Functional Analysis of Newly-found Actin Structure Involved in Chloroplast Photorelocation Movement

Masamitsu Wada
(Kyushu University, Faculty of Sciences, Professor)

【Outline of survey】
Chloroplasts move towards weak light to absorb more light to perform efficient photosynthesis but escape from strong light to avoid photodamage of chloroplasts. Arabidopsis plants deficient in the avoidance response cannot survive under direct sunlight in mid summer. Light condition, such as fluence rate and wavelength changes frequently according to the circumstances where plants live, so that to make full use of light is the first priority matter for plant life. Plants use light as information to monitor environmental conditions as well as energy source for photosynthesis. For that purposes chloroplasts monitor the light environment through blue light receptor, phototropin family proteins. Recently we found an actin fine structure that involves on chloroplast photorelocation movement. Our study will focus on the function of the actin structure for chloroplast movement.

【Expected results】
Actin filaments involve not only in muscle function in animals but also in various physiological phenomena such as organelle movement both in animal and plant cells. Two types of actin-dependent organelle movement are reported so far, one is dependent on molecular motor myosin and the other is depend on actin-network system mediated by ARP2/3 proteins. The actin structure that we found in chloroplast movement is the third category of actin-dependent systems. Our study will be able to clarify the mechanism of chloroplast movement, and, moreover, add a new concept on actin function how successfully organisms have evolved and developed the actin-dependent organelle movement.

【References by the principal investigator】

【Term of project】 FY2008–2012

【Budget allocation】 159,800,000 yen (direct cost)

【Homepage address】 None
Functional Diversity of Visual Pigments and Photoreceptor cells

Yoshinori Shichida
(Kyoto University, Graduate School of Science, Professor)

[Outline of survey]
Photoreceptor cells of animals contain visual pigments that absorb light and start a light-induced signaling cascade. The functional diversification of photoreceptor cells is thought to originate from the evolution and diversification of the various functional proteins involved in this signaling cascade. Several studies have been carried out to elucidate the molecular properties of the functional proteins and their relationships with the photoreceptor cell responses. However, *in vitro* analyses of functional proteins may sometimes cause these proteins to behave in a manner that is not directly related to physiological cell responses. Therefore, further research efforts should focus on elucidating what are the molecular properties of functional proteins that originate various cellular responses. In this study, by using knock-in mice exogenously expressing a visual pigment, we will identify the amino acids that give rise to the different molecular properties of visual pigments and functional diversity of photoreceptor cells. This approach will bring light to the mechanism of molecular diversification, that in turn gives rise to the diversification of cells and individual organisms. Our approaches will bring a new perspective into the filed of biodiversity research.

[Expected results]
Light signals from the outer environment are important for many living organisms, and the relationship of diversification of animal behavior and of their habitats have been examined through the analysis of photoreceptor function of various animals. In this study we use visual pigments of photoreceptor cells to test the relationship between functional modifications of proteins and functional diversification of cells and individuals by experimental means. This type of research may open a new field in molecular physiology on the basis of functional diversification of organisms and evolution.

[References by the principal investigator]

【Term of project】FY2008–2012

【Budget allocation】159,800,000 yen (direct cost)

【Homepage address】http://photo1.biophys.kyoto-u.ac.jp/
Structural basis for molecular mechanisms of substrate recognition and transport regulation by membrane transporters

Osamu Nureki
(The University of Tokyo, Institute of Medical Science, Professor)

【Outline of survey】
Membrane transporters maintain the intracellular circumstances by strictly regulating the import and export of metal ions, sugars, metabolites and drugs etc. To elucidate at an atomic resolution (A) how the transporters drive their transport, (B) how the transporters exclusively select their specific substrates and (C) how the transporters regulate their transporting activities, we will perform (1) structure determination by X-ray crystallography, (2) dynamic property analysis by MD simulation and (3) in vivo and in vitro complementary experiments, focusing on ion transporters of magnesium, iron and heavy metals, temperature-sensing cation channels, sugar transporters and multidrug transporters. Originality of this project is to uncover the essential molecular mechanism of membrane transport by comparing and integrating the functional mechanisms of transporters specific for various kinds of targeted solutes. Since Nobel prizes were awarded for investigations on aquaporin and potassium channel, more and more structural analyses of membrane transporters have been reported. In this project, we will first elucidate the three main unresolved mechanisms (A)-(C) by the above strategies (1)-(3).

【Expected results】
Plasma membrane defines the cellular boundary to maintain the distinct intra-cellular circumstances, which is essential for life. Transporters embedded in the membrane create the distinct cellular circumstances by regulating the transport of various substances. Therefore, structural and functional investigations of membrane transporters may elucidate the fundamental mechanisms of maintenance of life. Furthermore, since dysfunctions of transporter proteins are related to cardiac, renal, gastrointestinal and cranial nerve diseases, achievements of this research project may lead to medical application such as structure-based drug design (SBDD), in addition to the scientific significance.

【References by the principal investigator】

【Term of project】 FY2008−2012
【Budget allocation】 159,900,000 yen (direct cost)

【Homepage address】 http://www.x-ray.bio.titech.ac.jp/
Structure and dynamics of actin filament complex:  
mechanism of calcium regulation of muscle contraction

Yuichiro Maeda  
(Nagoya University, Graduate School of Science, Professor)

[Outline of survey]

The actin filament complex, consisting of actin, tropomyosin and troponin, plays the major roles in muscle contraction and its regulation. Transient increase of intracellular Ca\(^{2+}\) concentration induces the Ca\(^{2+}\) binding to troponin, whose signal is transferred through tropomyosin to polymerized actin, initiating the force generation (the Ca\(^{2+}\) regulation). We have so far elucidated for the first time the atomic structures of constituting proteins, troponin and tropomyosin. In this project, the atomic structure of the entire actin filament complex, as well as the structural dynamics should be elucidated, in order to elucidate the mechanism of the Ca\(^{2+}\) regulation. What are challenging in this project are, that the structure of the complex as large as 1MDa should be elucidated, and that the path should be found and established to go from the atomic structure to the structural dynamics, then further to our understanding the mechanism. Particularly, using troponin with cardiomyopathy-causing mutations, the relationship between the abnormalities of structural dynamics and the functional aberrations should be elucidated. This must be the path leading to our understanding the mechanism of calcium regulation.

[Expected results]

In this project, we are going to take innovative approaches which may be generally applicable in the biological study. First, we should construct not-naturally-occurring mini-actin filament complex of a uniform length. This is our effort to know the nature by analyzing objects which are artificially constructed, conferring the first step towards “engineering life”. Second, our project also includes developing novel procedures of analyzing structures of elongated protein complexes, which has remained under-developed in the structural biology. Third, we should establish what to measure for our understanding the structural dynamics, and find the path from the structural dynamics to our understanding the mechanism. Finally, we should shed lights on the cause of disease, based on our knowledge on the structural dynamics of the mutated protein. This may lead us to propose novel concept of structural dynamics-based drug design.

[References by the principal investigator]


【Term of project】 FY2008—2012  
【Budget allocation】 158,200,000 yen (direct cost)  
【Homepage address】 None
Dynamics of intrinsically disordered proteins and their functional roles

Yoshifumi Nishimura
(Yokohama City University, International Graduate School of Arts and Sciences, Professor)

Outline of survey
In this project we will examine dynamics of intrinsically disordered proteins by using NMR and reveal their functions based on the dynamics. In eukaryotes many nuclear proteins are intrinsically disordered in their free states and upon binding to their targets, the interacting region of each protein will be folded. Especially dynamics of chromatin-related proteins, histone proteins in a nucleosome core, transcription activators, transcription repressors, and general transcription factors will be investigated in their free and target-bound states to reveal the common role of intrinsically disordered structures in transcription. For example we have already established static tertiary structures of chromodomains of chromatin remodeling factors, Chd1 and Esa1, DNA-binding domains of two telomeric proteins, hTRF1 and hTRF2 in their free and DNA-bound states, a neural restrictive silencing transcription factor, REST bound to its corepressor, mSin3, a transcription activator, ATF2, and a complex between general transcription factors, TFIIE and TFIH by using NMR.

Expected results
Eukaryotic transcription factors, containing intrinsically disordered structures, regulate specific gene expression. Although classical proteins holding a specific tertiary structure interact with their targets by a simple key and lock model or an induced fit model, intrinsically disordered proteins interact with their targets by a coupled and folding mechanism. Recently some transcription factors are found to be essential for inducing iPS cell. It is very important to reveal dynamics and interacting modes of transcription factors for designing rationally iPS or ES cell. In addition histone modifications which are related to epigenetics should be revealed histone dynamics. Based on our study the basic phenomena of cell division and the maintaining mechanism of iPS or ES cell will be revealed.

References by the principal investigator

Term of project FY2008—2012

Budget allocation 138,000,000 yen (direct cost)

Homepage address http://www.tsurumi.yokohama-cu.ac.jp/stbiol/index.html
Molecular mechanism and regulation of assembly and remodeling of proteins

Hiroyuki Araki
(National Institute of Genetics, Department of Cell Genetics, Professor)

[Outline of survey]
Most biological reactions occur when many factors assemble on a specific region at a specific time period and express their functions. In chromosomal DNA replication, many proteins including DNA polymerases assemble on replication origins and then they are remodeled to start DNA synthesis for elongating DNA strands in a cell-cycle dependent manner. However, the molecular mechanism governing this process has not been well elucidated. In this project, we analyze chromosomal DNA replication in budding yeast as a model system, focusing on cell-cycle dependent protein assembly on replication origins and subsequent remodeling of these proteins. We purify the proteins assembling on replication origins and reconstitute an assembly in vitro. We further characterize how assembled proteins are remodeled for transition from the initiation step to the elongation step by in vivo analyses as well as reconstituted in vitro assays.

[Expected results]
We expect a break-through achievement for DNA replication research; the molecular mechanism of initiation in eukaryotic DNA replication will be revealed in this project. This is a big advancement and an epoch-making hallmark in molecular biology. We characterize protein-assembly on replication origins, which is regulated by a cell-cycle main-engine, CDK. Thus, the result leads to understanding of the cell-cycle regulation, which is often disordered in cancers and diseases. We hope that the results we will obtain in this project give clues to future characterization of cancers and genetic diseases.

[References by the principal researcher]

【Term of project】FY2008−2012
【Budget allocation】153,700,000 yen (direct cost)

【Homepage address】http://www.nig.ac.jp/section/araki/araki-e.html
Molecular Networks for the Regulation of Cell Polarization in Migrating Cells and Neurons

Kozo Kaibuchi
(Nagoya University, Graduate School of Medicine, Professor)

**Outline of survey**
In response to extracellular and intracellular signals, cell exhibits a polarized morphology with adhering neighboring cells and extracellular matrix. Cell polarization is a fundamental process that makes cells enable to exert specific physiological roles in tissues. A migrating cell has front-rear polarity for directional and persistent migration, and a neuron is highly polarized and comprised of two structurally and functionally distinct parts, an axon and dendrites. The molecular mechanisms by which cell polarization is regulated remain largely unknown. The purpose of our research is to clarify the signaling networks for the cell polarity formation and maintenance in migrating cells and neurons. Our study also aims to reveal the regulatory mechanisms of the cytoskeleton and adhesion, and a selective protein and vesicular transports involved in the cell polarization. We have been studying the Rho family small GTPases, Par complex and CRMP-2. Our research interests are focused on mode of actions of these molecules on the cell polarization.

**Expected results**
It is a fundamental issue in cell biology, developmental biology, and neuroscience to understand the control mechanisms of the cell polarization in migrating cells and neurons. Our study will provides us with whole pictures of the molecular mechanisms of the cell polarity formation, maintenance, and a selective protein and vesicular transports. We hope that our research on molecular regulatory mechanisms for cell morphogenesis sheds light on the therapeutic approaches of inflammation, atherosclerotic disease, nephritis, and psychiatric and neurological disorders.

**References by the principal investigator**

**Term of project** FY2008-2012
**Budget allocation** 150,000,000 yen (direct cost)

**Homepage address** [http://www.med.nagoya-u.ac.jp/Yakuri/](http://www.med.nagoya-u.ac.jp/Yakuri/)
Physiological substances and functions of ABC proteins involved in lipid transport

Kazumitsu Ueda
(Kyoto University, Institute for Integrated Cell-Material Sciences (iCeMS), Professor)

【Outline of survey】

Lipids, such as cholesterol, are essential components of the body. However, their aberrant accumulation due to an excess intake, etc., causes fatal disorders such as atherosclerotic vascular lesions. Dietary lipids are absorbed from the small intestine, transported throughout the entire body via the liver, and play important roles. Recently, many members of ABC proteins, an ATP-dependent transporter family, were revealed to be involved in lipid circulation in the body and play important roles in lipid homeostasis. However, despite extensive studies, their functions and mechanisms of regulation remain unclear due to difficulties in studying large membrane proteins.

We have been intensively investigating ABC proteins for about 20 years after we identified MDR1, the first of the ABC protein genes in eukaryotes. In this project, we will reveal the physiological substances and functions of physiologically important ABC proteins on the basis of our achievements in biochemical studies on them.

【Expected results】

The functional defects of 48 human ABC proteins can lead to a variety of pathological conditions, including cardiovascular diseases, diabetes, senile blindness, respiratory failure of infants, and skin diseases. Our research on ABC proteins will contribute to human health by exploring the cause of such diseases and finding ways to prevent them. The identification of food-related factors and chemicals affecting the functions and regulation of ABC proteins will be useful to maintain lipid homeostasis and prevent disease.

【References by the principal investigator】

* ABC proteins (edited by Ueda, K), Japan Scientific Societies Press, 2005

【Term of project】 FY2008—2012
【Budget allocation】 123,900,000 yen (direct cost)

【Homepage address】 http://www.biochemistry.kais.kyoto-u.ac.jp/
Studies on lipid peroxidation in human disease: its modulation from the view point of food chemistry

Teruo Miyazawa Ph. D.
(Tohoku University, Graduate School of Agricultural Science, Professor)

[Outline of survey]
Lipid hydroperoxide (LOOH) formation is well known in oxidative deterioration of edible food oils during storage and cooking. Miyazawa has considered that membrane lipid peroxidation in human body may be involved in cellular damage and senescence, life-style related disease, as well as in age-related disorders, and has carried out a series of fundamental investigations collaborating with clinical research groups.

Up to now Miyazawa et al have developed the CL-HPLC method and LC-MS/MS method for the selective and sensitive determination of LOOH present in human plasma and organ tissues, together with establishment of the synthesis method for pure LOOHs standard with high stability as to designed by protection of hydroperoxide group by the adduct formation with methoxypropene. Using these methodologies, Miyazawa et al have confirmed the membrane lipid peroxidation is closely association with several human diseases, and the evidence has been accumulated by cell culture studies, animal model researches and studies of patients with hyperlipidemia, diabetes, dementia, and skin-related diseases. To promote health benefit and disease prevention, the application of food constituents and their functionalities to modulate LOOH formation in human body is very important. We recently discovered that glycation of aminophospholipid occurs in human hyperglycemic plasma and red blood cells, and the glycated aminophospholipid causes membranous phospholipid peroxidation. We explained that the lipid glycation reaction is effectively inhibited by the presence of vitamin B6 (pyridoxal 5'-phosphate).

Considering our researches and findings, it is highly encouraged to further understand the “essential contribution of LOOH in human disease” and “prevention by food and food components of LOOH-mediated cytotoxicity and organ tissue injury”. This study is aimed 1) to develop comprehensive analytical methods of LOOH in human body, 2) to create the universal LOOH determination method by antibody with high selectivity for LOOH, 3) to clarify the molecular mechanism of LOOH-mediated cytotoxicity, organ tissue injury and disorders in atherosclerosis, diabetes, cancer, and dementia, and 4) to understand the functionality of food and food constituents which can control LOOH formation in human body.

[Expected result]
Applying our original techniques for the determination of LOOH and for the preparation of LOOH standards as well as our on-going research for anti-LOOH antibody, we will try to demonstrate the dream “real determination and visualization of LOOH in human body” in the world. The results have to contribute to understand the biological significance of LOOH formation in vivo, which will serve the discovery of new functions of food and food constituents and the prevention of human disease.

[References by the principal researcher]

[Term of project] FY2008—2012

[Budget allocation] 155,900,000 yen (direct cost)

[Homepage address] http://www.agri.tohoku.ac.jp/kinoubunshi/index-j.html
Improved breeding of fugu following whole genome sequencing.

Yuzuru Suzuki
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

[Outline of survey]
Following the release of the draft human genome sequence, it was decided that the next vertebrate genome to be sequenced was tora-fugu (*Takifugu rubripes*). Until now, fugu is only commercially valuable fish species to undergo genome sequencing. Here, we report the potential application of the fugu genome database to help improve breeding efficiency in this species. The technical merit of our study lies in the use of inter-species hybridization. Individuals belonging to the second generation (F2) of progeny bred via interbreeding between tora-fugu and kusa-fugu (*Takifugu niphobles*) independently exhibit significant diversity in many phenotypes. In this study, we plan to analyze both the genomic loci and the genes responsible for each phenotype, using fine genetic linkage mapping strategies which we already established. In particular, we will focus on the genes related to the characteristics beneficial for aquaculture, such as high growth rate, disease resistance, and manageability for the commercial fish farmer.

[Expected results]
The purpose of this study is to establish strategies and methods for improved breeding in fugu by exploitation of genomic sequence database available for this species. By the end of this project, we will be able to identify several genomic loci that are responsible for beneficial phenotypes, which characterize the species differences between tora- and kusa-fugu. Among the loci studied, we will determine a number of key genes. We will then survey the individual fugu possessing superior genes with the aim of selecting desirable traits. Interbreeding of fugu species may offer the additional possibility of studying the process of evolution that results in the production of key inter-species differences.

[References by the principal researcher]

【Term of project】FY2008–2012
【Budget allocation】146,600,000 yen (direct cost)

【Homepage address】http://www.se.a.u-tokyo.ac.jp/
Cell Turgor Measurement – Probe Electrospray Ionization (PESI) Mass Spectrometry for Molecular Profiling Techniques

Hiroshi Nonami
(Ehime University, Faculty of Agriculture, Professor)

【Outline of survey】
A cell pressure probe measures cell turgor of plants, and can extract cell solution directly from actively growing plants. The pressure probe technique and the probe electrospray ionization (PESI) mass spectrometry (MS) can be combined together in order to analyze cell molecular components in intact growing crops. By using physiological molecular information, environmental conditions can be adjusted optimally to grow crops in plant growth factories. Such a control method using physiological information to optimize energy efficiency and product quality control in plant growth factories is called as the speaking plant approach (SPA). The needle probe tip in PESI will be made to a scale as small as tens nanometers in tip diameter. The probe tip can be used to pick up molecules from cells. The thickness of cell walls of plants is about 200 nm, and if some molecules can be picked up from intact growing cells with the PESI probe, molecular components reflecting plant growth can be monitored. In the present project, the nano-precision PESI-MS technique will be developed for the purpose of introducing SPA in plant factories (i.e., Nano-Precision Agriculture).

【Expected results】
The pressure probe-combined PESI-MS will offer nano-scale resolution of molecular profiling in cells, leading to nano-precision agriculture for automated greenhouses. PESI can induce ionization of mixture samples with no special sample preparations. Nano-scale cell manipulation and MS analyses will make it possible to get physiological information for SPA in plant growth factories, resulting in high efficiency of energy usage and high quality production.

【References by the principal investigator】

【Term of project】 FY2008—2012
【Budget allocation】 124,300,000 yen (direct cost)

【Homepage address】 http://web.agr.ehime-u.ac.jp/%7Epbb/Grant-in-Aid%20(S).html
Studies on the phased immune-barrier systems in gut-liver axis focusing on immune responses of mesenchymal cells

Hiroshi Ozaki
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Outline of survey
Gut facing directly external environments and liver directly connected to this organ possess highly developed immune systems. “Gut-Liver Axis” has recently been proposed because these organs mutually defend against the external interferences. Many of the previous studies have focused on professional immune cells. However, it still remains unknown how mesenchymal cells, distributed around the immune cells with much larger size, behave against the interferences. In this study, we attempted to hypothesize that mesenchymal cells provide not only the spatial environment for immune cells but also play critical roles to provide immune responses after having initial activation with professional immune cells in the “Gut-Liver Axis”.

Expected results
In this study, we are focusing on the phenotypic changes and acquiring immune activity of mesenchymal cells (smooth muscle cells, myofibroblasts, endothelial cells, interstitial cell of Cajal etc. which line in the Gut-Liver Axis) after the inflammatory stimuli. We will produce new evidences that these mesenchymal cells might contribute to immune response by synthesizing broad range of inflammatory mediators and signaling proteins by communicating with professional immune cells. The results will provide newer strategies for the treatment of diseases characterized by inflammation of gastrointestinal tract and liver, such as inflammatory bowel disease (ulcerative colitis and Crohn’s disease: IBD), functional gastrointestinal diseases, virus hepatitis, alcoholic hepatitis, nonalcoholic steatohepatitis (NASH), and cirrhosis. The results will also pose useful method for the nutritional management and the treatment of chronic gastrointestinal diseases of domestic animals.

References by the principal investigator

Term of project FY2008—2012
Budget allocation 117,300,000 yen (direct cost)

Homepage address http://www.vm.a.u-tokyo.ac.jp/yakuri/kiban-s/
Finding of Regulatory Proteins of Microtubule Polymerization and Discovery of Natural Compounds against Dementia

Takafumi Uchida
(Tohoku University, Graduate school of Agricultural Science, Professor)

【Outline of survey】
Japan is the world’s top country for longevity, and the number of patients of dementia as Alzheimer’s disease is increasing. But we have no effective drug for the disease. We created prolyl isomerase Pin1-knockout mice and have found that Pin1 protects against Alzheimer’s disease. We think that appropriate regulation of microtubule polymerization should decrease the risk of dementia. We would like to find the proteins that regulate microtubule polymerization and elucidate their functions. The goal of this project is to discover natural compounds against dementia.

【Expected results】
We will find regulatory proteins of microtubule polymerization and elucidate the functions of them completely. In order to investigate the biological functions of them, we have been creating the knockout- and the transgenic- mice. We will use these mice to study the pathogenic mechanism of dementia. If the natural compounds that control the regulatory proteins are discovered, they will be the innovative drugs against dementia.

【References by the principal investigator】

【Term of project】 FY2008-2012
【Budget allocation】 80,800,000 yen (direct cost)

【Homepage address】 http://www.agri.tohoku.ac.jp/enzyme/index-j.html
Innovative Asymmetric Synthesis of Pharmaceuticals Through Strategic Development of Multifunctional and Multimetallic Catalysts

Masakatsu Shibasaki
(The University of Tokyo, Graduate school of Pharmaceutical Sciences, Professor)

【Outline of survey】
Development of pharmaceuticals involves state-of-the-art multidisciplinary researches and directly contributes to human health all over the world. Although a drug discovery research based on human genome sequence and in silico analysis have become more and more popular, a technology to produce complex small molecules with minimum environmental impact still constitutes a fundamental and indispensable research area. Our research group has developed several conceptually new multifunctional asymmetric catalysts to achieve highly efficient stereoselective synthesis of functionalized molecules. In the present research program, we envisioned an thorough elucidation of the origin of high catalytic activity and stereoselectivity of our multimetallic asymmetric catalysts through extensive spectroscopic analysis, leading to a new concept in the strategic development of multifunctional catalysts. These multifunctional asymmetric catalysts will boost practical synthesis of many therapeutic targets.

【Expected results】
Conventional design of asymmetric catalysts is based on the combination of one ligand—one Lewis acidic (or transition) metal. In contrast, we have designed asymmetric catalysts that constitute multi metalic center. Thus obtained multimetallic asymmetric catalysts activate multiple substrates simultaneously in the asymmetric environment, exhibiting extraordinarily high catalytic activity and stereoselectivity under mild reaction conditions. Our catalysts and newly developed catalysts in this research program will find a lot of opportunities to be applied in the practical synthesis of significant pharmaceuticals and smart materials. In particular, high catalytic activity allows for the use of non-activated substrates, which contributes to make a synthetic process atom-economical, environmentally benign and cost-effective in the industrial scale synthesis.

【References by the principal investigator】
reviews:

【Term of project】 FY2008-2012
【Budget allocation】 160,700,000 yen (direct cost)

【Homepage address】 http://www.f.u-tokyo.ac.jp/~kanai/index.html
**Single-cell on-time molecular analysis by hyper-sensitive video-mass scope**

*Tsutomu Masujima*

(Hiroshima University, Graduate School of Biomedical Sciences, professor)

**Outline of survey**

When we are able to analyze molecules of visualized reacting cells directly in real time, studies of molecular mechanisms of living systems will become more direct and fast. Thus we should seek a very sensitive and exhaustive molecular detection method for a single cell with simultaneous video-microscopic observation. We have developed the method to detect hundreds to thousands of small molecular MS peaks from a living single cell to extract and identify the key molecules specifically existing in a cell. We will further develop this new method as a quick methodology for findings of new medicinal molecules, new factor of differentiation for re-generative medicine and for finding new molecular mechanism of living systems.

**Expected results**

1. Acceleration of molecular mechanism analysis of living systems. It is already found that the results with many cells are not always true for a single cell level. It is also possible to show a full metabolomics in a organelle.
2. Accelerated finding of new medicinal molecules and its task in a cell.
3. The analyses of molecular mechanism of diseases and application to diagnosis.
4. Finding of new factor of cell differentiation for re-generative medicine.
5. Wide applications for nano-medicines and nano-technologies.

**References by the principal investigator**

4. JP PAT No.4129587 “Mass Filter for Mass Spectrometer” and 6 applications for Pat.

**Term of project** FY2008–2011

**Budget allocation** 160,700,000 yen (direct cost)

**Homepage address** [http://home.hiroshima-u.ac.jp/analytic/](http://home.hiroshima-u.ac.jp/analytic/)
Establishment of the basis for drug development by the analysis of molecular mechanisms of stress signaling

Hidenori Ichijo
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

**Outline of survey**
Stress response is one of the most fundamental cellular functions. Abrogation of the mechanisms of stress response leads to various human diseases including inflammation, cancer, neurodegeneration and autoimmunity. However, structural and spacio-temporal information of stress sensors as well as direct stress sensing mechanisms by proteins are largely unknown. In this study, we aim at elucidating the signaling mechanisms of stressors such as oxidative stress, Endoplasmic Reticulum stress and osmotic stress by focusing on the analysis of stress-activated ASK family kinases and their regulatory proteins. We would like to understand the mechanisms by which those physico-chemical and biological stressors are sensed by cells and converted to phosphorylation-dependent signals. The notable scientific features of this study are that we have been the front runner in the field of ASK family kinases and their regulatory proteins in stress responses and that we will perform these studies by using the forefront analytical techniques of stress signaling.

**Expected results**
We expect through this study to understand the molecular basis of stress signaling, especially sensing, recognition and conversion of cellular stressors. Our goal is also expected to lead to the development of lead chemical compounds of drugs based on our understandings of the molecular mechanisms of stress regulation of ASK family kinases. We hope accomplishment of our studies will produce fruitful results with novel principles in biology, pharmaceutical sciences and medicine.

**References by the principal researcher**

**Term of project** FY2008—2012

**Budget allocation** 160,400,000 yen (direct cost)

**Homepage address** [http://www.f.u-tokyo.ac.jp/~toxicol/index.html](http://www.f.u-tokyo.ac.jp/~toxicol/index.html)
Analysis of Methylome of Cancer by high throughput sequencer

Shinichi Nishikawa MD PhD
(RIKEN, Stem Cell Research Group, Group Director)

【Outline of survey】
Myelodysplasia syndrome (MDS) comprise of a diverse set of abnormalities in which both anaemia and leukemia coexist. In most cases, MDS is discovered as anaemia but eventually develop to leukemia. Currently, the radical cure of MDS is only attained by bone marrow transplantation, which is usually difficult to apply for an aged population. As its incidence increase as aging, MDS is an important problem in such rapidly aging countries as Japan. Recently, it was reported that a group of drugs that inhibit DNA methylation is effective for a significant proportion of MDS. As DNA methylation is an epigenetic mechanism to inhibit gene expression, this observation suggests that abnormal DNA methylation is involved in development of MDS. However, which genes are methylated during MDS development remains unclear. This is due to a technological difficulty in genome-wide analysis of DNA methylation in a quantitative manner. Recently, this problem was overcomed by a technology based on DNA array bearing entire human genome. Moreover, development of next-generation sequencer that allows sequencing of 10-100 million base pairs at one run is expected to boost the genome-wide analysis of epigenome. The major purpose of this project is to apply a high-throughput sequencers for genome-wide analysis of methylome of MDS cells who respond to the treatment with drugs inhibiting DNA methylation. Likewise, we will try to define tumor specific methylome of malignant melanoma cells.

【Expected results】
That some MDS patients undergoes remission in response to inhibitors of DNA methylation is the evidence for that abnormal methylation is involved in MDS development. Thus, comparison of the methylome of MDS cells and normal hematopoietic cells will allow us to define genes that are involved in MDS development. Through this analysis,
1) The oncogenic process of MDS and melanoma will be elucidated.
2) Genes that are involved in the development of these two tumors will be defined, which will lead to discovery of target molecules for tumor treatment.
3) The basic mechanisms underlying maintenance of stem cell systems will be elucidated.

【References by the principal investigator】
For all participants including the principal investigator, this is the first time of studying genome-wide epigenome of tumors. Hence, there is no papers directly related to this projects. However, the PI has been working on the developmental biology of hematopoietic and melanocyte stem cell systems. Followings are examples of contributions.


【Term of project】 FY2008—2012
【Budget allocation】 148,700,000 yen (direct cost)

【Homepage address】 http://www.cdb.riken.go.jp/scb/
Study of *Shigella* infectious strategy for the intestinal barrier

Chihiro Sasakawa  
(The University of Tokyo, The Institute of Medical Science, Professor)

**[Outline of survey]**

The intestinal lumen is covered by epithelial monolayer, which acts an intrinsic defensive barrier against microbial invaders. Nevertheless, many pathogenic bacteria, including *Shigella*, are capable of colonizing the intestinal epithelium by circumventing the various host barrier functions. In the present study, we investigate how the bacterial pathogens such as *Shigella* colonize the intestinal epithelium and how they can evade host innate defense system. In brief, we focus on the role of subset of effectors secreted via the type III secretion system from intracellular *Shigella* in the middle of stage of infection of intestinal epithelium, and investigate their biological activities and roles of each of the effectors in promoting bacterial survival and colonization. Based on the results with each of the effectors together, we envisage to unveil the novel bacterial infectious system, which will also provide some important insight into understanding the sophisticated bacterial infectious strategies shared with many other bacterial pathogens.

**[Expected results]**

When we will achieve the goals of the research proposal, we expect the following outcomes; (i) our study will provide clue and idea to elucidate other bacterial infectious systems and the host-cellular responses, (ii) our study will allow us to evaluate the impact of each barrier function lying in the intestinal epithelium on bacterial infection, (iii) we will get some insight into understanding the molecular basis for determining the human-specificity of *Shigella*, and (iv) we will translate the knowledge obtained through this study into development of safer *Shigella* vaccine, and animal model.

**[References by the principal researcher]**


**[Term of project]** FY2008—2012  
**[Budget allocation]** 152,800,000 yen  
(direct cost)

**[Homepage address]**  
http://www.ims.u-tokyo.ac.jp/bac/hp/mainpage.html
Roles of guidance factors in immune regulation

Hitoshi Kikutani

(Osaka University, Research Institute for Microbial Diseases, Professor)

【Outline of survey】

Semaphorins, which were originally identified as axon guidance factors, play critical roles in development of not only the nervous system but also of other organ systems. Recent studies from the principal investigator’s laboratory have also revealed that several semaphorins and their receptors are critically involved in regulation of immune responses. This study will be carried out in order to determine the mechanisms how semaphorins regulate immune responses, by 1) analyzing immune responses of gene targeted mice that are deficient in semaphorins or their receptors, 2) analyzing signals of semaphorins in immune cells and 3) analyzing effects of recombinant semaphorins or antibodies against semaphorins on various experimental animal models of immunological diseases.

【Expected results】

It is likely that semaphorins play roles in regulation of immune responses in the ways quite different from those used by known immunoregulatory molecules such as cytokines and co-stimulatory molecules, this study may reveal a novel mechanism of immune regulation. In addition, this study is expected to reveal new therapeutic molecular targets of various immunological disorders.

【References by the principal investigator】


【Term of project】 FY2008—2012

【Budget allocation】 159,300,000 yen (direct cost)

【Homepage address】 http://www.biken.osaka-u.ac.jp/
An integrative elucidation of the energy metabolism-regulating system and its disruption

Takashi Kadowaki
(The University of Tokyo, University Hospital, Professor)

[Outline of survey]
Glycemic, lipid and energy metabolism represents a critically important process in life’s manifestations, where insulin (Ins) and adiponectin (Ad) account for two major pathways for this metabolic process. An integrative conceptualization of the energy metabolism-regulating system and its disruption will hold the key to unraveling the causes of diabetes and the metabolic syndrome (MS) as well as to developing therapeutic modalities for these conditions. The proposed research therefore intends to draw fully on the resources of tissue-specific Ad receptor (AdipoR)-deficient mice for elucidation of the action of Ad in relevant tissues (Nature 2003;423:762, Nat Med 2006;3:247) as well as on tissue-specific Ins receptor substrate (IRS)-deficient mice for evaluating the effect of primary depletion of Ins action on relevant tissues (Nature 1994;372:72, J Clin Invest 2004;114:917) to set out to (1) elucidate the mechanisms of interorgan cross-talk in metabolic regulation; and (2) to unravel the mechanisms of cellular function and homeostasis in place in metabolic regulation, thus aiming to give a full picture of the action of Ins and Ad in central and peripheral tissues as well as in the whole body.

[Expected results]
Ours is the only laboratory in the world to have access to and draw on the resources of tissue-specific Ad receptor (AdipoR)-deficient mice for elucidation of the action of Ad in relevant tissues (Nature 2003;423:762, Nat Med 2006;3:247) as well as on tissue-specific Ins receptor substrate (IRS)-deficient mice for evaluating the effect of primary depletion of Ins action on relevant tissues (Nature 1994;372:72, J Clin Invest 2004;114:917) and thus is capable of integrative elucidation of the energy metabolism-regulating system. The proposed research is therefore expected to mark a milestone in that all resulting findings and insights will translate into novel therapeutic modalities for diabetes, the metabolic syndrome and associated cardiovascular disease.

[References by the principal researcher]

[Term of project] FY2008–2012

[Budget allocation] 174,800,000 yen (direct cost)

[Homepage address] None
Development of an innovative radiotherapy technologies for the improvement of treatment outcomes of intractable cancers.

Masahiro Hiraoka
(Kyoto University, Graduate School of Medicine, Professor)

Outline of survey
Lung Cancer, malignant pleural mesothelioma (MPM), esophageal cancer and pancreatic cancer are still formidable diseases to treat despite intensive efforts. Better control of local disease progression using radiation therapy is necessary to improve the prognoses for those patients. However, respiratory motion of these tumors and the adjacent risk organs make it difficult to focus the optimal radiation dose to the targets with accuracy and safety by conventional radiation techniques. To overcome these problems, we developed a novel irradiation system (TM series) in collaboration with the company; Mitsubishi Heavy Industries Ltd., which has the potential to track moving tumors continuously depending on respiratory motion. This system has some characteristic structures not only to have the ability to construct a tumor tracking system, but also have the potential to create the innovative irradiation techniques which has never been recognized.

In this project, we are going to develop a 4-dimensional (4D) system for radiotherapy, which responds to respiratory motion of each tumor of the individual patient, and to lead the established 3D-systems to the next 4D generation.

Expected results
The accomplishment expected in this project is to establish the methods of planning for radiation therapy which allow for the organ motion and deformation during breathing and to construct the methods how to evaluate them. In addition, by developing the novel irradiation systems maximizing the abilities of TM series, it is expected that the prominent progression from 3D to 4D generation in treatment planning and radiotherapy will be achieved, which would lead to innovative treatment strategies in radiation therapy.

References by the principal investigator

Term of project] FY2008—2012

Budget allocation] 159,100,000 yen (direct cost)

Homepage address] http://www.kuhp.kyoto-u.ac.jp/%7Erad_onc/Public/department_info/mission.htm
**Integrative Study of transcriptional network systems during enchondral ossification**

**Toshiyuki Yoneda**  
( Osaka University, Graduate School of Dentistry, Professor )

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### Outline of survey

In vertebrae, most of skeletons are formed by enchondral ossification. Endochondral ossification is a sequential and complex biological event that consists of condensation of mesenchymal cells, their differentiation into chondrocytes, maturation and apoptosis of chondrocytes, and replacement of cartilage tissue by bone. Transcription factors, Sox9 and Runx2 play an essential role in enchondral ossification and regulate the expression of genes necessary for chondrogenesis by cross-talking with numerous intracellular signalings. In this study, using molecular and cellular approaches and genetically engineered mice, we aim to understand transcriptional regulation and the network system spatially and temporally during enchondral ossification. Especially, we investigate the transcriptional factory which is a large protein complex assembled by Sox9 or Runx2. Thus, this study not only contributes to understanding of enchondral ossification but also makes breakthrough of the biology.

### Expected results

We would understand transcriptional network system, mainly of Sox9 and Runx2, at molecular, cellular and animal levels, thus spatially and temporally regulatory mechanisms of enchondral ossification. Moreover, our findings might contribute to development of novel and effective treatment for cartilage diseases such as osteoarthritis and rheumatoid arthritis. Therefore, our study would be important for not only advancement of science but also clinical fields.

### References by the principal investigator


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**Term of project** FY2008—2010  
**Budget allocation** 164,100,000 yen (direct cost)

**Homepage address**  
http://www.dent.osaka-u.ac.jp/~biochm/