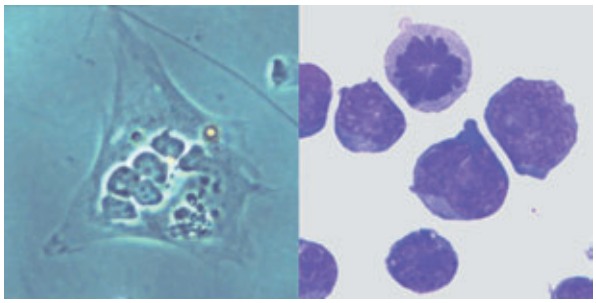

Figure 1

Intimate interaction of SPA-1-deficient leukemia cells with a bone marrow stroma cell

series of non-peptide antigens independent of MHC. These antigens are presented to γδ T cells ubiquitously by human tumor cells by yet undefined "presenting molecules". We are currently investigating the structural basis of the interaction between γδ T cell-antigen receptors and antigenic ligands including crystallographic analysis and intending to identify the antigen-presenting molecules. Through understanding of the unique antigen recognition modes, we expect to explore the immunological roles of γδ T cells, a long enigmatic T cell subset.

Study on the immune surveillance against cancer

Immune surveillance against cancer has been one of the most important concepts in immunology since 1960's, but still remains to be corroborated. The major hung-up has been the lack of feasible animal models. We have developed SPA-1-gene targeted mice, which develop age-dependent T cell-immunodeficiency and accelerated hematopoietic progenitor expansion by affecting the cognate cellular interaction between T cells and APC, and hematopoietic progenitors and stroma cells, respectively. By the collaborative effects, the mice ultimately develop lethal myelogenous leukemia of late onset. We intend to study the feasibility of immune surveillance against cancer by using this animal model.

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Laboratory of Molecular and Cellular Biology

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Kazuhiro Sakamaki (Associate Professor, D.Med.Sc.)
Kyung-Kwon Lee (Instructor, D.Sc.)

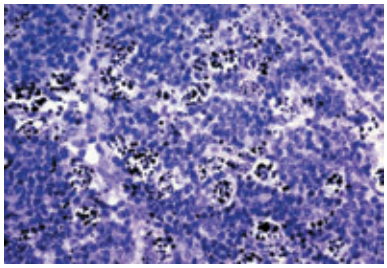
Shin Yonehara

Fas-mediated apoptotic cell death

Apoptosis, or programmed cell death, plays an important role in many biological processes, including embryogenesis, development of immune system, maintenance of tissue homeostasis, and elimination of virus-infected and tumor cells. We found cell surface Fas antigen (Fas) which can directly transduce apoptosis-inducing signals into cells by stimulation with agonistic anti-Fas mAbs (Yonehara et al., 1989; Itoh et al., 1991) or Fas ligand. Genetic and biochemical analyses of Fas and FasL have revealed that Fas plays an important role in the elimination of autoreactive peripheral T and B cells, and tumor and virus-infected cells. Our main purpose is to understand intracellular signals *via* Fas, intracellular regulatory (inhibitory) signals for Fas-mediated apoptosis and physiological role of Fas and its related signaling molecules. In conjunction with these studies, we have been trying to identify other apoptosis-related molecules which play a key role in immune system, embryogenesis or tumorigenesis.

Fas-mediated apoptosis-inducing signals

Fas-induced apoptosis (Figure 1) has been shown to be mediated by adaptor molecule FADD and caspase-8 that directly bind to Fas and FADD, respectively (Figure 2). We cloned cDNA encoding a novel protein FLASH, which can bind caspase-8 and FADD, and FLASH was suggested to be involved in Fas-mediated activation of caspase-8 (Imai et al., 1999).



B. Anti-Fas mAb RK8-induced in vivo apoptosis on lymphadenopathy

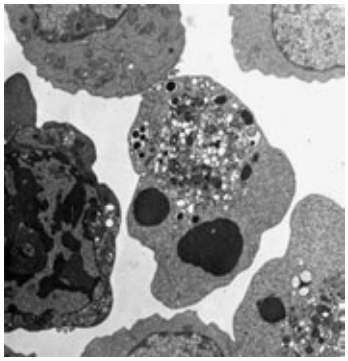


Figure 1

Fas-mediated apoptosis.

A. Transmission electron micrograph.

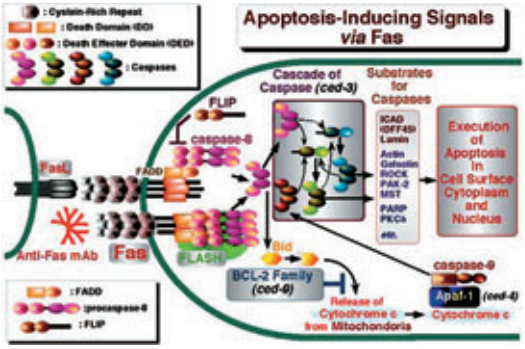


Figure 2

Fas-mediated apoptosis-inducing signals

Inhibition of Fas-mediated apoptosis

To elucidate inhibitory mechanisms for Fas-mediated apoptosis in tumor cells and autoreactive immunocytes, we showed that Raf/MAPKK/MAPK (ERK)/CREB cascade, which can be induced by bFGF, plays an important role in the inhibition of Fas-mediated apoptosis in fibroblasts (Kazama and Yonehara, 2000). Interestingly, viral oncoporotein p40Tax was also shown to inhibit Fas-induced apoptosis in fibroblasts through activating CREB, although p40Tax inhibits Fas-mediated apoptosis in T lymphoblasts by a different molecular mechanism with activation of NF-κB. In addition, we found that cell surface receptor Kit-induced signals negatively regulate Fas-mediated apoptosis by down-regulating Fas-expression through activating Akt. Thus, various oncogene products and growth factors inhibit Fas-mediated apoptosis in various cells by different and complicated molecular mechanisms, which must be clarified in the future.

Functions of caspase-8 and FADD in various vertebrates

We demonstrated that caspase-8-deficient mice died with deficiency in early embryonic development of heart, neural tube and yalk sac, and caspase-8 is indispensable for apoptosis induced through the death receptors such as Fas and TNF receptor. We examined function of caspase-8 and FADD which are derived from Xenopus levis and Zebra fish, and found that they show similar function as those of mammals. Therefore, we suggest that the apoptotic machinery using caspase-8 and FADD is conserved in vertebrates.

A novel scavenger receptor SR-PSOX with chemokine activity

We cloned a novel scavenger receptor SR-PSOX. We demonstrate that membrane-bound SR-PSOX/CXCL16 efficiently mediates adhesion and phagocytosis of not only OxLDL but also Gram-negative and Gram-positive bacteria. Strikingly, SR-PSOX was found to be identical to novel transmembrane-type chemokine CXCL16. SR-PSOX/CXCL16 is likely to play a role in both innate and adaptive immunity by mediating phagocytosis of bacteria as well as recruitment of T cells by dendritic cells.

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Laboratory of Immunobiology

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Immune regulation by the dendritic cell system

The physiologic function of the immune system is defense against infectious microbes. However, even noninfectious foreign substances can elicit immune responses. Furthermore, mechanisms that normally protect individuals from infection and eliminate foreign substances are themselves capable of causing tissue injury and disease in some situations. Dendritic cells (DCs) are the only antigen-presenting cells capable of stimulating naïve T cells. Present in all tissues, they engulf, migrate to draining lymph nodes, and present processed antigens to T cells. The DC system consists of heterogeneous populations in terms of differentiation stage and pathway. We focus on DC functions as a controller in innate and adaptive immune responses at inflammatory as well as steady states. Our goal is to provide a new insight for autoimmunity and chronic immune-based diseases.

Uptake of dying cells by DCs *in situ*

Although both DCs and macrophages sample various substances, only immature DCs continuously circulate through tissues and into lymphoid organs, capturing self-antigens as well as innocuous environmental proteins. We recently found that dying cells or its fragments were engulfed by $CD8^+$ $CD11b^{low}$, but not $CD8^-$ $CD11b^{high}$, DC subset, though both DC subsets capture protein antigens and microspheres (Fig 1). This ability was also confirmed in the experiment of uptake *in vitro*. We are now looking for responsible molecule(s) on $CD8^+$ DC subset to recognize dying cells.

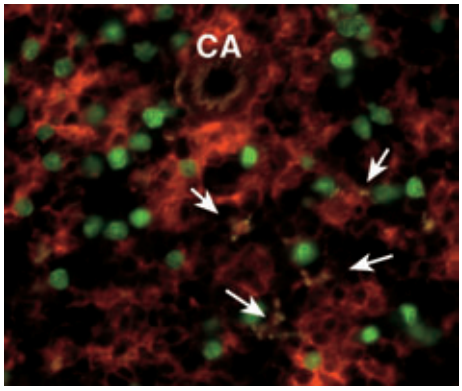

Figure 1

Dying cells phagocytosed by spleen DCs *in situ*.

Receptors involved in recognition and endocytosis of microbes

DCs could be specialized to respond to specific microbes by expressing distinct sets of TLRs and C-type lectins depending on their tissue localization and differentiation state. C-type lectins recognize carbohydrate profiles on pathogens and also interact with self- glycoproteins to mediate cellular processes, such as differentiation and migration. We cloned mouse Langerin (CD207) expressed primarily on epidermal Langerhans cells. Five mouse homologues to human DC-SIGN (CD209) and DC-SIGNR/L-

SIGN were also obtained (Fig 2). Studies are now carrying out to find ligands, as well as pathogen targets for these C-type lectins. We are also interested in signals derived from C-type lectins and from TLRs for DCs and macrophages to determine the possibility that pathogens escape immunity or are eradicated.

Antigen presentation and T cell activation

Formation of immunological synapse, a complex cluster of molecules organized at the contact area of cell conjugate, is the essential step to initiate immune responses. However, it remains to be determined what are unique characters restricted to DCs in addition to high expression of MHC and adhesion/costimulatory molecules. We are studying membrane microdomain formation using bone marrow-derived DCs at different stage of maturation.

Dendritic cell subsets in vivo

Different subsets of DCs localize in all tissues and continuously move to draining lymphoid organs in steady state (Fig 3). We have reported that DCs play an essential role to induce tolerance in periphery in the absence of inflammatory stimuli. However, functional differences of DC subsets for inducing deletion or inactivation of T cells and for maintaining regulatory/suppressor T cells are not fully elucidated. Furthermore, compositions of DC subsets in lymphoid and nonlymphoid tissues and their lifespans are largely unknown. These subjects are also investigating to clarify physiological role of DC subsets *in situ*.

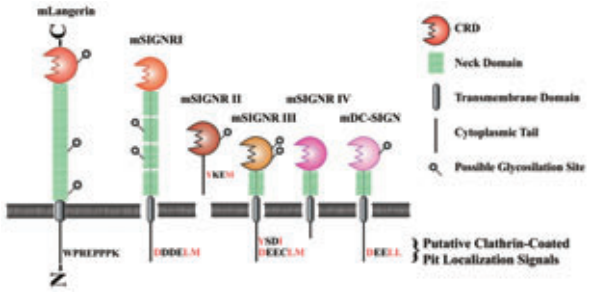

Figure 2

Structure of C-type lectins currently investigated.

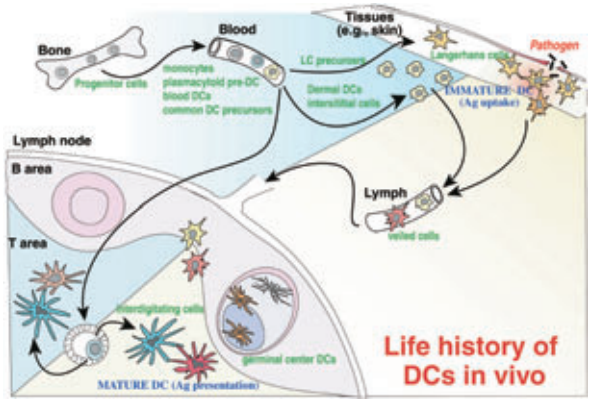

Figure 3

Life cycle of DCs in the family.

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Laboratory of Molecular Cell Biology

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Hisataka Sabe

Intracellular signaling for cell migration

Cell migration is a multifactorial process in which a number of distinct events occur simultaneously. The fundamental question in modern cell biology is, what are the molecular mechanisms involved in the temporal and spacial coordination of distinct processes, as well as orchestration of distinct events occurring at different parts within single cells, all of which result in unified, efficient, directional migration, and are also necessary for the stop of migration. Our main research interests are the molecular and cellular mechanisms involved in cell adhesion, migration and contact inhibition. We are also interested in the roles of membranes and cytoarchitecture, and their remodeling in growth control.

Endosomal recycling and cell migration

Arf proteins belong to the Ras-superfamily of GTPases, and are involved in membrane traffic and remodeling. We have found that several members of ArfGAP proteins are associated with paxillin, an integrin-assembly adaptor protein, and shown that Arf6 activity is essential for cell migration. We are analyzing the roles of the paxillin-associated ArfGAP proteins in controling cell motile activity.

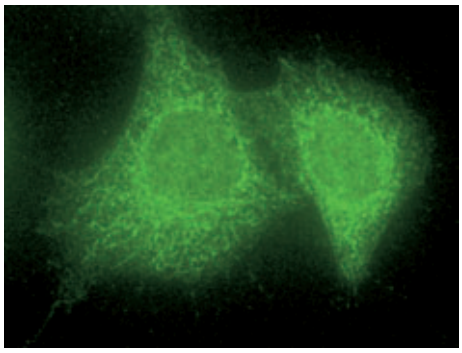

Figure 1
Subcellular localization of a paxillin-associated ArfGAP, AMAP2, in live cells.

Integrin signaling of protein tyrosine phosphorylation and cell motility

We have found that tyrosine phosphorylation of p130Cas and paxillin are the prominent events occurring upon integrin activation, and demonstrated that these proteins have the potential to exert opposing effects on several integrin-mediated cellular events. Paxillin tyrosine phosphorylation appears to be closely related to the mutually exclusive regulation of the activities of Cdc42, Rac1 and RhoA in cell migration, and we are analysing the downstream signaling pathways.

Membrane remodeling and cytoskeletal remodeling

Arfs are known to be involved in actin cytoskeletal remodeling, besides their function in membrane remodeling. Through extending our studies on paxillin-associated ArfGAPs, we aim to clarify the principal mechanisms

involved in the coordination between membranes and the cytoskeleton in motile cells.

LIM proteins in Drosophila development

Paxillin has multiple LIM domains at its COOH-terminus, and its homologue is present in Drosophila. We have found that Drosophila uses a unique system to generate a LIM-only protein from the paxillin gene locus. Drosophila, on the other hand, does not have other paxillin-related LIM proteins, such as Hic-5 and leupaxin, present in mammals. Our findings provide clues regarding the origin as well as the current functions of the LIM proteins and the LIM-only proteins, and we are currently investigating these issues using Drosophila.

Cell migration in neuronal network formation

Normal cells have the ability to detect their collision with other cells or extracellular materials and to stop migrating, that is, contact inhibition. Contact inhibition of cells has been believed to be essential for efficient and proper formation of neuronal networks as well as for the development of correct tissue morphology. We have started to address these issues using paxillin and paxillin-associated ArfGAP proteins, coupled with their gene manipulations in mice.

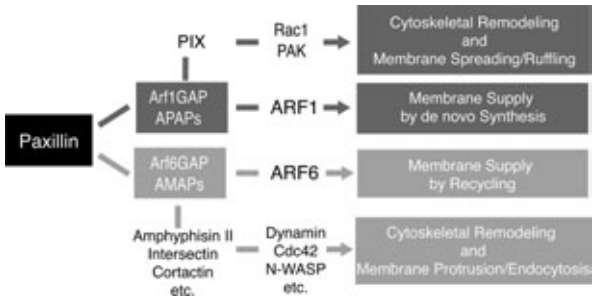

Figure 2

Biochemical circuitry of paxillin-associated ArfGAPs in motile cells

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Masatoshi Takeichi

Cell adhesion, recognition, and morphogenesis

During animal development, cells undergo dynamic morphogenetic movement and rearrangement, leading to the formation of complex tissue structures. We are studying the molecular basis of such cell behavior, focusing on the roles of a family of cell-cell adhesion molecules, the cadherins, and associated molecules. We are also seeking to unravel the mechanisms of synaptic junction formation; this project includes testing the idea that cadherin family members may play a role in interneuronal recognition during neural network formation. Furthermore, we hope to identify the molecules that regulate the lamination of cortical layers in the central nervous system, as a model of tissue patterning. Our studies are expected to benefit not only basic developmental biology but also a number of medical fields, such as those seeking to identify the mechanisms underlying neural circuit defects and cancer metastasis.

Regulatory mechanisms of cell-cell adhesion, and their roles in morphogenesis

Cadherin/catenin complexes are crucial for the establishment of stable cell-cell association. We are interested in identifying signaling mechanisms that modulate cadherin-mediated adhesion, which we consider to be essential for morphogenetic processes such as cell relocation and cell shape changes, and even in cancer metastasis. The cadherin cytoplasmic tail has regulatory functions, and our current studies include identifying novel molecules interacting with the cadherins and catenins, and analyzing their functions.

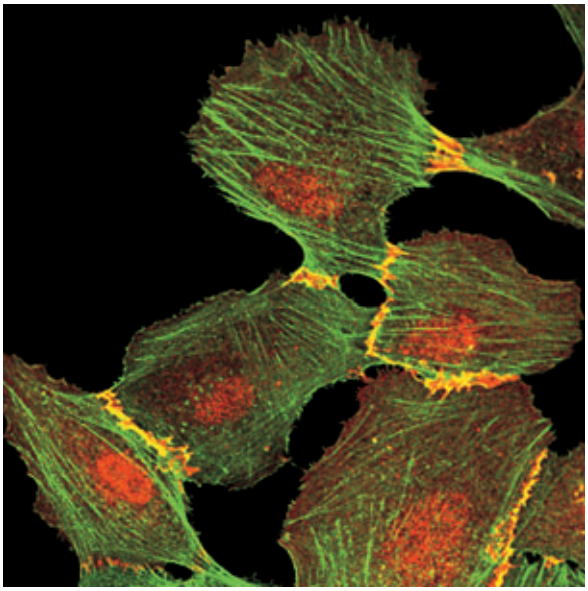

Figure 1

Double-staining for cadherin (red) and actin (green) in epithelial cells.

Synapse formation and interneuronal recognition

The neural network is generated through specific connections between neurons, but their mechanisms are poorly understood. Our group discovered that cadherin/catenin complexes are localized in synaptic junctions, playing

important roles in synapse formation. We are currently investigating the following problems: How the cadherin system regulates synapse morphology and function, how cadherin-dependent specific cell adhesion can contribute to interneuronal recognition, and how axons and dendrites can be distinguished from each other.

Seeking novel functions of cadherin superfamily genes

The cadherin gene superfamily comprises more than 120 members, as estimated from the human genome database, and this number seems to have dramatically increased after vertebrate evolution. The biological functions of these diversified molecules are, however, mostly undetermined. We have started attempts to uncover the roles of some of the cadherin superfamily members, including protocadherins and other newly identified molecules, expecting to shed light on their evolutional roles.

Identification of mechanisms controlling neuronal positioning and patterning

In the nervous system, during cortical development, each differentiating neuron is placed in a specific layer, resulting in the formation of laminated structures. We are investigating the mechanisms of neuronal positioning, using the neural retina as a model. Our recent studies revealed that a subtype of Wnt genes play a central role in controlling retinal cell differentiation and laminated layer formation.We are now working to uncover the molecular and cellular events downstream of this Wnt signaling. In addition, we are studying the role of αN-catenin signaling in cortical morphogenesis, as its loss causes cell migration defects in the brain.

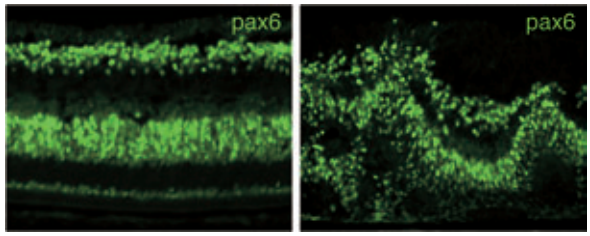

Figure 2

Blocking cadherin activity in the neural retina causes disruption of its laminated structures. Left, normal retina; right, cadherin-blocked retina, stained for Pax-6 to visualize the ganglion, amacrine, and horizontal cell layers.

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Laboratory of Molecular Neurobiology

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Manabu Negishi

Neuronal functions of Rho family GTPases

The mission of the lab is to understand the way in which neurons elaborate and guide their neurites and neural circuits are formed. In the developing nervous system, neurite outgrowth is an essential process underlying the formation of the highly specific pattern of connections between neurons.

One of our major focuses has been on the intracellular signal transduction systems involving the Rho family of small GTPases that allow extracellular guidance signals to instruct the neurite formation, elongation and guidance of the neurites. Rho family GTPases and their signaling partners play important roles in the reorganization of the actin cytoskeleton for neuronal morphological changes. Among them, Rho has been known to induce neurite retraction, while Rac and Cdc42 have been shown to be involved in neurite outgrowth.

Neuronal function of RhoG

We have examined a signal transduction pathway for Rho-induced neurite retraction, and we revealed that G12 family of heterotrimeric G proteins activates Rho and activation of Rho triggers neurite retraction through Rho-associated kinase. On the other hand, concerning the Rac and Cdc42-mediated neurite outgrowth, we revealed that RhoG, another Rho family GTPase, is a key regulator in NGF-induced neurite outgrowth in PC12 cells, acting downstream of Ras and upstream of Rac1 and Cdc42.

We have started two research projects in the neuronal functions of Rho family GTPases. The first is to understand the molecular basis of regulation of neuronal morphology by Rho, Rac and Cdc42: how G12 family transduces the signals via Rho activation and how RhoG extends the neurites through activation of Rac and Cdc42.

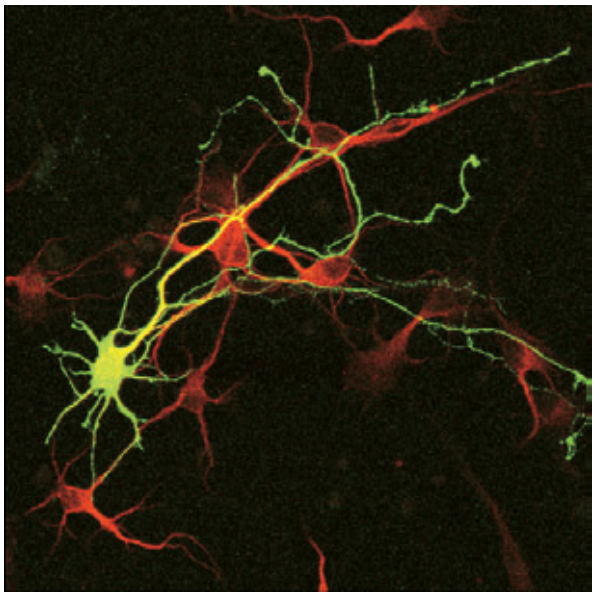

Figure 1

Primary cultured hippocampal neurons expressing GFP.

Neuronal function of Rnd subfamily

The second is to investigate neuronal functions of a novel type of Rho family GTPases, Rnd subfamily, two of them, Rnd1 and Rnd2, being expressed mainly in brain. We have recently revealed several downstream novel effectors of Rnd GTPases. We are now examining the molecular routes for the Rnd signaling in neuronal network formation.

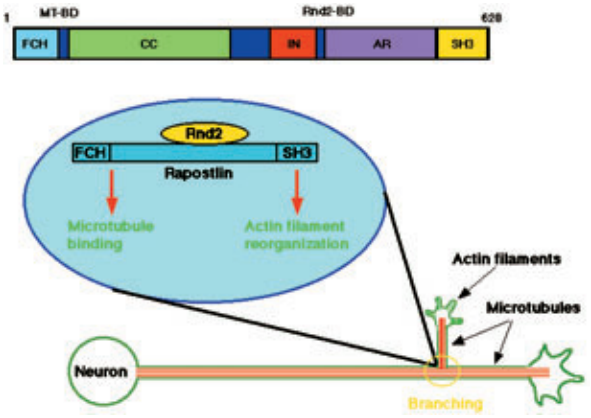

Figure 2

Structure and function of Rapostlin, an effector of Rnd2.

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Laboratory of Membrane Biochemistry and Biophysics

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Yasunori Kozutumi

Research on the biological function of membrane lipids and glycoconjugates

Outline of Research Activities are as follows:

1. Sphingolipid-mediated signal transduction in mammals and yeasts
2. Molecular biological studies on the sialic acid converting enzyme and its function using knockout mice
3. Elucidation of action mechanisms of antimicrobial self-defense peptides and the development of potent antibiotic peptides
4. Application of membrane-permeable peptides to intracellular drug delivery
5. Molecular recognition between membrane-spanning helices
6. Interactions of amyloid peptides with glycolipids

Biological function of sphingolipids

We are focusing on sphingolipids as potential regulators of the induction of multinuclear cell formation through the inhibition of cytokinesis. The first approach is to study pathologically occurring multinuclear cells. A sphingolipid, psychosine, was demonstrated to be a trigger lipid for the induction of multinuclear giant cells associated with a sphingolipid metabolic disease (Kanazawa et al., 2000) (Fig.1). The second approach is to investigate the action mechanism of a new immunosuppressant that inhibits the biosynthesis of sphingolipids. The inhibition was shown to induce multinuclear cells and apoptotic cells. A serine/threonine kinase potentially involved was identified using yeast (Sun et al., 2000).

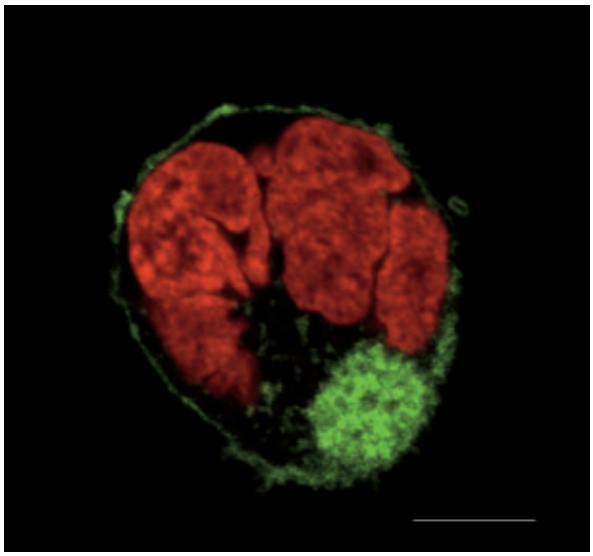

Figure 1

A typical multinuclear cell induced by psychosine. The psychosine-treated cells were stained with FITC-phalloidin (green) for actin filament and with PI (red) for nuclear staining. The bar corresponds to 10 μm.

Onset mechanism of Alzheimer's disease

The critical step in the development of Alzheimer's disease is considered to be the conversion of soluble, nontoxic

amyloid β-protein (Aβ) to aggregated, toxic Aβ rich in β-sheet structures. We have proposed that the conformational transition occurs in lipid rafts rich in sphingolipids and cholesterol. We discovered that soluble Aβ selectively binds to a ganglioside cluster, the formation of which is facilitated by cholesterol (Kakio et al., 2001), and that amyloid fibril formation is facilitated in the presence of GM1 ganglioside-bound Aβ in a β-sheet-rich structure, that works as a seed (Kakio et al., 2002) (Fig.2).

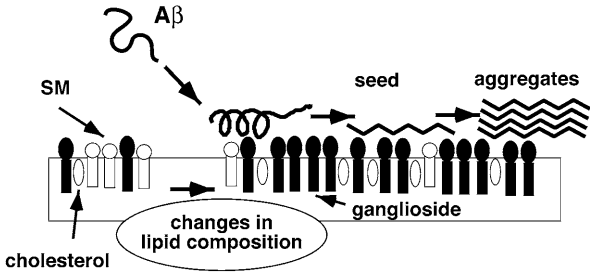

Figure 2

Our model for Alzheimer amyloid formation in lipid rafts. Soluble Aβ with unordered structures does not bind to rafts without ganglioside clusters. Once ganglioside clusters are formed by changes in local lipid composition, such as increases in cholesterol content, which is a risk factor for the disease, Aβ recognizes the cluster, forming a helix-rich structure. An increase in membrane-bound Aβ density induces a helix-to-sheet conformational transition of Aβ. Under conditions where β-sheet-rich Aβ is observed, especially in the presence of GM1, amyloid fibril formation is markedly accelerated.

Study on the biology of sialic acid mediated molecular recognition

Sialic acids comprise a family of sugars found at the termini of the cell surface glycoconjugates. N-Glycolylneuraminic acid (NeuGc) is one of the major sialic acids, and is a structural derivative of the most abundant N-acetylneuraminic acid (NeuAc). In contrast to NeuAc, the expression of NeuGc varies between animal species. NeuAc hydroxylase is the rate-limiting enzyme for the biosynthesis of NeuGc in mammalian cells. Recently, the human gene for CMP-NeuAc hydroxylase was reported to have suffered an exon-deletion mutation whereas the mouse and other mammals including our closest relative, the chimpanzee retain the active gene. In our laboratry, we are persueing the biological consequences of the targeted disruption of NeuGc biosynthetic pathway in mice.

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Laboratory of Functional Biology

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Akira Kakizuka

The molecular and biochemical basis of neurodegeneration

Many neuodegenerative disorders manifest disease-specific phenotypes, and thus it was thought impossible to deduce a common molecular mechanism underlying many, if not all, neurodegenerative disorders. However, protein aggregates and vacuoles are almost universally found in degenerating neurons, suggesting the existence of similar molecular processes in neuronal cell death. In 1994, we identified the gene responsible for Machado-Joseph disease (MJD), the most common inherited spinocerebellar ataxia. Since then we have elucidated common molecular mechanisms underlying neurodegeneration via molecular analysis of MJD. Not only MJD but also another 8 inherited neurodegenerative diseases, including Huntington's disease are caused by the expansion of CAG repeats encoding polyglutamines. Indeed, we have showed that polyglutamines have the ability to self-aggregate and induce neurodegeneration and neuronal cell death, leading to a proposal of 'polyglutamine disease'. Furthermore, we have identified ter94/VCP, a member of the AAA+ ATPase, as a key molecule in neurodegenration. Interestingly, VCP co-localizes not only with polyglutamine aggregates but also other protein aggregates and Lewy bodies. Moreover, profound deficits in its ATPase activity are found to severely affect ER quality control, leading to abnormal ER expansion and cell death. These lines of evidence indicate that VCP functions not only as a common sensor for abnormal protein accumulations but also as a mediator of neurodegenerative phenotypes; excessive accumulation of abnormal proteins may inactivate VCP's ATPase in several neurodegenerative disorders, eventually leading to the neurodegenerations. A proper regulation of VCP function is thus proposed to lead to novel treatments that are effective in a broad spectrum of neurodegenerative diseases.

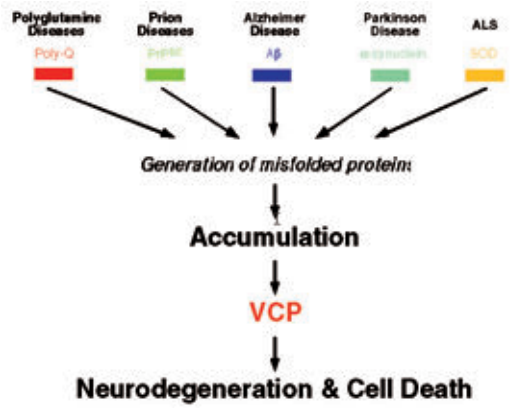

Figure 1
VCP is a potential key mediator of neurodegeneration caused by abnormal protein precipitation

The molecular basis of gene expression networks determining energy expenditure and obesity

A balanced body energy budget controlled by proper physical exercise is most effective against obesity and diabetes mellitus, but the precise molecular basis of this effect is still unclear. We are analyzing ERRL1 (for **ERR** **L**igand **1**), a protein related to PPARγ coactivator-1, which can bind and activate orphan ERRs (estrogen receptor-related receptors) *in vitro*. Consistently, ERRL1 transgenic mice exhibit increased expression of the medium chain

acyl coenzyme A dehydrogenase (MCAD), a known ERR target and a pivotal enzyme of mitochondrial β-oxidation in skeletal muscle. As a result, the ERRL1 mice show a state similar to an athlete; namely the mice are hyperphagic and of elevated energy expenditure, and are resistant to obesity induced by a high-fat diet or by a genetic abnormality. Indeed, expression of both *MCAD* and *ERRL1* is induced in the skeletal muscle after exercise in mice. These results demonstrate that ERRL1 can function as a 'protein ligand' of ERR and that its level contributes to the control of energy balance in vivo, and provide a strategy for developing novel anti-obesity drugs, or 'exercise mimics'.

KKAy mice (Yellow) KKAy (Yellow) + ERRL1 control mice (black)

Figure 2

ERRL1 expression can antagonize the obesity induced by a genetic mutation

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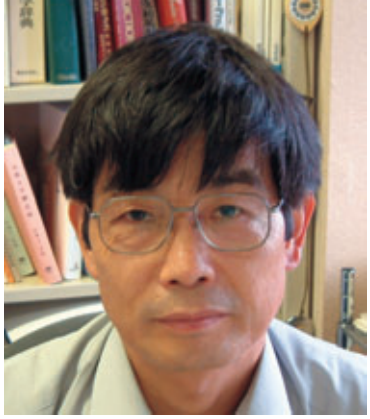
Laboratory of Viral Oncology

Kunitada Shimotohno (Professor, D.Pharm.Sc.)
Makoto Hijikata (Associate Professor, D.Med.Sc.)
Yasuo Ariumi (Instructor, D.Med.Sc.)
http://www.virus.kyoto-u.ac.jp/Lab/htv/jp/index.html



Kunitada Shimotohno

Molecular mechanisms of virus induced carcinogenesis

We focus on the carcinogenesis caused by infection with human oncogenic viruses, in particular, human T-cell leukemia virus type 1 (HTLV-1) and hepatitis C virus (HCV). Clarification of the molecular mechanisms of viral carcinogenesis may not only facilitate development of methods for disease prevention but may also contribute to our understanding of how cell proliferation is regulated by internal and external signals. Our current research projects are concerned with clarification of the role of virus proteins in virus replication and modulation of cell proliferation. We are investigating the processes by an HCV replicon system that can mimic HCV replication in cultured cells and by screening for oncogenic gene alteration(s) in hepatocellular carcinoma (HCC) and in adult T-cell leukemia (ATL).

Multiple functions of Tax-1 of HTLV-I

Tax-1, an oncoprotein encoded by the HTLV-1 genome, has potential to immortalize normal human T-lymphocytes. We have suggested that several physiological phenomena of HTLV-1 infected cells are associated with the modulations of NFκB, CREB, or p53 activation pathways by Tax-1. During analysis of the trans-acting function of Tax-1, we observed that Tax-1 was associated with transcriptional cofactors CBP/p300 and suppressed transcriptional activities of p53 and its family member p73 through competition with these transcription factors for the interaction with transcriptional coactivators CBP/p300. We are further extending our work to clarify molecular mechanisms of Tax-1 functions on the modulation of expression of various cellular genes.

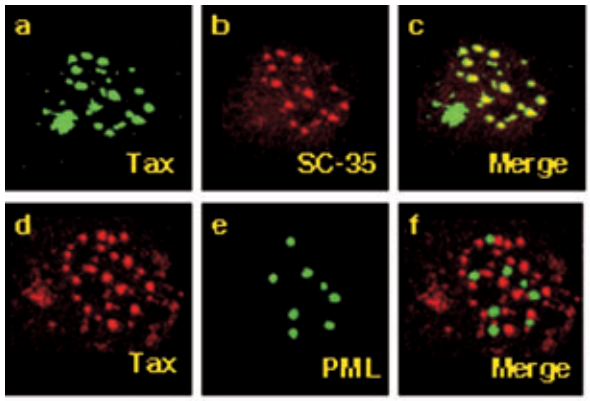


Figure 1
Co-localization of Tax-1 with the RNA splicing bodies
Tax-1 ectopically produced in COS7 cells was detected in the particular nuclear structures as well as SC-35 (panels a-c) but was not colocalized with coproduced PML (panels d-f), indicating that Tax-1 is located in the RNA splicing bodies in the nucleus.

HCV proteins that modulate cell proliferation

Persistent infection of HCV and resultant chronic hepatitis for a few decades is believed to be important for the pathogenesis of HCC. We have shown that some HCV proteins have functions in the modulation of cell proliferation which seems to be related with these events. The core, an HCV structural protein, has potential to modulate growth, transformation, and apoptosis by affecting the cellular signaling pathways, including Ras/Raf and NFκB pathways. NS5A, an HCV non-structural protein, has potential to interfere with the function of PKR, a double-

stranded RNA dependent protein kinase induced by interferon. We are studying the molecular mechanisms of these phenomena exhibited by the HCV proteins through the identification of cellular molecules targeted by these viral proteins.

Replication of HCV genome reconstituted in cell lines

The most effective way to prevent the development of virus-caused cancer is to block virus replication in carriers. Thus, development of anti-HCV agents is quite important for the cancer prevention. There was, however, no experimental system supporting efficient HCV proliferation which was likely to be essential for development of anti-viral agents. We have shown that some human-derived cell lines, including hepatocytes and mononuclear cells, support HCV replication, although not very efficiently. Using the subgenomic sequences from these culture cell-derived HCVs, we have recently established a highly efficient HCV replication system that mimics the replication of HCV using cell lines, and have been studying the molecular mechanism of HCV genomic replication. Based on this system we are now trying to establish an in vitro system reconstituting the whole HCV life cycle.

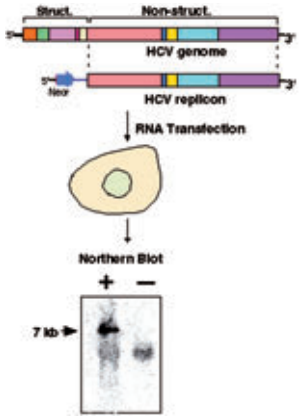


Figure 2

Establishment of the cell lines in which the HCV subgenome RNA is efficiently replicated

The HCV genome bearing the neomycin resistance gene (neor) instead of the region encoding the structural proteins (subgenomic RNA, upper panel) was synthesized in vitro and transfected into the hepatocellular carcinoma derived HuH-7 cells (middle panel). After selection by geneticin, the subgenomic RNA was found to be maintained in the total RNA from the selected cell lines (lower panel), suggested the subgenomic RNA was autonomously replicated in the cells and was supplying the neomycin resistance gene product.

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Laboratory of Genetic Information

Akira Shimizu (Professor, D. Med. Sc.)
Kei Tashiro (Associate professor, D. Med. Sc.)

Akira Shimizu

Molecular mechanisms underlying highly systemic functions

Vertebrate animals have highly organized systems, such as neural and immune systems, which are characteristic to higher animals. To understand the molecular basis of such highly systemic functions, we focused on the analyses of structure and regulation of genetic information responsible for such systemic functions using model animals, for example, transgenic or gene disrupted mice. In the immune system, genetic information of the antigen receptors is reorganized during the lymphocyte development in a cell stage specific manner. Cell to cell contact and signaling, cell movement, fine organization of multi cellular system are also known to be very important for immune and neural systems. Our recent subjects are genes and molecules involved in the regulation of such functions.

Accessibility of TCR Vγ genes are developmentally regulated by histone acetylation

Histone acetylation correlates with accessibility, since histone acetylation at the fetal-type Vγ3 gene in accord with germline transcription is relatively high in fetal thymocytes, but specifically becomes low in adult thymocytes within the entirely hyperacetylated locus. Furthermore, inhibition of histone deacetylation during the development of adult bone marrow-derived thymocytes by a specific histone deacetylase inhibitor, trichostatin A, leads to elevated histone acetylation, germline transcription, cleavage, and rearrangement of the Vγ3 gene. These data demonstrate that histone acetylation functionally determines the chromatin accessibility for V(D)J recombination *in vivo* and that an epigenetic modification of chromatin plays a direct role in executing a developmental switch in cell fate determination. (Figure 1)

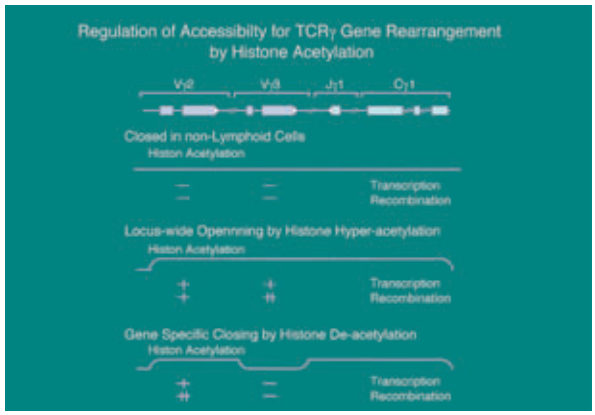

Figure 1
Accessibility regulation of TCRg genes.

Id2 is an essential negative regulator of class switch recombination to IgE

Class switch recombination (CSR) to IgE was specifically enhanced in B cells lacking Id2, through activation of ε germline transcription. This augmentation is because of the highly augmented activity of E2A. In contrast, Id2 is induced by TGF-β1 and suppresses IgE CSR in wild-type B cells, but this suppression did not occur in Id2 deficient B cells. Our results provide evidence for the inhibitory and selective role of Id2 in IgE CSR, especially in response to TGF-β1. Id2 might act as molecular safeguard to suppress the production of IgE to prevent serious compli-

cations such as allergic hypersensitivity during the normal course of immune response.

Th1 cells have higher trans-endothelial migration activity than Th2

The TEM abilities of Th1 cells from mice bearing autoimmune diseases and antigen specific Th1 cell lines were several folds higher than those of Th2 cells and lines of the same origin. These preferences were observed without exogenous chemoattractant. Antibodies against LFA-1 and ICAM-1 as well as CD44 markedly blocked TEM of Th1 cells. TEM ability was also blocked by pharmacological inhibitors for Src-family protein tyrosine kinases and phosphatidylinositol-specific phospholipase C. Cross-linking of CD44 strongly induced highly elongated morphology in Th1 lines but weakly in Th2 lines. These data indicate that there are signaling pathways for TEM independent of chemokine attraction but through adhesion molecules including CD44 and that preferences in TEM ability of Th1 over Th2 is formed, at least in part, by intrinsic differences in these pathways.

Other than above, Dr. Tashiro's research group is working on identification of novel regulatory proteins using high efficiency cDNA cloning systems for those encoding cell surface and secretary proteins.

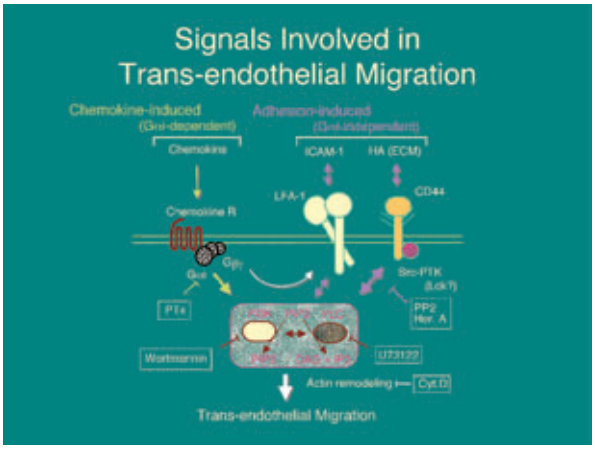

Figure 2
Two signaling pathways for trans-endothelial migration.

Figure 3
Members of Prof. Shimizu's research group.

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Laboratory of Cell Recognition and Pattern Formation

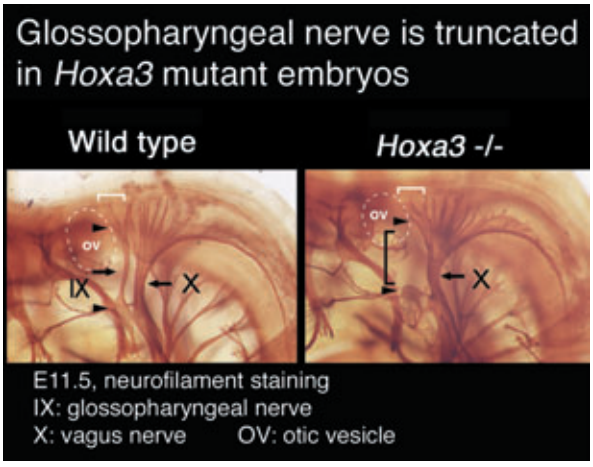
Osamu Chisaka (Associate Professor, D.Med.Sc.)

Cranial Neural Crests

In vertebrates, cranial neural crest cells contribute to organs/tissues such as thymus, parathyroid, great vessels, etc., that are indispensable for survival of an animal. We are interested in how these cranial neural crest cells find their way to the destined place and how they can differentiate into such a broad range of organs/tissues. To address these questions, we have been analyzing mutant mice which show cranial neural crest defects.

Hox code and Neural Crest Cells

Cranial neural crest cells arise from dorsal hindbrain where regional identity is governed by the "Hox-code". We found that in *Hoxa3* mutant mouse embryos, a specific population of neural crest cells show abnormal migration pattern and results in the truncation of the glossopharyngeal nerve (Watari et al., 2001). Sensory neurons of the glossopharyngeal nerve consist of two different populations, i.e., neural crest derived and epibranchial placode derived. We are currently investigating the cell autonomy of the nerve defect focusing on the interaction between Hoxa3 expressing neural crest cells and placode cells at the level of presumptive glossopharyngeal nerve forming area. Recently we found another type of neural crest defect (growth/differentiation) in *Hoxa3* mutant mice (Kameda et al., 2002).



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Laboratory of Molecular Neurobiology

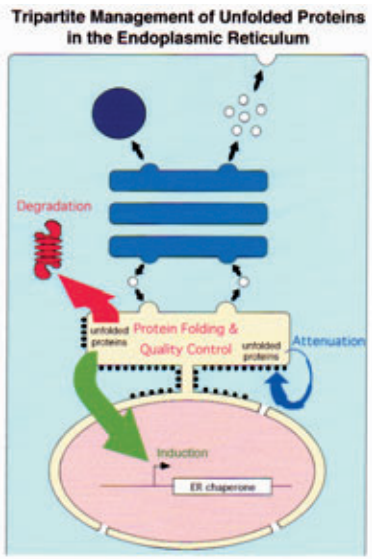
Kazutoshi Mori (Associate Professor, D.Pharm.Sc.)

Unfolding or misfolding of proteins constitutes a fundamental threat to all living cells. In eukaryotes, proteins can be unfolded or misfolded in a variety of subcellular compartments such as cytoplasm, mitochondria, and peroxisomes, but the risk of protein misfolding is particularly acute in the endoplasmic reticulum (ER), in which newly synthesized secretory and transmembrane proteins attain their proper tertiary structure. Efficient quality control systems have evolved to prevent incompletely folded molecules from moving along the secretory pathway. Thus, accumulation of misfolded proteins in the ER would detrimentally affect the function and/or localization of the approximately one-third of all cellular proteins that translocate into the ER after synthesis on membrane-bound ribosomes. Furthermore, misfolded proteins can exert proteotoxicity through hydrophobic and non-productive interactions with other cellular proteins. Eukaryotic cells have developed three different mechanisms for dealing with an accumulation of unfolded proteins in the ER: transcriptional induction, translational attenuation, and degradation (see the figure below). Our goal is to achieve comprehensive understanding of how eukaryotic cells control the quality of proteins in the ER by pursuing the molecular mechanisms of these homeostatic responses.

Current Projects

We have identified two intracellular signaling pathways from the ER to the nucleus important for the transcriptional induction in mammalian cells, which ultimately utilize the transcription factors ATF6 and XBP1 activated by regulated intramembrane proteolysis and frame switch splicing, respectively. We are currently investigating the roles of these transcriptional induction systems in the ER quality control.

Figure 1



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Yoshida, H. et al (2001). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107, 881-891.

Laboratory of Cell Regulation and Molecular Network

Yota Murakami (Associate Professor, D.Sc.)
http://www.virus.kyoto-u.ac.jp/virus.html

Nuclear/Chromatin structure and chromosome functions

Nuclear and chromatin structure is involved in the regulation of chromosome functions, such as transcription, DNA replication and recombination, but its molecular mechanism is unclear. To understand how such higher order structure regulates the chromosome functions, we mainly use fission yeast as a model system and focus on establishment and maintenance of heterochromatin, which is a structurally and functionally important constituent of the chromosome and nuclear structure.

Establishment and maintenance of heterochromatin in fission yeast

It has been unclear how heterochromatin is established at specific regions. Human CENP-B is a centromere specific DNA binding protein, and fission yeast has three CENP-B homologs. We indicate that the CENP-B homologs act as site-specific nucleation factors for the formation of centromeric heterochromatin by heterochromatin-specific modifications of histone tails. (Nakagawa et al. 2002, Figure). We are investigating the process of heterochromatin establishment by detail analysis of the nucleation of heterochromatin induced by CENP-B homologs. Little is also known about the heterochromaitn maintenance. Several proteins, which are involved in DNA replication, checkpoint and chromatin assembly, seem to be involved in this process. We start to tackle this subject by molecular analysis of these proteins.

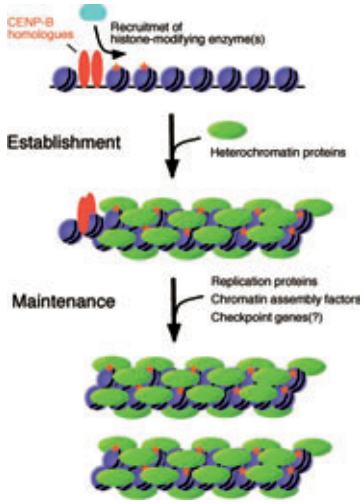
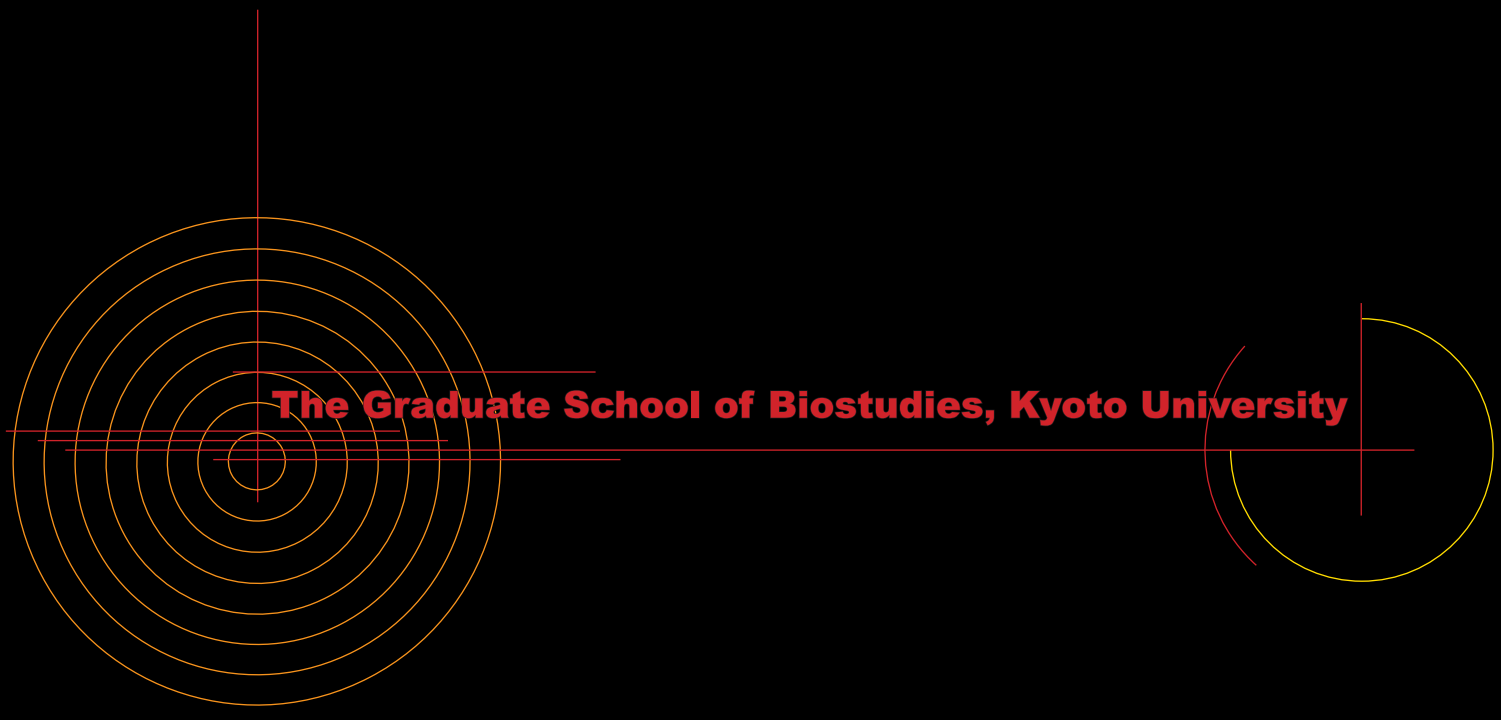


Figure 1

Establishment and Maintenance of heterochromatin

Nakagawa et al (2002). Fission yeast CENP-B homologs nucleate centromeric heterochromatin by promoting heterochromatin specific histone tail modifications. *Genes & Dev.* 16, 1766-1778


The Graduate School of Biostudies, Kyoto University

The Graduate School of Biostudies, Kyoto University

Bioscience: a key solution to creating a brighter future

– Aiming toward the Welfare and Happiness of Humanity in the 21st Century –

Preface

At the start of the 21st century, we are witnessing the beginning of a new era in life sciences: biological phenomena are finally being elucidated at an organismal level, and vast amounts of genetic, molecular, and cellular information is being generated. Anticipating such advancements, we founded the Graduate School of Biostudies at Kyoto University in 1999 by combining research groups from the Faculties of Science, Agriculture, Pharmaceutical Science, and the School of Medicine. As we were the first academic institution in Japan to combine these research fields to study bioscience, we have received much attention throughout Japan and abroad. Bioscience is a new concept and breakthrough beyond the traditional educational and research systems, and could not have been achieved within the conventional concept and fields of the life sciences.

As a research institution, day-to-day production of valuable research results and the future development of this research are the most important issues; however, considering our role as an educational institution, we seek to establish a long-term objective and vision to achieve our commitments. That is, we need to raise a specific awareness of future education and research activities. We will make every endeavor to realize our vision – "Aiming toward the Welfare and Happiness of Humanity in the 21st Century" –, which we set at the institution of the Graduate School of Biostudies.

Kayo Inaba
Dean, Professor

October 30th, 2004

About the Graduate School of Biostudies

– Institution and History –

It has taken approximately 10 years for our frontier scientists to realize their vision. The researchers who filled the full-time positions at the Graduate School spent these seeding years both to carry out academic planning and to establish mutual understanding and personal communications among the founding members. During this period, to attract researchers and graduate students, we invited internationally renowned researchers from Japan and abroad and held joint seminars on a regular basis. Our enthusiasm and strong desire to establish an institution with a novel vision of bioscience have been confirmed through these activities and efforts. In the beginning of 1998, we finally submitted the final proposal to institute the Graduate School of Biostudies at Kyoto University to the Monbusho (current Ministry of Education, Sports, Science, Culture and Technology); this proposal was readily welcomed and accepted. A total of 19 laboratories, including 16 existing labs (five labs each from the Faculty of Agriculture and the Faculty of Science, two labs each from the School of Medicine and the Faculty of Pharmaceutical Science, and one lab from the Institute for Virus Research and the Faculty of Integrated Human Studies) and three new labs, make up the Graduate School of Biostudies.

To achieve our mission, the Graduate School of Biostudies consists of two divisions, Integrated Life Science and Systemic Life Science. Laboratories in Integrated Life Science investigate fundamental life mechanisms at the levels of gene, chromosome, cell, muticellular organization and surrounding environment. Research topics include gene mechanisms, chromosome segregation, cell cycle control, cell and developmental biology, plant gene and totipotency, applied molecular biology, environmental signals and stresses. Education and research in Systemic Life Science are focused on the elucidation of the fundamentals of molecular and systemic biology, cell biology and immunology, and neurology for the recognition of self-and non-self, in response to various factors. Research areas include molecular and system biology, animal development and physiology, signal transduction, functional biology, mammalian regulatory network. The two divisions work closely together, while each division maintains its own specific viewpoint and engages in unique independent research projects and educational activities.

To enrich education at the Graduate School, we added in-school cooperative laboratories (four labs from the Institute of Virus Research, one lab from the Developmental Biology Research Center at the Graduate School of Science, and one lab from the Genetic Experiment Facility), and off-campus cooperative labs for joint reseasrch (two labs from the Osaka Bioscience Institute). After an organizational restructuring, we currently have 6 in-school cooperative labs (4 labs from the Institute of Virus Research, one lab from the Radiation Biology Center, and one lab from the Center for Genomic Medicine), and three off-campus cooperative labs (one lab from the Osaka Bioscience Institute and two labs from RIKEN). In addition, a new lab that studies Bioscience communication has joined the graduate school in 2004. In this way, we have made every endeavor to take "Life" science to new heights.

The primary aim of the Graduate School of Biostudies was to integrate laboratories that were scattered throughout the campus of Kyoto University. A building for advanced comprehensive research that will house the Graduate School and the School of Medicine is currently under construction on the South campus, and the construction of a new building for joint use with the Faculty of Agriculture is also underway on the North campus; both buildings are to be completed in the spring of 2005. As described above, the Graduate School of Biostudies has a bright future.

Division of Intergarted Life Science
- Department of Gene Mechanisms
  - Laboratory of Chromosome Transmission
  - Laboratory of Gene Dynamics
  - Laboratory of Cell Cycle Regulation
- Department of Cell and Developmental Biology
  - Laboratory of Cell Recognition and Pattern Formation
  - Laboratory of Signal Transduction
- Department of Plant Gene and Totipotency
  - Laboratory of Plant Molecular biology
  - Laboratory of Molecular and Cellular Biology
- Department of Applied Molecular Biology
  - Laboratory of Biosignals and Response

Laboratory of Applied Molecular Microbiology
Laboratory of Molecular Biology and Bioresponse

Department of Responses to Environmental Signals and Stresses
Laboratory of Plant Physiology
Laboratory of Plasma Membrane and Nuclear Signaling

Department of Molecular and Cellular Biology
*Laboratory of Cellular and Developmental Genetics
*Laboratory of Mammalian Molecular Biology

Department of Genome Stability
*Laboratory of Genome Stability

Division of Systemic Life Science

Department of Molecular and System Biology
Laboratory of Neuroscience
Laboratory of Immunology and Cell Biology

Department of Animal Development and Physiology
Laboratory of Developmental Gene Regulation
Laboratory of Immunobiology
*Laboratory of Molecular Cell Biology
*Laboratory of Molecular Cell Biology and Development

Department of Signal Transductions
Laboratory of Molecular Neurobiology
Laboratory of Membrane Biochemistry and Biophysics

Department of Functional Biology
Laboratory of Functional Biology

Department of Biostudies and Society
Laboratory of Science Communication and Bioethics

Department of Mammalian Regulatory Network
*Laboratory of Cell Regulation and Molecular Network
*Laboratory of Viral Oncology
*Laboratory of Genetic Information

(*cooperate laboratories)

Our Mission

The outcomes of new life sciences are increasingly permeating into our daily lives; therefore, it is very important to achieve a proper prospective of living in harmony with nature and the environment, in addition to pursuing the development of technologies. Thus, it is our commitment to develop frontier researchers, who are not bound only to their own specific field, but have profound understanding of the multi-dimensional advancement of the life sciences, the ability to recognize the importance of the environment surrounding human society, and the ability to study the relationships between the environment and society from a broader point of view. Our mission is to develop human resource who are capable of the following tasks:

(1) To advance novel biostudies to the highest level in the world.
In order to meet the demands of industry, universities, and research institutions, our rigorous program educates students so that they will be equipped with advanced knowledge, master the technologies of novel biosciences, and establish a sense of “self” in society.

(2) To apply novel biosciences to protect the global environment for human welfare and happiness.
By integrating knowledge and technologies of conventional sciences, agriculture, medicine, and pharmaceutical science, we aim to develop researchers who can understand the complex biosphere and become leaders in 21st century human society.

(3) To observe and elucidate various biological phenomena of living organisms as systemic functions.
In a 21st century society seeking human welfare and happiness, we promise to develop human resource who will become leaders in the global community and who will promote harmony between the human race and other living organisms.

Research Activities

The number of grants-in-aid for Scientific Research and other competitive funds the Graduate School has received truly reflects our research achievements. For the academic years of 2002, 2003, and 2004, the Graduate School of Biostudies received grants-in-aid that have surpassed a billion yen. Furthermore, the number of grants per faculty member has been at the top in Kyoto University. In addition, among our publications, eight papers have been cited as references more than 1,000 times, and 300 papers have been cited more than 100

times. Many of the lab heads play leading roles in their research fields. Also, many of the lab heads have served as editors of academic journals. Two professors have been nominated as members of the National Academy of Science, USA and the Royal Society of London, suggesting that they are indeed world-renowned leaders in their research fields. Moreover, since the institution of the Graduate School in 1999, five professors have received prestigious prizes, such as the Japan Academy Prize, the Imperial Award, the Asahi Award, the Osaka Science Prize, the Toray Science and Technology Prize, and the Inoue Prize for Science.

We also have strong ties to industries: two of our professors and nine other faculty members have experience working at institutes attached to private corporations. We have up to 100 contract research agreements and academic grants-in-aid, which truly indicate that our research direction meets social needs.

In order to seek further development, and disclose the outcomes of our research projects to the public, we maintain high standards, and are ready to accept evaluation from outside sources. As one component of sharing our research results, all of the labs at the Graduate School have participated in an annual two-day open-to-the-public symposium. Many people from inside and outside the University have participated in the symposium, which has become a valuable forum for the exchange of intellectual information. In addition to the annual symposium, we have invited renowned researchers from Japan and abroad in December 2002 and 2003 (three from abroad, four from Japan in 2002; two from abroad, five from Japan in 2003) and held research conferences. We received meaningful opinions and comments as well as evaluations from the outside the University.

Organization and Management

At the Graduate School, Faculty Council meetings, which include the professors from both divisions, and Department meetings, which include faculty members who hold positions above lecturer in all participating labs, are held monthly. The dean presides over both meetings. At Faculty Council meetings, most issues regarding activities at the graduate school are closely and carefully examined, and decisions are made. Department meetings deal with educational issues, such as the entrance examination and the comprehensive examination and thesis defense for the Master's and doctoral degrees. In addition to these routine meetings, lab meetings, which are called and presided over by the head of each lab, are held to discuss the daily operation and management of each lab, as well as personal matters regarding young researchers working in the lab, prior to the Faculty Council and department meetings. Furthermore, all topics and reports that are to be discussed at the Faculty Council and Department meetings are to be discussed a priori by the steering committee.

To date, six young researchers from our department have become professors at other universities or research institutions, and one was promoted to associate professor. Two professors have retired since our institution began. As a result, (number) professors have been newly recruited from inside and outside the University. Furthermore, the number of female faculty members has doubled since the institution began (two at the time of institution: Professor (1), Assistant Professor (1); four at present (Professor (1), Associate Professor (1), and Assistant Professor (2)). However, the employment of foreign researchers has not increased (currently we have only one researcher working as an Assistant Professor). We would like to increase the employment of female and foreign researchers in the future.

Education

The Graduate School of Biostudies aims to accomplish the following objectives to establish day-to-day operations, provide outstanding education, and promote research studies:

(1) To develop human resource who are equipped with knowledge of next-generation, advanced life sciences. We aim to develop a new type of human resource who possesses unique and creative qualities to deal with various unknown issues that the next generation of humanity must face.

(2) To develop human resources who establish a sense of “self” in society.
We value the unique academic background and future vision of each member of the Graduate School, and we aim to establish a system to assess the effects of an unconventional diversified education and help students

acquire healthy and fair critical minds.

(3) To encourage active and flexible personnel management. We encourage active information exchange between labs, and aim to advance and develop unique research projects to explore novel fields of life sciences.

(4) To maximize the post-doctoral program and establish a method to assess and evaluate achievement. In order to intensively develop internationally active life scientists, we aim to maximize the existing system, and maintain a faculty to student ratio lower than at the former graduate school.

The Graduate School of Biostudies offers a two-year Master's degree program, and a three-year doctoral degree program. Students require 30 units to graduate from the Master's program; attending lectures, seminars, submitting a Master thesis based upon the student's research project, and passing the comprehensive examination and thesis defense are required for graduation. Doctoral candidates are obligated to attend seminars that credit more than 8 units per year, conduct a unique research project, publish original academic papers, and submit a Ph.D. dissertation. A comprehensive examination and thesis defense are mandatory to qualify for the Ph.D. degree.

It has been six years since the Graduate School of Biostudies was founded. As expected, many important outcomes have already been produced through our research activities. The selection of our institute for the 21st century COE program in 2002 has further enhanced our programs and broadened and deepened the horizons of our research activities. In this new environment, graduate students are given more opportunity to closely work with the instructors; this results in an encouraging atmosphere of cooperativity and helps foster a unique character that values diversity and the frontier spirit.

We make the most of the 21st century COE program funds to educate graduate students and young researchers. For example, to be active in the global research community, researchers need the linguistic skills for oral presentation and writing manuscripts in English; therefore, we have designed special classes where students can learn English conversation directly from native instructors. Reduced class size allows active involvement. In 2002 and 2003, almost 400 students were enrolled in these classes. Furthermore, we designed a special opportunity for the students to learn bioscience in English: we offered intensive lectures on molecular biology in English. To refine the students' linguistic ability, we supported an International Student Seminar that was hosted by the graduate students. We also invited renowned researchers from abroad to present in the lecture series. We hosted an open forum with guest speakers twice. Through these experiences, we have provided opportunities for the students to be exposed to the most advanced research. In 2004, at the third International Student Seminar, two native English speaking researchers and four outstanding Japanese researchers from other research institutions were invited and asked to evaluate the students for their oral presentation skills in English. Ten students will be given the opportunity to give oral presentations at the Kyoto-Singapore International Symposium, which will be held in January 2005. The 21st century COE program provides further financial support to the graduate students; the students are employed as research and education assistants. Also, in certain research fields where further promotion is thought to be necessary, we are actively involved in employment of young researchers as "post-doctoral fellows."

To date, accounting for some fluctuation in each academic year, 80 to 85 Master's students and 45 to 55 Ph.D. candidates enroll in the Graduate School of Biostudies each year. As a result, the Graduate School of Biostudies has a total enrollment of 326 hard working graduate students, 162 Master's students (of whom 53 are women), and 164 Ph.D. candidates (of whom 37 are women). In April 2004, 17 students, who were among the first graduates of the doctoral program at the Graduate School of Biostudies, received their Ph.D. degrees. More students have applied to defend their Ph.D., and about 25 students will receive their degrees at the end of this academic year.

In March 2001, at the time of the first graduation from the Master's program, we initiated an alumni association "Ibuki" of the Graduate School of Biostudies. Thereafter, two issues of the alumni bulletin have been published. We hope the alumni association will become an open forum where the graduates and students can freely exchange opinions and information.

Laboratory of Plant Molecular Biology

Takayuki Kohchi (Professor, D.Agr.)
Hideya Fukuzawa (Associate Professor, D.Agr.)
Katsuyuki Yamato (Instructor, D.Agr.)
http://www.lif.kyoto-u.ac.jp/labs/plantmb/

Takayuki Kohchi

Molecular Mechanisms of Plant Growth and Development

All organisms have to acclimatize themselves to their environment that undergoes continuous change over both short and long time periods. Plants are exquisitely sensitive to their environment, and have developed well-refined sensing and signaling systems to modulate their growth and development. How do plant cells recognize and integrate ambient conditions such as light and CO_2? The research in this laboratory focuses on the molecular basis of developmental plasticity and genetic control of growth and differentiation using *Arabidopsis*, moss *Marchantia*, and green alga *Chlamydomonas* as model photosynthetic organisms (Figure 1). Topics include 1) photo-sensing and signal transduction by phytochrome; 2) CO_2-sensing and signal transduction; 3) hierarchical regulation of plant development by transcription factors; 4) sex chromosome and sexual development of plants; and 5) polyunsaturated fatty acid metabolism.

Figure 1 Model photosynthetic organisms used in the laboratory

Top left; *Arabidopsis thaliana* is a small weed of no economic use, but the primary model organism for genetic studies of higher plants. Top right; *Chlamydomonas reinhardtii* has developed a carbon-concentrating mechanism (CCM) to acclimate to CO_2-limiting or high-light stress conditions to maintain photosynthesis. Bottom; *Marchantia polymorpha* is dioecious plants with sex chromosomes - male (left) and female (right) plants.

Structure-function assays of phytochrome chromophore for photo-sensing in plants

The phytochrome family in plants is a major photoreceptor that has the linear tetrapyrrole molecule, phytochromobilin as its chromophore. Genes for tetrapyrrole metabolism were identified by a molecular genetic approach with *Arabidopsis* photomorphogenic mutants (Figure 2) and by a comparative genomics. We have developed a genetic modification system that is suitable for evaluating the

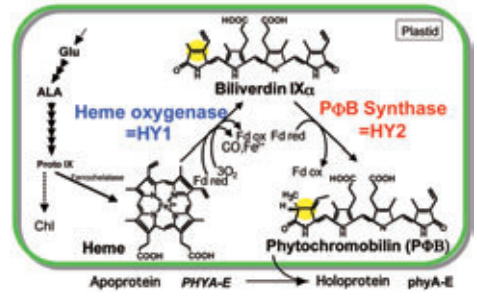


Figure 2 Phytochrome and chromophore biosynthesis pathway in plants

effects of chromophore structure on photobiological activities *in planta*. We found that the structural requirement of phytochrome chromophore is diverged in the modes of action and that the selection of phytochromobilin as a chromophore might have an ecological significance for red/far-red light responses in land plants (Kami et al 2004).

Molecular mechanisms of CO_2-sensing and acclimation to CO_2-limiting conditions

Aquatic photosynthetic organisms adapt to CO_2-limiting conditions by inducing a set of genes for carbon-concentrating mechanisms (CCM). The *Ccm1* gene encoding a protein with a novel zinc-finger motif is a master regulator of CCM in Chlamydomonas. Transcriptome and genetic analyses of CO_2-insensitive regulatory mutants revealed that cells acclimate to low-CO_2 conditions by inducing CCM-related genes through CCM1 and MYB-transcriptional factor LCR1, which binds to CO_2-resposive DNA elements (Yoshioka et al 2004, Miura et al 2004).

Hierarchical regulation of growth phase transition by receptor kinase and transcription factors

Intercellular communication mediated by receptor-like kinases (RLK) is important for diverse processes in plant development. A MADS-box protein, AGL24, that plays a role in promotion of floral transition was identified as a substrate for mersitematic receptor-like kinase, MRLK (Fujita et al 2003).

Structure of sex chromosomes and sexual development in liverwort

We determined the draft sequence of the Y chromosome from the dioecious liverwort, *Marchantia polymorpha*. Currently we are investigating its genetic program of sex determination and differentiation by functional genomics utilizing genomic information.

Lipid metabolism in liverwort and application to plant molecular breeding

Liverwort contains high levels of polyunsaturated fatty acids (PUFA), such as arachidonic and eicosapentaenoic acids. We have isolated the PUFA-synthesizing enzyme genes encoding fatty acid elongase and desaturase from liverwort, and applied to PUFA production in higher plants (Kajikawa et al 2004).

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Laboratory of Science Communication and Bioethics

Kazuto Kato, (Associate Professor, D.Sc.)

Kazuto Kato

Biostudies and society

Due to the rapid progress of biostudies over the last few decades, our society faces many problems in the interface between scientific research and society. First, many areas of biostudies are highly specialized so that it has become difficult for non-specialists to understand current state of research. Second, there are increasing numbers of socially controversial issues, such as cloning, stem cell research, genetically modified food etc. The aim of our research is to analyze these problems through practical and theoretical approaches. We also try to propose possible actions and solutions. Current research topics include 1) communication of biostudies to non-specialists, 2) analysis of ethical and social issues in the areas of biostudies, and 3) modern history of biostudies.

Communication of research to non-specialists

Today, science communication is considered to be one of the most important activities in the interface between biostudies and society. It contains communication among and within various groups in the society such as communities of scientists, private companies, mass media, educational institutes, policymakers, and the general public. We are particularly interested in activities organized by scientists or scientific communities. We organize programs or seminars in which scientists meet non-specialists and explain their research. Then effects of the activities on the participants are analyzed. One example is the “Genome Square” organized by us together with genome scientists in Japan. The event was held in two to three cities every year from 2002 to 2004. It contained poster exhibitions by 30 to 40 laboratories for the general public. We have analyzed the effect of the events on visitors and researchers. One of the findings is that having conversations with members of the general public provides researchers with opportunities to think deeply about aims and meanings of their own research.

Analysis of ethical and social issues

For the balanced development of biostudies, it is important to identify ethical, legal and social issues (ELSI) and find appropriate ways of dealing with them. Our research focuses on the ELSI surrounding recently advanced research fields including genomics, human ES cells and others. In particular, we have been analyzing ethical and social issues arising from International HapMap project. We have also conducted survey of how various countries particularly in Asia are handling ELSI problems and found that many countries in Asia are rapidly developing frameworks for bioethics. Analyses of international projects and situations in other countries would facilitate reflections on the current system in Japan.

Modern history of biostudies

Our third research topic is the analysis of modern history of biostudies. We plan to focus on several research fields

that were important for the development of current biostudies. To carry out the research, we conduct literature surveys and interviews with scientists.

Lectures for graduate students

We organize lectures on biostudies and society for graduate students of the School of Biostudies. Topics of current lecture courses include science communication, bioethics and other issues on social and cultural aspects of biostudies. These lectures are expected to help students to consider issues around their research and take responsible actions.

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Laboratory of Cell Regulation and Molecular Network

Masahiko Sugita (Professor, D.Med.Sci.)

Masahiko Sugita

CD1: A new paradigm for antigen presentation

A salient feature of our immune system is its ability to respond specifically against invading pathogens and cancer cells. The molecular and cellular basis for the antigen-specific immune response appeared fully established by studies carried out for the past two decades, focusing on the organization and the function of major histocompatibility complex (MHC)-encoded molecules. MHC class I and class II molecules bind peptide antigens and present them to specific T lymphocytes. We now know, however, that the universe of antigens recognized by the immune system includes non-peptide lipid antigens, which are presented by CD1, a novel lineage of antigen-presenting molecules. Our recent studies indicate that CD1 mediates MHC-independent pathways for host defense against pathogens and cancer cells, thus encouraging us to develop a new class of lipid-based vaccines.

Dendritic cells and CD1 immunity

Dendritic cells (Figure 1) comprise the major cell population in our body that expresses CD1 molecules. Lipid antigens taken up by dendritic cells are transported to endocytic compartments where CD1 molecules capture antigens. Subsequently, lipid antigen bound CD1 molecules traffic to the plasma membrane and activate lipid antigen-specific T cells. Most of these T cells are $CD8^+$ cytotoxic killer T cells bearing $\alpha\beta$ T-cell receptors, but a fraction of $\gamma\delta$ T cells also responds to lipid antigens presented by CD1 molecules. These CD1-restricted T cells are likely to mediate elimination of microbe-infected cells and cancer cells.

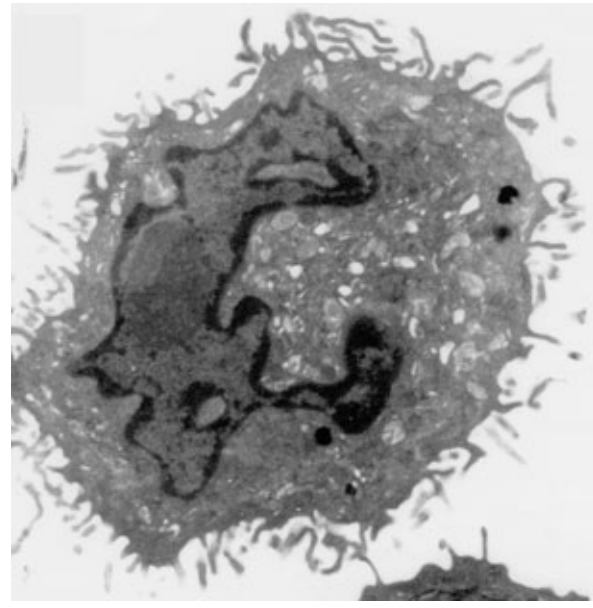

Figure 1
Dendritic cells isolated from human epidermis

Cell biology of CD1

Molecules of the human CD1 family (CD1a, CD1b, CD1c, CD1d) are differentially expressed in endocytic compartments of dendritic cells (Figure 2). CD1b molecules are internalized from the plasma membrane via clathrin-coated

pits and then interact with a cytosolic adaptor protein complex, AP-3, which directs their trafficking to lysosomes. In contrast, CD1a molecules fail to bind AP-3 and follow a recycling pathway of the early endocytic system. The differential distribution of CD1a and CD1b is important for efficient sampling of lipid antigens because lipids with short carbon chains and those with long carbon chains are transported to recycling endosomes and lysosomes, respectively (Figure 3).

Identification of a human genetic disease with impaired CD1 function

Patients with Hermansky-Pudlak syndrome type 2 (HPS-2) carry mutations in the AP-3 genes. In cells derived from HPS-2 patients, CD1b molecules are unable to gain access to lysosomes, resulting in a profound defect in lipid antigen presentation. Since MHC molecules function normally in AP-3 deficient cells, defects in CD1b antigen presentation may account for recurrent bacterial infections in HPS-2 patients.

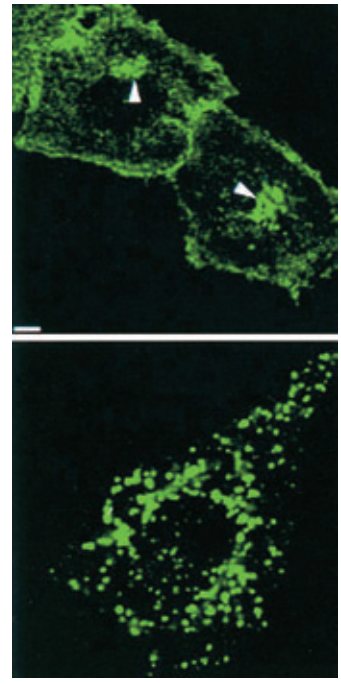

Figure 2
Distinct intracellular localization of human CD1 molecules
CD1a-containing vesicles (upper panel) and CD1b-containing vesicles (lower panel) represent recycling endosomes (arrowheads) and lysosomes, respectively.

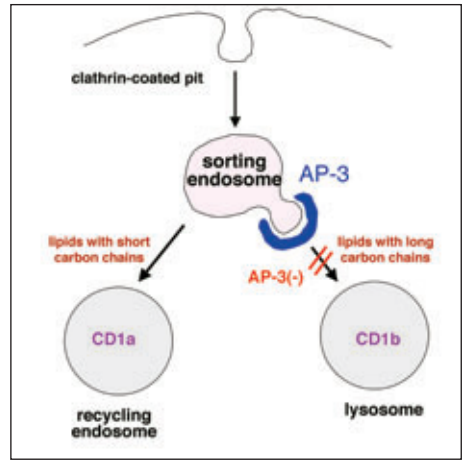

Figure 3
Intracellular trafficking of CD1 molecules and lipid antigens

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Sugita, M. et al (1999). Separate pathways for antigen presentation by CD1 molecules. *Immunity* 11, 743-752

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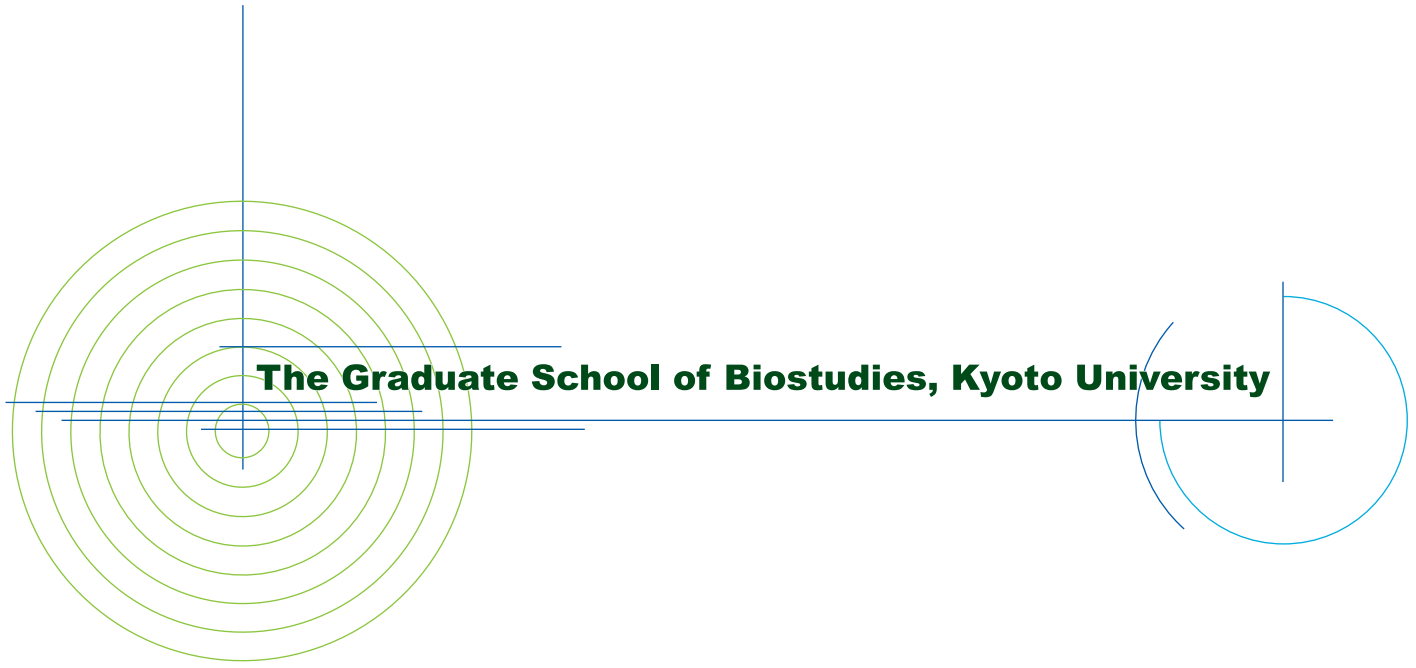
The Graduate School of Biostudies, Kyoto University