Department of Biological Sciences

# Department of Biological Sciences

The Department of Biological Sciences, Graduate School of Science, offers a variety of graduate programs including lectures on a wide range of current biology, research training and advanced seminars focusing on specific topics. The programs are guided by researchers at the Department of Biological Sciences, Institute for Protein Research, Institute of Scientific and Industrial Research, Research Institute of Microbial Diseases, Genome Information Research Center, Frontier Biosciences, and three affiliated institutes outside the university.

The Department of Biological Sciences, Graduate School of Science, was reorganized in 1996 by combining two departments, Physiology and Biochemistry, both of which had been founded in 1953. As a result of reorganization and together with the cooperation of three institutes outside the university, the research conducted at the department covers a wide range of the research fields of current biology and life sciences, which have been rapidly expanding.

When the Department of Biology for undergraduate studies started with three laboratories in 1949, two of them were protein chemistry (Professors Akabori and Okunuki) and one for biophysical cell biology (Professor Kamiya). The department was different from other biology, zoology and/or botany departments at that time. We follow this unique tradition in a modernized way and are always aiming at developing research fields in biology, especially in new "supramolecular biology" which might surpass ordinary molecular biology and ultimately elucidate the life and living organisms at the atomic and supramolecular levels. To pursue this task, we welcome students who have backgrounds other than biology, such as chemistry and physics. Of course, we also welcome students with a solid background in biology. New research fields will be established in this kind of amalgamating atmosphere.

Postgraduate students enrolled in the Master and/or Doctoral programs are requested to conduct their own researches in the laboratories to be conferred with their degrees. The research is supervised by one of the supervisors of the Graduate School. In the doctor course, two advisors in addition to the supervisor will guide your research. For detailed information of research topics, see the following pages and http://www.bio.sci.osaka-u.ac.jp/en



Home Page http://www.bio.sci.osaka-u.ac.jp/en/index.html

#### Department of Biological Sciences Laboratory of Cellular & Structural Biology Members Takahide KON (Professor), Ryosuke YAMAMOTO (Assistant Professor), Hiroshi IMAI (Assistant Professor)

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#### [Research interests]

In the cells that make up our bodies, a wide variety of macromolecules including proteins move quickly at the velocity of several meters per second using thermal energy. However, that is not useful for the long-distance transportation to the specific direction in the cells because the direction of the thermal motion is random. For example, in an elongated neuron with the length of 1 m, it will take more than 100 years to transport an average-sized protein from the cell body to the nerve terminal by the thermal motion. Eukaryotic cells manage this problem by establishing intracellular transport systems that powers a wide variety of fundamental biological processes including ciliary beating, cell division, cell migration and active transport of numerous cargoes. The partial loss of the function has been implicated in neurodegenerative disease, infertility and developmental abnormality. Our laboratory aims to elucidate the molecular mechanism underlying the intracellular transport system by means of atomic-level structural analysis and single-molecule functional

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analysis. Recently, we have focused on a huge motor protein complex, dynein, which is the heart of the transport system toward the center of the cells, and determined its atomic structures. We have also started research projects to achieve a comprehensive understanding of mRNA transport systems in neurons.

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#### Upper panel: Atomic structure of "Dynein", the heart of the transport system. Lower panel: Single-molecule observation of dynein moving along a microtubule track.

Department of Biological Sciences

# Laboratory of Single Molecule Biology

 

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#### [Research interests]

Living cells are complex but well-organized systems comprising various kinds of biomolecules. Because biomolecules operate stochastically under the strong influence of thermal fluctuations, living cells can be referred to as stochastically-operating biomolecular computation systems. Through the dynamic processes in reaction networks of biomolecules, cells can respond flexibly and adaptively to environmental changes. Recent progress in single molecule imaging techniques has made it possible to monitor directly the stochastic behaviors of biomolecules in living cells, in which the locations, movements, turnovers, and complex formations of biomolecules can be detected quantitatively at the single molecule level, providing powerful tools to elucidate molecular mechanisms of intracellular signaling processes. Our laboratory develops quantitative single-molecule imaging methods, computational modeling methods and biochemical synthetic methods to reveal the molecular mechanisms of cellular chemotaxis with singlemolecule resolution.

#### [Research projects]

- 1) Development of automated in-cell single-molecule imaging system (AISIS)
- 2) Single-molecule biology of chemotactic signaling system
- 3) Synthetic biology of chemotactic signaling system



Left: Chemotaxis of *Dictyostelium discoideum* amoebae to cyclic AMP gradients.

Middle: Total internal reflection fluorescence microscopy (TIRFM) for single molecule imaging.

Right: Single molecule imaging of PTEN molecules on the membrane of living cells. Individual white spots represent single molecules of PTEN.



#### [Research Interest]

# Molecular mechanism for genetic and epigenetic inheritances in mammalian cells

The ability of cells to store, retrieve, and translate the genetic information is essential for making and maintaining living organisms. The genetic information of mammalian cells is preserved in the nucleus, in which DNA together with proteins and RNA form a complex called chromatin. Different types of cells in our body are originated from a one cell embryo. Thus, such different types of cells still possess same genetic information, but their cellular identities are determined by each cell-type



specific gene expression. This cell-type specific gene expression is controlled by epigenetic information including DNA methylation, histone post-translational modifications and chromatin structure. These epigenetic information or epigenomes can be changed during differentiation or by environmental factors, but are also maintained and inherited by the next generation if cellular identity is fixed. We are interested in genetic and epigenetic mechanisms to inherit the genetic information and utilize it properly. In addition, we want to understand how cell-type specific epigenome can be switched or maintained through cell division at molecular level. We employ omics approaches using mass spectrometry and next generation sequencer, as well as molecular biological and genetical, biochemical, and cell biological approaches, to elucidate these issues.



Mass spectrometer for proteomic analysis

Next generation sequencer for genomics

Department of Biological Sciences

# Laboratory of Plant Development

 

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### [Research interests]

Plant development relies on coordinated division, differentiation and expansion of cells. In order to understand the underlying mechanisms, we study both inter-cellular communication and cellular events. Plant hormones play pivotal roles in almost all processes of plant development. We have identified biosynthetic enzymes and receptors of cytokinins, and clarified the role of cytokinins in plant development. We are also working on the roles of secretory signaling peptides. We identified several peptides that regulate plant development, including EPF1, which regulate spacing of stomata; EPF2 and stomagen, which regulate epidermal cell number, and CLE9/10 peptide that regulates stomatal number and vascular cell pattern. We are also working on transcription factors that regulate the development of epidermis, vascular cells, and pericycle. We would like to understand regulatory networks, consisting of transcription factors and intercellular signaling molecules, that regulate plant development.

We are also trying to understand how plants modulate regulatory systems for development depending on environments plants are facing.



Figure. The signaling molecule CLE9/10 regulates two different developmental processes, depending on which large protein receptor interacts with the peptide. When it interacts with HSL1 receptor in the leaf, it regulates how many leaf pores (stomata) develop. When it binds to BAM receptor in the root, it regulates the development of the cells that form the water-conducting vessels (xylem).

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# [Research Topics]

We are dissecting cellular processes from the sensing of environmental fluctuations, for example, in light condition,  $CO_2$  concentration, metal ions distribution, to the final physiological responses in plants. Ongoing subjects include intracellular positioning and movement of organelles (we found that chloroplasts, mitochondria, and nuclei in *Arabidopsis* mesophyll cells change their distribution patterns under different light conditions: see Figure), dynamic behavior of cytoskeleton and nucleoskeleton, and circumnutation of stems. Our goal is to disclose the sensing mechanisms, roles of cytoskeletons, and signaling factors, together with biological significance of these intriguing responses.

Furthermore, we recently identified long unrevealed candidate genes for plant nuclear lamina components. The mode of action of these candidates in regulating gene expression via intranuclear chromosome rearrangement is under investigation.



Department of Biological Sciences

# Laboratory of Developmental Biology

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### [Research: Mechanisms of animal embryogenesis ]

We all have developed from fertilized eggs of 100  $\mu$ m in diameter. Have you thought about how it can be possible? Our laboratory is working on mechanisms how eggs develop into a well organized adult body using micromanipulative and molecular approaches.

In animal development, embryonic cells not only proliferate, but also generate various types of cells such as epidermis, muscle, neuron, and blood cells. All of these cells are originally derived from a fertilized egg. What kinds of mechanisms are involved in these processes in which some cells are fated to become muscle and other cells to become neuron? Namely, cellular and molecular mechanisms of cell fate determination during embryogenesis are the theme of our laboratory.

We use embryos of ascidian (sea squirt, *Halocynthia roretzi*) as an experimental material. Ascidian has been regarded as a primitive chordate that evolved to basic vertebrates. Fertilized eggs develop into tadpole larvae within 35 hours of development (Figure). Its embryogenesis has been intensively described in details so that we can predict which cells of the early embryo give rise to which cells of the tadpole larva (Figure, bottom).

Ascidian embryos provide us the unique possibility of understanding various mechanisms of fate determination in every cell type, because the tadpole consists of a small number of cells, and of a few types of tissue. Understanding fate determination mechanisms using this simple model organism with the basic body plan of Chordates would contribute to our knowledge in Developmental Biology.



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# [Research Area]

### 1. Left-right asymmetric development in Drosophila

2. Mechanisms of Notch signaling

The internal organs of many animals show directional left-right (LR) asymmetry. However, mechanisms of LR asymmetric development remain largely unknown in most animals. *Drosophila*, a fruit fly, is a good model organism for studying developmental biology. We aim to understand the mechanisms of LR asymmetric development, such as chirality of cells, using combinations of genetics, computer simulation, and bioimaging.

Development and homeostasis require cell-cell interactions in

multicellular organisms. Notch is a receptor and transduces cellsignal through a direct cell-cell interaction. We are studying cellsignaling through the Notch receptor using *Drosophila* as a model system. We aim to understand the mechanisms of Notch signal transduction and find ways to control the Notch signaling.

# Cell chirality induce LR asymmetry



Department of Biological Sciences

# Laboratory of Comparative Neurobiology

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### [Research Interests]

Animals and plants are organized on a daily and seasonal schedule. By using endogeneous clock system organisms anticipate and prepare for environmental harsh period to change their physiological conditions. We study neuronal mechanisms underlying biological timing system.

#### 1) Photoperiodism and diapause

For seasonal adaptation animals and plants read day length to change their morphs or development. This is called photoperiodism. We study photoperiodic mechanisms using insects (flies, true bugs, a silkworm) and a mollusk. The blowfly *Protophormia terraenovae* develop the ovaries under long days but suppress their development to enter diapause under short days (Figure). We have identified circadian clock neurons involved in photoperiodic response and different types of brain neurosecretory neurons controlling diapause in *P. terraenovae*. However, it remains unknow how the photoperiodic clock system discriminates between short and long days (time measurement) and counts number of days required for switching diapause and nondiapause states (day counting). We investigate into time-measurement and day-counting system in the brain.

#### 2) Circa'bi'dian rhythm

The large black chafer *Holotrichia parallela* have a unique two-day periodicity called circa'bi'dian rhythm. In the field they appear on the ground to forage and mate every two nights. In the laboratory the rhythm continues under constant darkness with a period about 48h. Phase responses of the rhythm to light pulses suggest the circadian clock (ca 24 h) produces circa'bi'dian rhythm (ca 48 h). We propose a novel function of the circadian clock characterized by the release of an output signal every two cycles to produce the 2-day rhythm. We are interested in proximate and ultimate causation of the circabidian rhythm.





### [Research Area]

Mitochondria, double membrane-bound organelles with tubular network structures, are essential for oxidative ATP production and play pivotal roles in regulating calcium homeostasis, ROS production and apoptosis. Mitochondria dynamically change their morphology by frequent fusion and fission, and three types of high molecular-weight GTPase proteins have been identified as core components of the fusion and fission machineries. We are analyzing their molecular mechanism and the physiological roles in mammals, and found that the regulation of mitochondrial dynamics coupled with a quality control system is essential for cellular homeostasis, mtDNA regulation, and tissue differentiation. To analyze mitochondrial dynamics, we characterize purified proteins, observe live imaging, and analyze in vivo phenotypes in mitochondrial-dynamics defective cells/animals.



#### Department of Biological Sciences

# Laboratory of RNA Biofunction

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#### [Research Interests]

Transcriptome analyses in the beginning of the 21st century have revealed that large portions of the eukaryotic genomes produce numerous numbers of non-coding RNAs (ncRNAs), which expectedly play important regulatory roles in various biological events. Our laboratory aims to elucidate the functions and the underlying new genetic code, thereby reconstructs the basic concept of genome function. Among thousands of ncRNAs in mammalian cells, we particularly focus on "architectural ncRNAs (arcRNAs)" that play architectural roles to build membraneless organelle through inducation of intracellular phase separation. We attempt to elucidate the specific biological functions of the arcRNAs that require to form the massive phase separated stretures.





#### [Research Area]

Organisms are quite small, but the most complicated creations in nature. My lab is interested in reading the meaning of animal forms and uses comparative anatomical approaches to understand the morphological evolution and adaptation. We are studying how the animal form had evolved in the life history using the dicyemid mesozoans (Phylum Dicyemida), a unique group of animals that inhabit the renal organ of cephalopod mollusks. The dicyemid body consists of only 20 to 40 cells and represents the smallest number of cells in the animal kingdom. Dicyemids have neither body cavities nor differentiated organs, and were named "Mesozoa" for the dicyemids as an intermediate between Protozoa and Metazoa in body organization. However, some zoologists regard the simple organization of dicyemids to be the result of specialization of parasitism. Recently we have revealed that dicyemids are not truly primitive animals, that deserve the name of "mesozoans", but that they belong to the metazoans. It still remains to be explored how such a simple body organization has evolved. Dicyemids are subjected to a number of selecting pressures due to their unique habitat with the renal organs of cephalopod hosts. In terms of morphological and ecological adaptation, this microenvironment could afford a space for a simple natural experiment.



Department of Biological Sciences

# Laboratory of Interdisciplinary Biology 1

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### [Research Area]

1) Molecular mechanism of photosynthetic light energy conversion (Hirozo OH-OKA)

Photosynthetic light energy conversion system is a key process carried out by pigment-associated protein complexes. In order to understand molecular mechanisms how light energy is converted into chemical energy, we have three research subjects; (a) molecular structure of type 1 reaction center and (b) photosynthetic electron transfer mechanism, and (c) molecular architectures for biological hydrogen productions.



2) Mechanisms of cell's division-plane selection and tissue formation in plants (Tetsuhiro ASADA)

Cell arrangement in plant bodies largely depends on the selection of the plane of cell division. As an effort to understand the mechanisms of controlling the plane of cell division for plant tissue formation, we analyze the tendencies of division plane selection in isolated cells and developing tissues from plants.





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### [Research Area]

1) Theoretical Biology (Koichi FUJIMOTO)

Using physics and mathematics our laboratory tries to understand the underlying mechanisms of biological processes in a wide spectrum ranging from microbes to animals and plants. We study how gene networks regulate animal and plant morphogenesis, and chemical and mechanical communications of cell populations. By combining theoretical prediction derived from computer simulations with quantitative analysis of experimental data, our missions are to uncover the principles for the evolution of gene regulatory network (Top left figure), the robust determination of organ numbers (Bottom left) and the cell mechanics (right).

For example, we found that the plant root tip outlines of many species are best fitted by the catenary curve seen in mechanically stable architectures such as bridges (center).



#### Departmen of

Sciences

# Laboratory of Interdisciplinary Biology 3

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### Initiation of DNA Replication in Eukaryotic Cells – Yumiko KUBOTA–

e-mail

One of most fundamental feature of life is reproduction. All living organisms are made up of a cell or cells and DNA in cell nuclei carries the genetic information of the organisms to construct and maintain them. Therefore, the precise duplication and distribution of DNA to daughter cells during cell division cycle is the basis of keeping an organism alive. The failure of these processes would cause the severe defect in the organisms, such as cell death or serious diseases like cancer. To understand the faithful duplication of DNA, we investigate the basic mechanisms and the regulations of initiation of DNA replication using the cell-free DNA replication system of *Xenopus* egg extracts, which contain plentiful proteins for DNA replication to support the rapid early cell division cycle of embryo.



Replicated DNA visualized by incorporation of labeled nucleotides.

#### Molecular Mechanisms of Chromosome Rearrangements – Takuro NAKAGAWA –

Human genome consists of 3.2 billion base pairs of DNA. DNA is fragile and keeps suffering various kinds of DNA damage including DNA double-strand breaks (DSBs) and stalled replication forks. Faithful repair of these damage is required to maintain genome integrity. When these damages are not properly repaired, gross chromosomal rearrangements (GCRs) such as translocation, truncation, and isochromosome formation take place. Chromosomal abnormality sometimes causes human genetics diseases including cancer. However, it remains unclear how GCRs occur in cells. To gain insights into the mechanism behind these events, we perform genetic studies using fission yeast and human cell lines.



Fluorescent image of the fission yeast Schizosaccharomyces pombe : Localization of the centromere region of chromosomes that consist of DNA repeats.

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### [Research Theme]

Most living things on the earth have the circadian clock (biological clock) to adapt to the daily environmental changes. In mammals, the master circadian clock is located in the suprachiasmatic nucleus (SCN) in the hypothalamus. The master clock synchronize its autonomous oscillation with the environmental changes corresponding with the day-night cycle, and adjusts the internal environment with the external environment by regulation of the peripheral clocks in other brain regions and the body (right figure). We aim to elucidate how various environmental factors including light affect the biological clock at the molecular level. In addition, we are studying the aftereffects of those factors, that is, plasticity of the biological clock.



Department of Biological Sciences

# Laboratory of Organic Biochemistry

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### [Research Interests]

- 1) Synthesis of dinuclear and multinuclear metal complexes activating molecules
- 2) Synthesis of heterometallic multinuclear complexes activating molecules
- 3) Synthesis of novel metal complexes as a photosensitizer
- 4) Development of artificial metalloenzymes activating molecules
- 5) Investigation of relationships between structures and functions of metalloprotein
- 6) Synthetic and mechanistic study on metal complexes with anticancer activity

The photosynthetic energy transduction and metabolism involve photo-excitation coupled with electron transfer process, and activation of substrates including small molecules such as  $O_2$  and  $N_2$ . The 1<sup>st</sup> transition metals are frequently essential trace elements for organisms, working at the active sites in the proteins. The metal complexes sometimes show pharmaceutical activity. We study on metals in biology, and we newly develop relevant metal complexes and metalloenzymes.



VIIII

Nano-sphere

**Bouquet Micelle** 

Vesicle

Flower Necklace

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#### [Research Interests]

The intremolecular attractive force among polymer molecules is necessary to construct polymer assemblies. Different kinds of attractive forces or interactions act among polymer molecules, the solvophobic interaction, electrostatic interaction, hydrogen bonding, and so on. Furthermore, the atomic groups, or functional groups, producing the attractive forces are arranged on the polymer chain in different ways. The variety of those polymer chemical structures provides a number of morphologies of polymer assemblies, as shown in the right scheme. Our laboratory aims at (a) synthesizing novel polymers forming unique assemblies, (b) characterizing the structure of polymer assemblies, (c) understanding the mechanism of the assembly formation, and (d) establishing the relationship between the structures and properties of polymer assemblies. Our final goal is to understand the structures and properties of assemblies constructed by both synthetic and biological macromolecules in a unified way.

The followings are the research themes which we are studying:

- 1) Polymer micelles formed by amphiphilic polymers.
- 2) Polymer complexes formed by amphiphilic polymers and various substances.
- 3) Polyion complexes formed by oppositely charged polyelectrolytes.
- 4) Conformation and molecular recognition of polysaccharides.

Department of Biological Sciences

# Laboratory of Macromolecular Structure

Star Micelle Cylindrical Micelle

**Flower Micelle** 

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#### [Research Area]

Biological process is driven by complex molecular machines composed of biological macromolecules. The flagellum, which is an organelle for bacterial motility, is one of those molecular machines. The flagellum is a huge protein assembly composed of a helical filamentous screw, a molecular universal joint, a highly efficient iondriven motor and a protein export apparatus for self-construction. The Laboratory of Macromolecular Structure aims to provide fundamental understanding of operating principles and the selfassembly mechanism of biological macromolecular machines, such as the bacterial flagellum, through the studies of molecular structure at atomic resolution and reconstitution of the molecular machines. We also study structures of polymer complexes with low molecular weight compounds and the relationship between their structures and functions.

- 1) Rotation mechanism of bacterial motor systems.
- 2) Self-constitution mechanism of bacterial motility machines.
- Structural and functional studies on bacterial infection apparatus.

- 4) Structural and functional studies on environmental sensing units.
- 5) Study on the structure of polymer complex with small molecule and its formation mechanism.



Schematic drawing of the bacterial hook basal-body architecture

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#### [Research Interests]

- 1) Development of a high performance sensing element.
- 2) Construction of energy conversion and catalytic systems using hybrids of biomacromolecules with synthetic molecules.
- 3) Creation of functionalized supramolecular materials based on self-assembly of bio-related and/or synthetic polymers.

In biological systems, life processes are led by the unique behavior of macromolecules such as proteins and DNA. Molecular recognition by macromolecules plays an important role, for example, in substrate specificity of enzymes and antigen-antibody reactions in human life. Selective molecular recognition among macromolecules is achieved through a large number of weak interactions. We have focused our attention on the special behavior of antibodies, especially monoclonal antibodies, because they can recognize a larger and more complex compound with high specificity. In this laboratory, we develop novel supramolecular materials and construct functionalized sensing, catalytic, and energy conversion systems via specific molecular recognition of biomacromolecules and selective assembly of bio/synthetic molecules.



# Laboratory for Molecular and Developmental Biology (Institute for Protein Research)

 

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[Research Interests]

Our laboratory studies molecular mechanisms underlying the development and function of the vertebrate central nervous system(CNS) using various research methods of molecular biology, mouse genetics, biochemistry, cell biology and neural physiology. We use the retina as a model system to understand how DNA encodes programs to generate various neurons and glial cells, form precise neuronal circuits, and enable complicated neuronal function. We also focus on how abnormality of biological processes in development and maturation leads to human diseases. We are eager to contribute to development of diagnosis and cure of human diseases. Together, our lab aims to elucidate mechanisms and principles underlying the CNS development from DNA programs to physiological function and human diseases.

#### [Research Project]

1) Molecular analysis of synapse formation in the CNS.

2) Elucidation of functional roles of microRNAs (miRNAs) in CNS development.

3) Analysis of molecular mechanisms underlying transcription and chromatin regulation in neuronal differentiation.



Figure. We previously identified Pikachurin, an extracellular matrix–like retinal protein, and observed that it localized to the synaptic cleft in the photoreceptor ribbon synapse. Pikachurin KO mice showed improper apposition of the bipolar cell dendritic tips to the photoreceptor ribbon synapses, resulting in alterations in synaptic signal transmission and visual function. WT (left), Pikachurin KO (right)

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#### [Research Programs]

- (1) Metabolism and function of dipeptides in mammals
- (2) Proteomic analysis of protein and peptide breakdown

In living cells, there are numerous short peptides such as dipeptides and tripeptides, some of which are produced by protein degradation, while the others synthesized from amino acids by specific enzymatic reactions. Regardless of their route of production, short peptides are essential to protein metabolism and its homeostasis. We have been studying the function and metabolism of short peptides, especially focusing on dipeptide-hydrolyzing enzymes or dipeptidases, CN1 and CN2.

We have also been trying to develop proteomic procedures to analyze protein degradation, and apply them to study disease-induced changes in protein degradation products



# Image: Anomalic Structure Laboratory for Molecular Biophysics (Institute for Protein Research) Image: Members Toshimichi FUJIWARA (Professor), Yoh MATSUKI (Associate Professor), Yohei MIYANOIRI (Associate Professor), concurrent)

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### [Research Interests]

Signal transduction and energy conversion play very important roles in the human body. Many these functions are performed by supramolecular systems across biomembranes. These systems are also responsible for forming networks of integrated biological activities. We are elucidating these essential functions of proteins on the basis of structures and the interactions revealed mainly by nuclear magnetic resonance spectroscopy (NMR).

Tel • Fax

NMR reveals structure and function of biologically important molecular complexes that not amenable to X-ray crystallography and electron microscopy. These systems include proteins bound to lipid bilayers and noncrystalline large molecular complexes, such as membrane protein pHtrII for the transmission of light signal, halorhodopsin for lightdriven ion pumping, amyloid protein fibers, and model Gprotein-receptor complexes. We are also developing NMR methods by using advanced technologies for NMR experiments, bioinformatics and molecular biology. Two NMR spectrometers features high-power terahertz wave sources, gyrotron for enhancing the sensitivity by using electron spin polarization.

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### [Research Interests]

In plants and algae, the eukaryotes, photosynthesis is carried out in a specialized organelle called chloroplast. It is now widely accepted that virtually all chloroplasts in today's photosynthetic eukaryotes derive from one fairly rare primary endosymbiotic event with a cyanobacterium-like ancestor thought to have occurred more than a billion years ago. Extant higher plants can synthesize only ~100 proteins inside the chloroplast but must import such 2000-3000 different cytosolically-synthesized nuclear-encoded proteins, across the double envelope membranes surrounding this organelle, to fulfill their complex physiological roles including photosynthetic functions. Two successive protein translocons at the outer and inner envelope membranes, termed TOC and TIC, respectively, are responsible for the task of protein import into chloroplasts. Our recent discoveries of the genuine TIC translocon (SCIENCE 2013) and associated import motor (PLANT CELL 2018) both of which are well conserved among most land plants as well as green algae could provide us an entirely revised view on the molecular mechanisms of protein translocation across the inner envelope membrane of chloroplasts and also novel insights on the evolution of the chloroplast protein import system.



#### Department of Biological Sciences

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Home Page http://www.protein.osaka-u.ac.jp/rcsfp/synthesis/index.html

#### [Research Interests]

- 1) Elucidation of mammalian signal transduction mechanism via structural study of ligand-receptor complexes
- 2) Structure-based design of "novel therapeutic proteins" through state-of-the-art protein/antibody engineering
- 3) Near-atomic resolution structural analysis of biologically important macromolecules by using cryoelectron microscopy (cryo-EM)

Cellular response to the extracellular environment depends on the "sensing" the extracellular cues by use of the receptor-ligand system. Binding of ligands to the extracellular domain of the receptors transduce signals into cells that initiates various cellular events, ultimately changing the cell fate. Most of the "signal transduction researches" deal with cytoplasmic events such as phosphorylation/dephosphorylation of signaling molecules and subsequent recruitment of adapter molecules, but mechanism for the "signal transmission across the membrane", the very first step in the signaling pathway is poorly understood.

Our study focuses on questions such as how receptors recognize their specific ligands, how this recognition leads to structural change in the receptor complex, and how the information cross the plasma membrane without transporting chemical entity. Using structural as well as chemical approach, we would tackle on this difficult problem to obtain insights into the mechanism of transmembrane signaling. Such information would eventually be used for drug development and benefit medical as well as biological research in general.



Crystal structure of human Wnt3 (left) and cryo-EM structure of LRP6 (center), combined to give hypothetical structure of the entire signaling machinery on cell surface (right).

Department of Biological Sciences	Laboratory for Computational Biology (Institute for Protein Research)	
	Members Kenji MIZUGUCHI (Professor), Kosuke HASHIMOTO (Associate Professor), Chioko NAGAO (Assistant Professor)	
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	Home Page https://mizuguchilab.org/	
[Research Inte	rests]	
1) Data integration higher-order biolo	For relating molecular-level events to gical systems.	

- 2) Understanding and predicting molecular interactions involving proteins, and modelling biological responses
- 3) Transcriptome analysis of early human embryos

We aim to increase our understanding of biological systems and diseases by combining computer science and computational biology approaches, with applications to drug discovery and other research areas. Artificial Intelligence (AI) is expected to play major roles in many domains. Recognizing that the availability of a large amount of data in a computer-friendly format is key to the successful development of AI models, our research is focused on integrating a wide array of data, including genes, proteins, chemical compounds and diseases. We also develop methods for predicting protein structure, function and interaction, and apply them to specific biological problems.



Department of Biological Sciences

# Laboratory of Supramolecular Crystallography (Institute for Protein Research)

Members Atsushi NAKAGAWA (Professor), Mamoru SUZUKI (Associate Professor), Eiki YAMASHITA (Associate Professor), Makoto MATSUDA (Specially Appointed Assistant Professor) Home Page http://www.protein.osaka-u.ac.jp/physical/

#### [Current Research Projects]

- 1) X-ray structure determination of biological macromolecular assemblies and proteins.
- Development methodologies for X-ray structure determination of biological macromolecular assemblies using synchrotron radiation and X-ray free electron laser.
- 3) Development of data processing algorithm of diffraction data from micro-crystals.

Macromolecule assemblies, consisting of proteins, nucleic acids, and other substances, play key roles in all living system. Our laboratory works on structure determination of biological macromolecular assemblies using X-ray diffraction technique. Development of tools for X-ray crystallography of biological macromolecular assemblies, including the synchrotron radiation beamline at SPring-8, is also one of our main works.



# Laboratory of Protein Profiling and Functional Proteomics (Institute for Protein Research)

Members Toshifumi TAKAO (Professor), Toshiki TAKEI (Assistant Professor), Qiuyi WANG (Specially Appointed Assistant Professor)

Home Page http://www.protein.osaka-u.ac.jp/rcsfp/ profiling/

#### [Research Programs]

- 1) Development of chemical/analytical methods and software for analysis of protein primary structure by mass spectrometry
- 2) Mass spectrometric analysis of post-translational modifications
- 3) Development of chemical and analytical methods for proteomics
- Study on fragmentation of peptides and carbohydrates in mass spectrometry
- 5) Hardware development for high-sensitivity and high-accuracy mass spectrometry

Mass spectrometry (MS) is a well-accepted technique for the analysis of chemical structures of biological compounds. For the last four decades, we have been working to develop methods for determining primary structures and post-translational modifications of proteins using MS. In conjunction with accumulating protein and gene sequence databases, we are using state-of-the-art MS for largescale protein identification, which is indispensable for understanding biological events. We apply the following developed methods to the structural analysis of micro quantities of peptides, proteins, and their related substances.



MALDI-TOFMS can reveal the peptide C-terminus.

#### Department of Biological Sciences

# Laboratory of Genome and Chromosome Functions (Institute for Protein Research)

embers Akira SHINOHARA (Professor), Asako FURUKOHRI (Associate Professor), Masaru ITO (Assistant professor), Yurika FUJITA (Assistant professor)

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#### [Research Area]

#### [Research Subjects]

1) In vivo and in vitro analysis of recombination reactions

- 2) Analysis of proteins working with RecA homologues in recombination
- 3) Analysis of the roles of chromatin modification in meiotic recombination
- 4) Mechanisms of choice of DSB repair pathways
- 5) Analysis of recombination in human cells
- 6) Mechanism of chromosome dynamics in mouse meiosis

Homologous recombination, an exchange between DNA strands, plays a role in the maintenance of genome stability and the production of genome diversity. While, in mitosis, it is required for the repair of DNA damage, it is for the segregation of homologous chromosome at meiotic division I. Meiotic recombination is coupled with chromosome of the recombination leads cancer and infertility in human. To reveal molecular mechanism of the recombination, we have been analyzing genes/proteins involved in the process using molecular, genetical, biochemical, and structural methods.



Synaptonemal complex (SC) formation. Immuno-staining analysis of the SC components, Zip1 (red) and Red1 (green) in the budding yeast. In SCs, paternal and maternal chromosomes are fully paired along chromosomes. Blue shows DNA, thus chromosomes.



#### [Research Interests]

- 1) Structural studies of photosynthetic energy-transducing membrane protein complex and related redox enzymes
- 2) Crystal structure analyses of dynein motor
- 3) Damage-free crystal structure analysis of metalloproteins at high resolution

Three-dimensional protein structure brings us a deeper insight into the biological function. X-ray crystallography combined with Cryo-TEM is the best method to determine atomic coordinates of protein molecules. The main aim of our group is the structure determination of the biological macromolecular assemblies including membrane protein complexes, in order to elucidate the molecular mechanism of the highly organizes biological process at atomic level.



Crystal Structure of the dynein motor domain

#### Department of Biological Sciences

# Laboratory of Protein Organic Chemistry (Institute for Protein Research)

 Members
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#### [Research interests]

- 1) Establishment of a method for protein synthesis
- 2) Chemical synthesis of glycoprotein, modified histone, and membrane protein

Chemical methods enable the synthesis of proteins, which can not be prepared by the recombinant method, such as site-specifically labeled, glycosylated and phosphorylated proteins. Our laboratory is aiming to promote new protein researches using these synthetic proteins. Thus, our laboratory is developing facile methods for protein synthesis based on ligation chemistries. In addition, the synthetic method is applied for the preparation of membrane proteins and their partial sequence to elucidate the signal transduction mechanism by solid state NMR and IR. Modified histones and their partial sequences, glycosylated proteins are also synthesized for the functional analyses.



General procedure for the chemical protein synthesis.

#### Department of Biological Sciences Laboratory for Advanced Brain Functions (Institute for Protein Research) Members Takatoshi HIKIDA (Professor), Tom MACPHERSON (Assistant Professor), Takaaki OZAWA (Assistant Professor) TEL FAX TEL 06-6879-8621 /FAX 06-6879-8623 e-mail hikida@protein.

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### [Research Interests]

Our laboratory studies neural circuit mechanisms underlying various advanced brain functions such as cognitive learning and decision making behaviors using molecular techniques for neural circuit specific manipulation. We use several mouse models to reveal molecular pathologies of neuropsychiatric diseases. Especially, we focus on molecular mechanisms of gene-environment interaction in the pathogenesis of mental disorders. We also promote translational research for targeting mental disorders in collaboration with clinical departments and pharmaceutical companies.

#### [Research Programs]

- 1. Analysis of neural circuit mechanisms in advanced brain functions
- 2. Molecular analysis of neuropsychiatric pathologies
- 3. Translational research of mental disorders



Department of Biological Sciences

# Laboratory of CryoEM Structural Biology (Institute for protein research)

embers Takayuki KATO(Professor), Jun-ichi KISHIKAWA(Assistant Professor), Hiroko TAKAZAKI(Assistant Professor)

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Page http://www.protein.Osaka-u.ac.jp/cryoem/index.html

### [Research Interests]

- (1) Study of energy conversion mechanism of molecular motor
- (2) Structural analysis of olfactory receptors
- (3) Study of molecular dynamics by cryo-electron microscope
- (4) Development of high-resolution structural analysis method
- by cryo-electron microscope

The biomolecules such as protein and nucleotide are responsible for life activity, its function is relative to the structure. In our laboratory, we will clarify the molecular mechanism by structural analysis using a cryo-EM. In particular, we are analyzing the energy conversion mechanism of motor protein such as flagellar motors and ATPase, and the mechanism of olfactory receptors. We are also developing of the method for analysis of molecular dynamics by cryo-EM and for high-resolution and high-resolution structural analysis by cryo-EM.



Structure of Flagella Hook



Cryo-EMs

### Department of Biological Sciences Laboratory for Cell Systems (Institute for Protein Research) Members Mariko OKADA (Professor), Keita IIDA (Assistant Professor), Ayaka ICHIKAWA(Assistant Professor), Sho TABATA (Specially Appointed Associate Professor), Ulrike MÜNZNER (Specially Appointed Assistant Professor), Suxiang ZHANG (Specially Appointed Researcher) TEL: FAX TEL: 06-6879-8617 • FAX:06-6879-8619

Home Page http://www.protein.osaka-u.ac.jp/cell\_systems/index.html

# [Research Theme] Understanding the dynamics of cells as time and space network of molecules

Cells carry out various molecular controls, and determine their own fate corresponding to the living environment. Upon the procedure, characteristics of cells occur not only by the property of molecule itself but also by the property of intercellular dynamics. In our laboratory, we quantitatively analyze interaction network of intracellular molecules such as protein, RNA and DNA in order to reveal the mechanism of intracellular process and cell fate control. We particularly focus on the regulation of gene expression dynamics by intracellular signal transduction and transcription factor. We thoroughly analyze the molecular mechanisms by combination of quantitative experimental analysis of molecular activity against input of the cell, mathematical modeling and simulation analysis. We also study data driven cell systems to understand how the original stimulus influences the whole cell and activate self-control by using the comprehensive measurement techniques such as genome, transcriptome, epigenome and proteome.

Studying time development process of cells in proliferation and differentiation, we want to understand the logic structure to induce cellular specificity by focusing on solving the signal transduction and transcription responses. Our laboratory conducts wet experiments along with dry approaches such as computational science, mathematics and bioinformatics.



Department of Biological Sciences

# Laboratory of Nanobiology (Institute for Protein Research)

bers Yoshie HARADA (Professor), Madoka SUZUKI (Associate Professor), Shingo SOTOMA (Assistant Professor)

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[Research Interests]

# Development of intracellular heat production (temperature) measurement technology

Heat is a type of energy, and the temperature that changes with heat is one of the basic parameters that express the state of matter. However, it is not clear how heat and the resulting temperature change can alter cell function. We developed various methods for measuring the heat production and temperature changes that occur within a single cell by using temperature-sensitive fluorescent polymers, fluorescent dyes, fluorescent nanoparticles, fluorescent nanodiamonds, combine various fluorescence imaging techniques such as fluorescence lifetime microscopy and ratio measurement to generate heat within a single cell. These new measurement methods aim to elucidate the significance and universality of local heat production and temperature changes in cells that affect cell functions and higher-order life phenomena constituted by them. We are also conducting joint research on applications in the biomedical field, such as evaluation at the cell level.



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Intracellular local temperature measurement image using fluorescence lifetime imaging microscope. The difference in color represents the difference in temperature. There was a large temperature difference inside the cell.



### [Research Themes]

1) Insect's feeding preference and speciation: Molecular mechanism of the host plant selection of swallowtail butterflies.

2) Vertebrate body patterning: How does the well-patterned morphology is generated in terms of ontogeny and phylogeny?

3) Interaction and evolution between insects and plants: Evolutionary and ecological study of the fig and fig wasp mutualism.

4) Evolution of developmental programs and cell structure/function: Experimental study using the *Drosophila* and spider models.5) Science communication and production: Presenting and sharing

biological research among many people.

BRH performs the studies on evolution and development of organisms, and the study of how to spread science throughout society. The abovementioned themes are carried out in five research groups. The staffs and other BRH members including Dr. Keiko Nakamura (Director General), Dr. Shin-ichi Nishikawa (Advisor) and researchers work together in supervising graduate students.



Department of Biological Sciences

# Laboratory of Oncogene Research (Research Institute for Microbial Diseases)

 

 Members
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### [Research Projects]

- 1) Studies on the roles of proto-oncogene products in the development of multicellular animals.
- Studies on the roles of Src family kinases in the metastasis and/or invasion of cancer.

The primary focus of this department is to understand the functions and regulatory mechanisms of proto-oncogene products, which play crucial roles in the cell signaling pathways involved in the development and differentiation of animal cells. Understanding the critical functions of these proto-oncogenes would provide insights into the molecular basis of normal cell development as well as oncogenesis, which can be considered as an aberrant form of differentiation. Presently, we are focusing on the proto-oncogenes encoding protein tyrosine kinases, particularly the Src family of tyrosine kinases (SFK). SFK is known to be involved in regulating cell-cell and cell-substrate adhesion and cell migration. Malignant cancer role of SFK in the progression of cancer metastasis. To elucidate the principal functions of SFK and to search for new molecules that can be targeted by drugs to clock

SFK-mediated cancer progression, we are currently engaged in the above projects.



Potential roles of Src and its regulator Csk in the regulation of metastasis and invasion of cancers. In various human cancers, it is known that the kinase activity of Src is elevated. The activation of Src leads to disruption of cell-cell interaction, enhancements of substrate adhesion and cell mobility, and increased secretions of MMPs and cytokines. As a consequence of these events, the metastatic and invasive actions of cancer cells are greatly promoted.



#### [Research Interests]

Most cancers originate from epithelial cells that normally form tight cell-cell adhesion. Accumulating gene mutations in epithelial cells promote their malignant progression, expanding their territory from the original epithelial tissue to the surrounding tissues and finally metastasizing to other organs via blood vessels. While a number of oncogenes and anti-oncogenes that are involved in the regulation of cell proliferation and survival have been identified, the molecular mechanisms governing the phenotypical changes of cancer cells in the threedimensional space, such as cancer invasion and metastasis that accompany the dynamic change of the tissue architecture, remain to be determined. How do the cancer cells escape from the tightly-connected epithelial tissue where they arise? How do the cancer cells expand their territory by invading into the surrounding tissue? To tackle and solve these important questions in cancer biology, we are investigating the molecular mechanism of cancer progression using mice and mammalian cultured cells.



In the intestine of mice that spontaneously form multiple polyps, gene disruption of CNNM4 stimulates malignant progression of the tumor cells in the epithelial tissue layer to cancers that have invaded into the muscle tissue layer (arrows in the right panel).

Department of Biological Sciences

# Laboratory of Homeostatic Regulation (Research Institute for Microbial Diseases)

lembers Tohru ISHITANI (Professor), Yuki AKIEDA (Assistant Professor), Masayuki OGINUMA (Assistant Professor),

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### [Research Interests]

- 1. A new concept of tissue homeostasis "Morphostasis"
- 2. Aging programs and their regulation
- 3. Robustness supporting signal transduction systems
- 4. Molecular basis underlying "Diapause", the systems suspending vital activities

In our body, cells recognize their position and role and behave accordingly via cell-cell communication. Such behavior supports tissue morphogenesis and homeostasis, while its dysregulation is involved in congenital malformation, cancer, and aging. We focus especially on the cell-cell communication and behavior supporting tissue homeostasis and explore unknown molecular systems controlling embryonic development, organogenesis, regeneration, aging, and disease, using in vivo imaging, animal model genetics, molecular and cell biology, and biochemistry techniques.



Molecular and cellular imaging



#### Department of Biological Sciences

# Laboratory of Biomolecular Science and Reaction

(The institute of Scientific and Industrial Research)

Members Shun'ichi KURODA (Professor), Toshihide OKAJIMA (Associate Professor), Yoh WADA (Associate Professor), Kenji TATEMATSU (Assistant Professor), Masaharu SOMIYA (Assistant Professor)

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### [Research Interests]

The aims of this laboratory are the analysis of intermolecular reactions found in various biological phenomena, and the development of bio-industrially useful technologies by utilizing these reactions.

1. *In vivo* pinpoint DDS nanocarriers using virus infection machinery.

2. Single cell analysis by using automated single cell analysis and isolation system.

3. Olfactory receptor repertory analysis by using human OR cell array.

4. Elucidation of biogenesis of novel built-in type cofactors.

5. Structural and functional analysis of bacterial twocomponent system.



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Malignant tumor-specific DDS nanocarrier utilizing sugar-lectin interactions.

(Left, high malignancy; Right, low malignancy)

Department of Biological Sciences Laboratory of Biomolecular Informatics (RIKEN Center for Biosystems Dynamics Research)

 Members
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### [Research Interests]

#### 1) Mechanisms of blood vessel formation (Phng).

We investigate molecular and mechanical mechanisms of how endothelial cells shape blood vessels. We employ the zebrafish embryo as a model system, and perform highresolution fluorescent live imaging, genetic engineering, chemical and optical perturbations with numerical simulations to understand how endothelial cells behave during vessel morphogenesis (Fig. 1) and respond to haemodynamics during development and homeostasis.



Fig 1: Actin cytoskeleton in a zebrafish vascular network.

### 2) The understanding and regulation of morphogenmediated patterning (Inomata).

Developmental processes take place through the exchange of information by cells within the constrained spatial environment of the embryo. In our research we will seek to gain a understanding into process of pattern formation via morphogen gradient. Further, we are also working to develop methods for regulating the morphogen distribution in vivo. By using such methods, we plan to gain a deeper understanding of developmental systems (Fig.2).



Fig 2: Spatiotemporal regulation of morphogen distribution

# Department of Biological Sciences Laboratory of Nuclear Dynamics Members Yasushi HIRAOKA (Professor), Tokuko HARAGUCHI (Professor) TEL·FAX Tel: 06-6879-4620, Fax: 06-6879-4622 Home Page http://www.fbs.osaka-u.ac.jp/labs/hiraoka/

# [Research Subjects]

### (1) Chromosome

Chromosome is a complex structure in which DNA binds to proteins such as histones. We are examining dynamic organization of chromosome structure and its function during the progression of meiosis.

### (2) Nuclear envelope

Nuclear envelope is important for maintain chromosome functions. We are examining molecular and structural bases for nuclear envelope dynamics during the progression of mitosis using mammalian cells and fission yeast.

### (3) Nuclear pore complex

The nuclear pore complex is a structure responsible for transport between the nucleus and the cytoplasm. We are examining the relationship between nuclear pore complex and nuclear function using fission yeast.

### (4) Development of imaging technology

We have developed a method of CLEM (correlative light-electron microscopy) combined with live cell imaging, called "Live CLEM", to observe the molecular dynamics in living cells at high-resolution.





Chromosome structure in meiosis

Nuclear pore complex