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 new research fields in biology, especially in "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
Laboratory of Cell Motility

Members
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[Research interests or Research Area]
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 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 a project to achieve a comprehensive understanding of mRNA transport systems in neurons.

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

Laboratory of Single Molecule Biology
(Graduate School of Frontier Biosciences)

Members
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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 single-molecule resolution.

[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.
### Laboratory of Genome Structure and Function

**Research interests**

Molecular mechanism of genetic and epigenetic inheritances

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.

![DNA carrying genetic information associates with proteins including histones and non-coding RNAs to form chromatin.](image1)

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### Laboratory of Plant Development

**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. So far we have discovered key enzymes for biosynthesis of cytokinins and receptors of cytokinins. We are also studying intracellular membrane trafficking system, which regulates plant development through regulation of auxin transporters. We are also searching for novel inter-cellular signaling molecules, and have identified several molecules that regulate plant development (Figure). Transcription factors play key roles in cell-type specification. We are searching for key transcription factors that govern cell-type identities, and examining the regulation of such transcription factors. Another interest of us is how plants adapt themselves to environments by regulating developmental programs.

![Facilities for comprehensive omics analyses;](image2)

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Figure. An example of intercellular signaling molecules that regulate cell-positioning. The peptide mediator EPF1 is produced in cells that are specified for stomatal cells, and prevent neighboring cells from being specified for stomatal cells, ensuring spacing between stomata (Green GFP signals in the developing leaf epidermis indicates EPF1 expression).
Laboratory of Plant Cell Biology

Members
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[Research interests or Research Area]
Plant cellular responses to environmental fluctuations

Plants respond to environmental fluctuations very sensitively and quite tactfully to regulate their life cycle for survival. Such behavior provides us two different types of questions; HOW plants behave in such an efficient way, and WHY plants have to behave in such an efficient way. Both types of questions strongly stimulate our research mind. We are interested in various aspects of plant behavior and aiming to get deeper understanding for such behavior based on our own individually posed questions.

We are dissecting cellular processes from the sensing of environmental fluctuations, for example, in light conditions, CO₂ concentration, mechanical stress, to the final physiological phenomena. On-going 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: Figure), dynamic behavior of cytoskeleton, 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.

Chloroplasts (green), mitochondria (red), and nuclei (blue) in Arabidopsis mesophyll cells change their distribution patterns when exposed to blue light of different intensity.

Laboratory of Developmental Biology

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Project: Mechanisms of animal embryogenesis

We all have developed from fertilized eggs of 100 μm 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 experimental materials. Ascidian has been regarded as a primitive chordate that evolved to basic vertebrates. Fertilized eggs develop into tadpole larvae within 55 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.
### Laboratory of Cell Biology

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**[Research Area]**

**Left-right asymmetric development in *Drosophila***

The internal organs of many animals show directional left-right (LR) asymmetry. For example, directional LR asymmetry is found in various internal organs of human. The mechanisms of LR asymmetric development are evolutionarily diverged among species. Except for some of vertebrates, these mechanisms remain largely unknown in most animals.

*Drosophila melanogaster*, a fruit fly, is a good model organism for studying developmental biology. The mechanisms of LR asymmetric development in *Drosophila melanogaster* are largely unknown and different from those found in vertebrates. Thus, we aim to find out such novel mechanisms responsible for the formation of the LR axis in the body of *Drosophila* and for the LR asymmetric morphogenesis of its organs, using combinations of genetics, computer simulation, and mechanobiology. Figure shows the LR asymmetric structures of the embryonic gut in *Drosophila melanogaster*.

2. The mechanisms of Notch signaling

Development and homeostasis require cell-cell interactions in multicellular organisms. Organized behavior of cells relies on such cell-cell communications. Although recent studies have revealed molecular basis of such cell-cell interactions, there are still many unsolved problems. Transmembrane receptors interact with signaling molecules at cell-surface. These receptor proteins receive and transduced cell-signals.

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

### Laboratory of Comparative Neurobiology

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**[Research interests or Research Area]**

How do the brain and nervous system encode temporal signals such as days and hours in biological timing system? It is suggested that animals use the circadian clock to measure day length and to count the number of days in photoperiodism. We study biological timing mechanisms using circadian clock system in invertebrates.

1) Photoperiodism and diapause

We keep flies, bugs and snails in the laboratory for years. These animals show photoperiodic response at a constant temperature. The blow fly *Protophormia terraenovae* develop their ovaries under long day conditions but arrest ovarian development under short day conditions to enter diapause. We have found two distinct groups of brain neurosecretory cells controlling diapause and identified circadian clock neurons involved in the photoperiodism. However, it still remains unsolved in any species how day length is measured and the number of days is counted to change diapause and nondiapause program. We study these mechanisms focusing on circadian clock neurons and the neurosecretory cells.

2) Circadian rhythm

The large black chafer *Holotrichia parallela* has a unique two-days rhythm called circadian rhythm. They emerge on the ground every 2 nights foraging and copulation. We are interested in proximate causation and ultimate causation of the circadian rhythm.
Laboratory of Interdisciplinary Biology 1

Members
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[Research interests or 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) Biology of dicyemid mesozoans (Hidetaka FURUYA)
Dicyemid mesozoans (Phylum Dicyemida) inhabit the kidney of cephalopod mollusks. The dicyemid body consists of only 20 to 40 cells and represents the smallest number of cells in the animal kingdom. We pursue a synthetic biology of dicyemids, which includes systematics, phylogeny, development, ultrastructure, coevolution with cephalopod hosts.

3) Developmental mechanisms of neural crest cells (Kazuo ITO)
We study developmental mechanisms of neural crest cells of which migration and differentiation are attributable to characterization of the vertebrate body plan. We analyze their developmental mechanisms by using the mouse as a model system and the lamprey, the most primitive living vertebrate, from the viewpoints of molecular developmental biology and evolutionary developmental biology.

4) Pattern formation in plant development (Tetsuhiro ASADA)
Tissue and organ developments in plants are accompanied by pattern formation. We are asking the mechanisms of developmental pattern formations in plants across scales, as well as their origins and significances. Current focus is on the patterning of cell arrangement, and study uses an original single cell system for identifying the most basic information for cell division plane selection.

Laboratory of Interdisciplinary Biology 2

Members
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[Research interests or Research Area]
1) Function of neural circuit (Kotaro KIMURA)
Our research focuses on the function of the nervous system of a model animal, the nematode Caenorhabditis elegans. There are a number of advantages to using the C. elegans nervous system for functional analyses, including the small size of the entire nervous system of the organism (only 302 neurons), the description of all neuronal connections, and its various types of behavioral plasticity. In addition, many sophisticated genetic and optical techniques are available to analyze and manipulate the animals' neural functions (Upper figures).

2) Theoretical Biology (Koichi FUJIMOTO)
Using physics, mathematics and bioinformatics, our laboratory tries to understand the underlying mechanisms of biological processes in a wide spectrum ranging from microbes to animals and plants. Dr. Fujimoto studies how gene networks regulate animal and plant morphogenesis, and chemical and mechanical communications of cell populations. By computer simulations of mathematical models consistent with molecular genetics and bioimaging, our missions are to uncover the principles for the evolution of gene regulatory network (Bottom left figure), the collective decision making of cells (Bottom center), and the robust determination of organ numbers (Bottom right).
1) Initiation of DNA Replication in Eukaryotic Cells (Yumiko KUBOTA)

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.

2) Molecular Mechanisms of Chromosome Rearrangements (Takuro NAKAGAWA)

Human genome consists of 3.2 billion base pairs of DNA present on 22 autosomes plus sex chromosomes. 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 the damage is 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. Homologous recombination (HR) and Non-homologous end-joining (NHEJ) are the major pathways to cure DSBs and replication problems. However, it remains unclear how GCRs occur in cells. To gain insights into the mechanism, we currently perform genetic studies using the fission yeast S. pombe (Figure) and human cell lines.

Figure: Replicated DNA visualized by incorporation of labeled nucleotides.

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1) Chemical synthesis of oligosaccharides
2) Chemical synthesis of glycoproteins and glycopeptides
3) Elucidation of oligosaccharide functions

The oligosaccharides of protein have been thought to concern with protein conformation, dynamics, protein trafficking and glycoprotein lifetime in blood. We have examined synthesis of homogeneous glycoproteins having human complex type oligosaccharide in order to evaluate oligosaccharide functions. We have synthesized several small glycoproteins (amino acids 40-76 residues), erythropoietin analogue (amino acids 166 residues), and co-stimulate glycoprotein of T-cell (amino acids 120 residues). In order to synthesize these glycoproteins, the polypeptide sequence of target glycoprotein were divided into several segments and these were synthesized by solid phase peptide synthesis. After prepared both glycopeptide-thioester and peptide, these were coupled by repetitive Native Chemical Ligation (NCL). After construction of the glycosylated polypeptide chain, we examined folding experiments and evaluated effect of oligosaccharide during protein folding process. In addition, glycoproteins folded was analyzed its structure by NMR and CD spectra in order to evaluate conformational differences between glycosylated and nonglycosylated proteins. In our laboratory, we would like to elucidate oligosaccharide functions by use of such chemical approach.
Laboratory of Polymer Assemblies  
(Department of Macromolecular)  

**Members**  
Takahiro SATO (Professor), Ken TERAO (Associate Professor)  
[Home Page](http://osku.jp/k072)

Polymers often form aggregates in solution and polymer aggregates play important roles in industries of foods, cosmetics, paints, and pharmaceutics, as well as in living cells where proteins and nucleic acids associate and dissociate during biochemical reactions. Polymer aggregates, or polymer assemblies, usually take very complex conformations and their characterization is not easy. Our group aims at understanding the structures and properties of polymer assemblies through the following projects.

**[Research Projects]**  
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  
5) Molecular conformation and liquid crystallinity of cyclic and branched polymers

Laboratory of Macromolecular Structure  
(Department of Macromolecular)  

**Members**  
Katsumi IMADA (Professor), Fumitoshi KANEKO (Associate Professor), Tatsuya KAWAGUCHI (Assistant Professor)  
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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 ion-driven motor and a protein export apparatus for self-construction. The Laboratory of Macromolecular Structure aims to proved fundamental understanding of operating principles and the self-assembly 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.  
4) Structural and functional studies on environmental sensing units of bacteria.  
5) Study on the structure of polymer complex with small molecular and its formation mechanism.

A plausible model for rotational switching in bacterial flagella.
Laboratory of Supramolecular Functional Chemistry  
(Department of Macromolecular)

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**[Research Projects]**

1) Preparation of functionalized supramolecular complexes using host molecules such as cyclodextrins or monoclonal antibodies.
2) Creation of stimuli-responsive and self-healing materials based on self-assembly of bio-related and/or synthetic molecules.
3) Development of a high performance sensing element.
4) Construction of energy conversion and catalytic systems using hybrids of biomacromolecules with synthetic molecules.

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 molecular recognition events of host molecules such as cyclodextrins and monoclonal antibodies. In this laboratory, we create unique supramolecular complexes or novel materials through molecular recognition of these molecules or hybridization of bio-related macromolecules with synthetic molecules. Functionalized sensing, catalytic, and energy conversion systems are also constructed.

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

**Members**  
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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 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).
Laboratory of Proteins Involved in Homeostatic Integration  
(Institute for Protein Research)

[Members]  
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[Research Projects]
1) Molecular mechanism of circadian rhythms.
2) Autonomic regulation of homeostasis.
3) Signal transduction in neuronal cells.

In mammals, the master circadian clock is located in the suprachiasmatic nucleus of the hypothalamus. We have been searching for molecules involved in generation of the circadian clock and its synchronization to the environment, and studying their functions in the circadian clock and the homeostatic integration. In addition, we are studying molecular functions of signal transduction molecules such as protein kinases and NO synthases in these neuronal systems.

Laboratory of Molecular Biophysics  
(Institute for Protein Research)

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[Research interests]
Signal transduction and energy conversion play very important roles in the human body. Many of these functions are performed by supramolecular systems across biomembranes. These systems are also responsible for forming networks for integrated biological activities. Reports on structures of these systems increase rapidly in number recently. We are elucidating these important functions of proteins on the basis of structures revealed by NMR (Nuclear Magnetic Resonance).

NMR elucidates structure and function of biologically important molecular complexes that are not amenable to X-ray crystallography and solution NMR. These systems include proteins tightly bound to lipid bilayers and noncrystalline large molecular complexes. For example, we study membrane protein pHiII for the transmission of light signal, transmembrane domains of proton ATP synthase, and model G-protein-receptor complexes. We are also developing NMR methods by using advanced technologies for NMR experiments, molecular biology and bioinformatics.
Laboratory of Organelle Biology
(Institute for Protein Research)

[Research interests or Research Area]
Molecular studies on chloroplast biogenesis
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. In the course of evolution, massive transfer of genes from the endosymbiont to the host’s nuclear genome occurred, accompanied with the development of the protein transport system that allows these nuclear-encoded chloroplast proteins back into the endosymbiont. 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 discovery of the genuine TIC translocon published in Science in 2013 provides an entirely revised view on the molecular mechanisms of protein translocation across the inner envelope membrane of chloroplasts and also novel insights into the evolution of the chloroplast protein import system. We are keen to hear from undergraduate or graduate students who wish to do research in our laboratory!

Laboratory of Protein Synthesis and Expression
(Institute for Protein Research)

[Research Interests]
1) Structural determination of extracellular proteins
2) Biochemical/biophysical analysis of receptor-ligand interactions
3) Ultrastructural analysis of proteins using electron microscopy/electron tomography
4) Development of high-quality recombinant protein expression system

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.
**Laboratory of Protein Folding**

*Institute for Protein Research*

**Members**
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[Current Research Programs]
1) Observation of folding processes and clarification of the mechanism of protein folding.
2) Analysis of structural stability and dynamics of protein molecules.
3) Analysis of structural stability and the mechanism of formation of amyloid fibrils.

Protein folding is a process in which an extended polypeptide chain acquires a unique folded conformation with biological activity. However, the exact molecular mechanism remains unknown. Clarifying the mechanism of protein folding is essential to improve our understanding of the structure and function of proteins. It is also important to design engineered proteins with improved functions.

Moreover, protein folding plays important roles in many biological phenomena. For example, the deposition of amyloid fibrils has been suggested to play a central role in over 20 degenerative disorders including Alzheimer's and prion diseases. Because the amyloid fibril deposition is often caused by misfolding of an originally functional protein, these diseases are called “folding disease”. In order to establish therapeutic treatments, clarifying the molecular mechanism of folding diseases is essential.

We are studying the conformational stability of proteins, molecular basis of folding reaction, and structures and formation of amyloid fibrils. These studies are performed using various observation methods, including spectroscopies (NMR, CD, IR), physicochemical methods (calorimetry, ultracentrifugation), and fluorescence microscopy, as well as gene manipulations for recombinant proteins by using the *E.coli* and yeast expression systems.

![Fig. An image of amyloid fibrils of amyloid-β peptide obtained using total internal reflection fluorescence microscopy.](attachment:image1)

**Laboratory of Supramolecular Crystallography**

*Institute for Protein Research*

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[Research Area]
1) X-ray structure determination of macromolecular assemblies and proteins
2) Development of 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 and X-ray Fee-Electron Laser, is also one of our main works.
[Research Programs]
1) Development of chemical/analytical methods and softwares for analyses of protein primary structures.
2) Hardware development for high-sensitivity MS.
3) MS analysis of post-translational modifications.
4) Development of a chemical derivatization method for high sensitive detection of sugar chains of glycoproteins.
5) Development of chemical and separation methods for proteomic analysis.
6) Study on fragmentation of peptides and carbohydrates in MS.

Mass spectrometry (MS) is a well accepted technique for the analyses of chemical structures of biological compounds. We have been working to develop methods for determining primary structures and post-translational modifications of proteins by using MS. In conjunction with accumulating protein and gene sequence databases, we are using state-of-the-art MS for large-scale protein identification which is indispensable for proteomics research. We also apply the above developed methods to the structural analysis of micro quantities of peptides, proteins, and their related substances.

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.
Laboratory for Protein Informatics

(Institute for Protein Research)

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**Home Page**

[Research Interests]
1) Bioinformatics studies focused on protein structures and protein-protein interactions.
2) Development of new algorithms and softwares for large scale simulation calculations by parallel computers and GPU clusters to examine free energy landscape of biomolecular systems for structure and energetic analysis and their prediction.

Our laboratory constructs and manages the international protein structural database (PDB), and develops the advanced database, as PDB Japan (PDBj). The aim of our laboratory is to elucidate the relationship between structures and functions of biological macromolecules, and mutual interactions by molecular simulation and structural bioinformatics.

Composite Structural Motifs of Binding Sites for Delineating Biological Functions of Proteins (C). They are defined by integrating the elementary motifs (A) associated with individual subunits having binding sites for ligands including small molecules, proteins and nucleic acids. The binding site atoms that constitute the elementary motif are shown in ball-and-stick representation with CPK coloring and ligands are shown in green wireframes (non-polymers) or tubes (proteins). These 3 composite motifs share the same elementary motif for FAD binding (labeled N2 in B). (Kinjo & Nakamura (2012) PLoS One 7, e31437)

Laboratory of Protein Crystallography

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[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 is the best method to determine atomic coordinates of protein molecules. The main aim of our group is the X-ray structure determination of the biological macromolecular assemblies including membrane protein complexes, in order to elucidate the molecular mechanism of the highly organized biological processes at atomic level.

Crystal Structure of the dynein motor domain
Laboratory of Protein Organic Chemistry

(Institute for Protein Research)

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[Research interests or Research Area]
1) Establishment of a method for protein synthesis
2) Chemical synthesis of glycoprotein, modified histone, and membrane protein
3) Elucidation of structure and function of a single transmembrane receptor

Chemical methods enable the synthesis of proteins, which cannot be prepared by the recombinant method, such as site-specifically labeled, glycosylated and phosphorylated proteins. Laboratory of Protein Organic Chemistry 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 sequences to elucidate the signal transduction mechanism by solid state NMR and IR. Modified histones and their partial sequences, glycosylated proteins are also being synthesized for the functional analyses.

Laboratory of Nuclear Network

(Institute for Protein Research)

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[Research Interests]

Elucidation of the functions of chromosome ends

Chromosomes contain genetic information and regulate the bases of biological activities. Deletion or duplication of chromosomal regions results in cell death, tumorigenesis, or serious diseases. Therefore, it is important not only for the elucidation of principals of life but also for the clarification of the mechanisms of human diseases to study chromosomal functions. Telomere, which exists at the end of a linear chromosome, plays important roles in chromosome integrity. Recent studies have revealed that telomere regulates cell senescence and life span, and that it is important for meiosis and preservation of species. We are studying the molecular mechanism of the functions of telomere-binding protein complexes using genetics, molecular biology, biochemistry and cell biology.

Subtelomere is a telomere-adjacent chromosomal domain. The knowledge about telomere has recently accumulated, but the functions of subtelomere are largely unknown. However, it is thought that subtelomere is important for human health because minute deletion or duplication of subtelomere DNA causes human severe diseases. Furthermore, the structure of human subtelomere is remarkably different from those of big apes, such as chimpanzee, bonobo and gorilla, suggesting the possibility that subtelomere plays some role in evolution. We are analyzing the structure and functions of the subtelomeres in fission yeast, humans and apes.
Laboratory of Membrane Protein Chemistry  
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[Research Interests]

Intracellular membrane fusion is a fundamental and conserved biological reaction which is vital for vesicle trafficking between cellular compartments, organelle morphology, hormone secretion, and neurotransmission. Fusion is regulated by specific proteins and lipids: SNAREs, SNARE chaperones, Rab GTPases, and phosphoinositides. However, it is unclear how they act together to drive membrane fusion. We have been studying this vital membrane fusion machinery in eukaryotic cells and recently developed reconstituting proteoliposomal fusion with purified components. Our current projects attempt to dissect the ternary synergy of two SNARE chaperone systems and phosphoinositides which is essential for physiological fusion through catalyzing the SNARE complex assembly and reorienting the assembled SNARE complexes. In our future directions, we will further explore the molecular machinery of not only membrane fusion but membrane fission/budding and deformation, by this powerful system of reconstituting proteoliposomes with defined components.

Figure: Yeast vacuoles as a model for intracellular membrane fusion. Yeast vacuoles (lysosomes in mammals) change their organelle morphology through membrane fusion (from C to A) and fission (from A to C) processes to respond to the extracellular environments and/or cell cycles.

Laboratory of Cell Systems  
(Institute for Protein Research)

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[Project]

Understanding of the cell as dynamic molecular networks

Mammalian signal transduction pathways transfer a variety of extracellular information to transcription factors in the nucleus to regulate gene expression and cell fate. Signaling pathways often control these processes in a nonlinear manner through spatio-temporal regulation of molecular networks. The aims of the laboratory are to define the general regulatory logics in signal transduction-transcriptional networks to apply this knowledge of regulatory principles to the understanding and treatment of human diseases. For this purpose, we perform quantitative measurements of the target biological system using genome, transcriptome, epigenome, and proteome (so-called Omics) approaches and integrate these heterogeneous data by means of mathematical modeling and computer simulation. Particularly, we focus on the time-course process on signal-dependent early transcriptional regulation in proliferation or differentiation of cancer and immune cells. Our laboratory uses both wet-lab (cell biology, molecular biology, biochemistry) and dry-lab approaches (bioinformatics, computation, mathematics) to solve this question.

Computer simulation (right 4 graphs) and experimental validation (left 4 graphs) of NF-kB signaling pathway in B cell.

By changing the parameter (simulation) and the sequence of the protein (experiment) (↑ up-regulation, ↓ down-regulation), dynamics of nuclear NF-kB activity is changed (Oscillation behaviors; 1st and 3rd rows, threshold response; 2nd and 4th rows).
[Research interests]
What make you different from others: the molecular mechanism of gene expression and intra-cellular temperature change.

Inside the cells, many kinds of bio-molecules are involved in the biological process. Although the basic design, the genome, is almost identical, the tiny gene expression differences in the level, the timing and the spatial area make us different from other 7.4 billions of peoples. To understand the molecular mechanism underlying these differentiations, we have developed new technologies, especially in the field of biological imaging. Currently, we focused on the single molecule imaging of gene expression at molecular and super-complex layer, and revealed some secrets of the gene expression control. For higher biological layer, the cell layer, we recently developed a super-resolution imaging technique for intracellular temperature, which allowed us to detect unexpected temperature difference in the nucleus and the cytoplasm, leading us to open the door of thermal biology.

[Projects]
We are imaging biological process at three different biological layers: the molecule, the super-complex and the cell.

1) Analysis of interaction between biomolecules using zero-mode waveguides (ZMWs)
2) Reconstitution of transcription super-complex using DNA origami
3) Imaging of intracellular temperature

Figure : Local temperature measurements

Using fluorescence lifetime imaging microscopy (color differences represent temperature difference). There was a large temperature difference inside the cell.

Laboratory for Advanced Brain Functions
(Institute for Protein Research)

[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

Figure. We use cognitive learning tasks for mice to reveal molecular and circuit mechanisms in various advanced brain functions and the pathophysiology of neuropsychiatric disorders (Ref. Morita et al., Mol Neuropsychiatry 2: 124-132, 2016).
In the intestine of mice that spontaneously form multiple polyps, gene disruption of CNNM4 stimulates malignant progression of the polyp cells in the epithelial tissue layer to cancers that have invaded into the muscle tissue layer (arrowheads in the right panel).

Disorganization of epithelial tissue architecture during cancer progression

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 three-dimensional 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 experimental animals, such as mice, and mammalian culture cells.

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 cells often have elevated SFK activity, suggesting the potential 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 block 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.
Cancer Biology

(Research Institute for Microbial Diseases)

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[Research interests or Research Area]
Understanding the molecular mechanisms linking aging and cancer

Cellular senescence is the state of irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli and has therefore long been considered to suppress tumorigenesis, acting as a guardian of homeostasis. Emerging evidence, however, reveals that senescent cells also promote secretion of various inflammatory and pro-proliferative factors. This newly identified senescence-associated phenotype termed SASP is likely to be associated with homeostatic disorders including cancer. It is therefore quite possible that accumulation of senescent cells during aging or obesity in vivo may contribute to aging- and/or obesity-associated cancers. By conducting the following studies, we aim to clarify the molecular mechanisms underlying aging- and/or obesity-associated cancer.

Laboratory of Biomolecular Science and Reaction
(The Institute of Scientific and Industrial Research)

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[Research interests or Research Area]
Analysis and Application of Biomolecular Reactions

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. In particular, we develop an in vivo pinpoint DDS (drug delivery system) nanocarrier (bio-nanocapsule) by mimicking the function of viruses, single cell-related technologies by utilizing an automated single cell analysis and picking up machine, an oriented immobilization technology for various biomolecules, and a bio-missile for selective degradation of pathogenic proteins in vivo. And, the active-site structures and catalytic mechanisms of various enzymes are being investigated by site-directed mutagenesis, various spectroscopies, and X-ray crystallography. Furthermore, we are conducting structural and functional analysis of bacterial two-component systems, which are involved in biofilm formation, pathogenicity, and drug resistance, to develop novel antibiotics against bacterial signal transduction.

[Current Research Programs]
1) In vivo pinpoint DDS nanocarriers (Bio-nanocapsule)
2) Single cell-related technologies by utilizing an automated single cell analysis and picking up machine
3) Oriented immobilization technology for various biomolecules
4) Bio-missile for in vivo selective degradation of pathogenic proteins
5) Mechanisms of biogenesis of novel built-in type cofactors
6) Structural and functional analysis of bacterial two-component system aiming at novel drug development

Pharmacokinetics of malignant tumor-specific DDS nanocarrier utilizing sugar-lectin interactions. (Left, high malignancy; Right, low malignancy)
Laboratory of Biohistory

(JT Biohistory Research Hall [BRH])

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[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) Phylogeny and evolution: (i) molecular phylogeny and evolution of arthropods; (ii) co-evolution and co-speciation between figs and fig wasps.
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 studies on the evolution and development of organisms, and the study of how to spread science throughout society. The above-mentioned themes are carried out in seven research groups. The staff and other BRH members including Dr. Keiko Nakamura (Director General) and researchers work together in supervising graduate students.

Laboratory of Cellular Structure and Function
(Advanced ICT Research Institute)

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[Research Interests]
This laboratory is studying functional organization of the cell nucleus using mammalian and fission yeast cells. Toward this end, we have developed the computer-controlled microscope system that is capable of recording living fluorescently-stained cells.

[Research Subjects]
1) Chromosome organization in fission yeast meiosis
We have found that telomeres and centromeres greatly change their nuclear positions upon entering meiosis. This phenomenon has been confirmed in a wide variety of eukaryotes from yeasts to humans. We are now trying to understand molecular mechanisms for such nuclear reorganization during meiosis.
2) Dynamic organization of the nuclear structures in mammalian cells
We are trying to understand how the cell nucleus is organized to achieve their functions. The nucleus provides a physical framework for gene expression. We are examining molecular and structural bases for nuclear functions with a special interest in chromosome dynamics, chromosome-nuclear membrane interactions and in nuclear membrane dynamics during the progression through mitosis.
3) Dynamic organization of the nuclear structures in Tetrahymena
The ciliated protozoa have two functionally and structurally distinct nuclei in a single cell. A somatic macronucleus (MAC) is transcriptionally active while a germ line micronucleus (MIC) is inert during vegetative growth. We are examining molecular and structural bases of such “nuclear dimorphism”.

82 Graduate School of Science
Laboratory of Biomolecular Informatics  
(RIKEN Center for Developmental Biology)

[Research interests]
1) The Mechanism of Chromosome Segregation in Oocytes (Kitajima).
   Chromosome segregation in the oocyte, which forms an egg through meiosis, is thought to be achieved by mechanisms different from those of other types of cell division. We recently reported the first complete chromosome tracking throughout meiosis by high resolution imaging of live mouse oocytes. We take advantage of this cutting-edge technique of live imaging and mouse genetics, to reveal the specialized molecular mechanism underlying chromosome segregation in mammalian oocytes. (Fig.1)

2) The Understanding and Reconstruction of Developmental System (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 "reconstructing" and "controlling" the morphogen gradient in vivo. By using such methods, we hope to gain a deeper understanding of developmental systems. (Fig.2)