Title of Project: **Dissection of Mammalian Biological Clock System at a Molecular Level**

Yoshitaka Fukada
(The University of Tokyo, Graduate School of Science, Professor)

Research Area : Basic Biology
Keyword : Biological clock, circadian rhythm, signal transduction, neuroscience, photobiology

【Purpose and Background of the Research】

Most living organisms show a variety of rhythms by virtue of having an intrinsic time-measuring system, called circadian clock, which can be entrained to daily variation of the environmental conditions such as light-dark cycle. In mammals, the central clock system resides in the hypothalamic suprachismatic nucleus, and dysfunction of the central clock leads to mental diseases such as bipolar disorder and to instability of memory and emotion, suggesting that output signals from the central clock play unraveled roles in maintenance of brain functions. On the other hand, the light input pathway to the central clock plays a major role for its entrainment. The mammalian clock system employs intrinsically photosensitive retinal ganglion cells, together with rod and cone visual cells, as the photoreceptors. The molecular mechanism of how the light signals captured in those cells are converted to phase shifting signals remains to be elucidated. Lastly, the core oscillating machinery in the circadian clock involves a number of clock genes and their encoded proteins, which generate a molecular oscillation with a transcription/translation-based feedback regulation. A key question is the mechanism of how these clock genes and proteins generate the very slow and stable molecular oscillation with a period of approx. 24 hours. The goal of this study is to understand the molecular and neuronal mechanisms that underlie the circadian clock function with its input and output regulation.

【Research Methods】

To understand the mechanism of circadian clock in terms of both behavioral rhythms and molecular oscillation, we will focus on the following three projects on the clock components. [1] Input pathway: The circadian clock generates "robust" 24-hour rhythms, while its phase can be "flexibly" controlled by environmental factors. To understand the mechanism underlying the robustness and flexibility, we will dissect molecular basis of clock phase regulation by the input pathways. [2] Output pathway: Higher-ordered functions in the brain are likely to be controlled by output signals of the circadian clock system. We will explore physiological roles of the clock output signals that are essential for brain functions. [3] Molecular Oscillator: Most of the clock proteins essential for circadian oscillation are regulated by post-translational modifications. We will investigate the roles of the modifications in spatiotemporal regulation of the clock proteins in order to understand how the slow and stable molecular oscillation is achieved.

【Expected Research Achievements and Scientific Significance】

In humans, nocturnal activities and midnight meals are known to disturb biological clock system, leading to obesity and other metabolic disorders. Shift works often elicit mood disorder. These facts suggest an intimate interaction between the circadian clock and normal physiological functions. However, their molecular links are largely elusive. The researches in this project will provide clues to understanding the chronobiological basis of human health and diseases.

【Publications Relevant to the Project】

Kon, N. *et al.* (2008) Activation of TGF-ß/activin signalling resets the circadian clock through rapid induction of *Dec1* transcripts. *Nature Cell Biol. 10*, 1463-9.

Hatori, M. *et al.* (2011) Light-dependent and circadian clock-regulated activation of sterol regulatory element-binding protein, X-box-binding protein 1 and heat shock factor pathways. *Proc. Natl. Acad. Sci. USA. 108*, 4864-9.

Yoshitane, H. *et al.* (2012) JNK regulates the photic response of the mammalian circadian clock. *EMBO Rep. 13*, 455-61.

【Term of Project】 FY2012-2016

【Budget Allocation】 167,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.biochem.s.u-tokyo.ac.jp/fukada-lab/index-j.html
sfukada@mail.ecc.u-tokyo.ac.jp

Title of Project : Studies on the oxygen-evolving reaction mechanism of photosystem II at an atomic resolution

Nobuo Kamiya
(Osaka City University, The OCU Advanced Research Institute for Natural Science and Technology (OCARINA), Professor)

Research Area : Structural Biological Chemistry
Keyword : X-ray crystallography, Photosynthesis, Oxygen evolution, Enzymatic reaction Artificial photosynthesis

【Purpose and Background of the Research】

In natural photosynthesis, photosystem II (PSII) performs light-induced electron transfer and water-splitting reactions, which lead to the formation of molecular oxygen. PSII from thermophilic cyanobacteria consists of seventeen membrane-spanning subunits, three hydrophilic, peripheral subunits, and many cofactors with a total molecular weight of 700 kDa for a dimer. X-ray crystal structures of PSII have been reported at 3.8-2.9 Å resolutions for PSII, which provide arrangement of protein subunits and most of the cofactors involved in the electron transfer reactions. However, the detailed structure of Mn_4Ca-clulster, the catalytic center of light-induced oxygen evolution, has not been resolved. We have improved the resolution and diffraction quality of PSII crystals significantly, and succeeded in solving the crystal structure of PSII from *T. vulcanus* at a resolution of 1.9 Å as shown in Figure 1. Electron density distributions for each of the five metal ions in the Mn_4Ca-cluster are clearly separated, allowing us to locate the individual metal ions and all of the ligands to the metal cluster unambiguously. This work was highly evaluated and selected in the breakthrough of the year, 2011, *Science*.

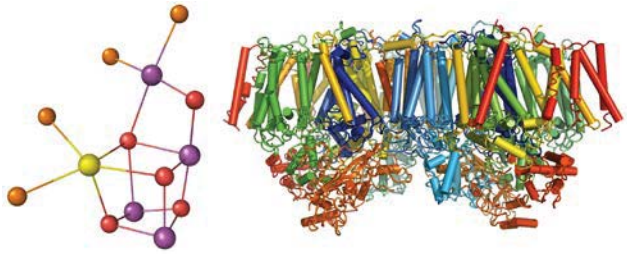

Figure 1 Mn4Ca cluster and PSII dimer

The light-induced water oxidation is catalyzed by the Mn_4Ca-cluster, changing its Si-states (with i=0-4) in the Kok cycle upon extraction of each electron by the PSII reaction center. When 4 electrons and 4 protons are extracted from 2 molecules of water, 1 molecule of di-oxygen is formed. In order to elucidate the water oxidation mechanism of PSII, crystallographic analyses for all Si-states are inevitable. At present, however, we have only the precise structure of the Mn_4Ca-cluster at the S1-state.

【Research Methods】

In order to obtain structural information for the S0-state, we will analyze the crystal structures of iodine-substituted PSII, herbicide complexes, and PsbM deletion mutant at an atomic resolution. The S2-state will be realized by laser light and resolved its structure also by X-ray crystallography.

【Expected Research Achievements and Scientific Significance】

The experimental proposals mentioned above are highly challenging, and if the structures of S0 and S2 states in the Kok cycle are uncovered, the real water oxidation mechanism proposed will provide important information to design water oxidation catalysts inevitable to realize artificial photosynthesis for a sustainable world on our planet.

【Publications Relevant to the Project】

・Umena, Y., Kawakami, K., Shen, J.-R., Kamiya, N., Crystal structure of oxygen- evolving photosystem II at a resolution of 1.9 Å, Nature, 473(7345), 55-60 (2011).
・Kamiya, N. and Shen, J-R., Crystal Structure of oxygene-evolving photosystem II from Thermosynechococcus vulcanus at 3.7 Å resolution, Proc. Natl. Acad. Sci. USA 100, 98-103(2003).

【Term of Project】 FY2012-2016

【Budget Allocation】 167,400 Thousand Yen

【Homepage Address and Other Contact Information】

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Title of Project： Identification of natural ligands for olfactory receptors and elucidation of biological function

Kazushige Touhara
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Area：Biological sciences
Keyword：olfaction, odorant, receptor, ligand, biological active substance

【Purpose and Background of the Research】

In mammals, detection of numerous volatile signals in the external world is mediated by several hundred odorant receptors (ORs) expressed in olfactory sensory neurons. In the past several years, ~10% of ORs have been paired with cognate odorants by screening compounds that are commercially available or can be obtained from fragrance companies. However, the ligands for ORs in a natural environment are distinct from odorants available in a laboratory, but are odorants derived from food or enemy, or from urine, feces, or various secretions of other individuals or species. In addition, ORs are also expressed in non-olfactory tissues such as testis, muscle, developing heart, brain, and spleen, and presumably these ORs are sensing small metabolic compounds for specific biological functions. Thus, natural ligands received by ORs expressed in a nose or in non-olfactory tissues are largely unknown. In this study, we will develop an OR assay with no background that could be utilized to purify endogenous natural ligands for ORs. We will identify natural ligands for mouse ORs that are secreted from various exocrine gland extracts. We will also identify endogenous natural ligands for ORs expressed in various non-olfactory tissues.

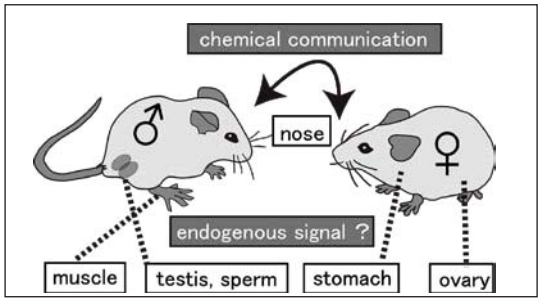

Fig. 1 Overview

【Research Methods】

We first develop a highly efficient OR assay with no background that could be utilized for crude extract samples. By OR assay-guided fractionation and chemical analysis, we will identify natural ligands for mouse ORs from various exocrine gland extracts. Using the same assay method, we will also identify endogenous natural ligands for ORs expressed in various non-olfactory tissues.

【Expected Research Achievements and Scientific Significance】

Identification of natural ligands for ORs will lead to understanding of socio-sexual behavior in mice. Biological function of a natural ligand as a chemosignal may be useful upon considering regulation of mouse reproduction. Functional analysis of ORs in non-chemosensory tissues will provide a new target of drug development. This study will give a high impact in the fields of environmental sciences, pharmacology, and behavioral and brain research.

【Publications Relevant to the Project】

・ Touhara K. & Vosshall, L.B. Sensing odorants and pheromones with chemosensory receptors. Annu. Rev. Physiol. 7, 317-332 (2009)
・ Haga S, Hattori T, (7 authors) & Touhara K. The male mouse pheromone ESP1 enhances female sexual receptive behavior through a specific vomeronasal receptor. Nature 466, 118-122 (2010)

【Term of Project】

FY2012-2016

【Budget Allocation】

165,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/biological-chemistry/

Title of Project : Structural basis for molecular mechanisms of membrane transporters.

Osamu Nureki
(The University of Tokyo, Graduate School of Science, Professor)

Research Area : Structural Biology, Biophysics, Biochemistry, Molecular Biology
Keyword : Transporter, X-ray crystallography, Computer simulation, Electrophysiology

【Purpose and Background of the Research】

Plasma membrane defines the cellular boundary and maintains the intracellular circumstance distinct from extracellular one, which is essential for life. Membrane transporters embedded in the membrane transport various substances to maintain the cellular homeostasis. The main points of the transporter research are: (A) how the transporters drive their transport, (B) how the transporters recognize their specific substrates and (C) how the transporters regulate their transporting activities. Although full understandings of molecular mechanisms of transporters are limited due to the difficulty of their structure determination, we have gained pioneering achievements by solving the crystal structures of six transporters. Based on the achievements, our research goal is to comprehend molecular mechanisms of membrane transporters, focusing on the above three main topics.

【Research Methods】

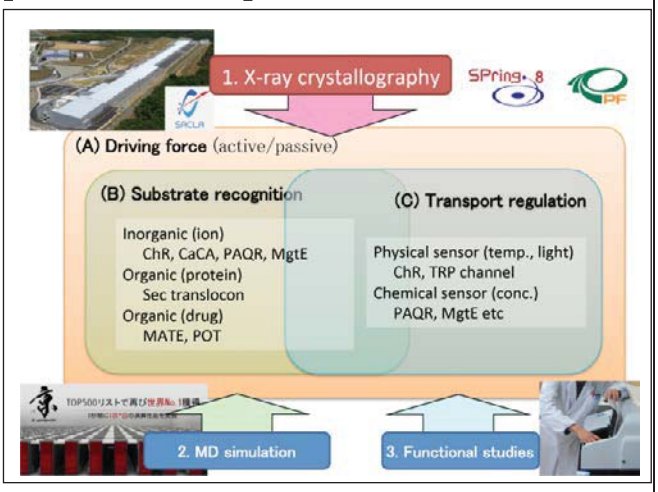

Fig. 1 Strategy

To clarify the molecular mechanisms of membrane transporters,

1. We will first determine their static structures by X-ray crystallography
2. We will then clarify their dynamic motions by MD simulation
3. We will finally verify the hypothesis derived from their structures by *in vitro*/*in vivo* functional analyses.

We will mainly focus on

1. Transport mechanisms by divalent cation transporters
2. Transport regulatory mechanism of physical sensors
3. Transport mechanisms of organic substances such as amino acids, sugars, proteins and drugs

【Expected Research Achievements and Scientific Significance】

In this project, we will take the world leadership in uncovering the comprehensive and general molecular mechanisms of membrane transporters by structural and functional studies shown in Fig. 1. Genetic dysfunction of membrane transporters often causes various diseases. Therefore, the achievements of this research not only contribute to basic science, but also to medical application such as structure-based drug design.

【Publications Relevant to the Project】

・"Crystal structure of the channelrhodopsin light-gated cation channel" H. E. Kato（12 authors） K. Deisseroth and O. Nureki ***Nature* 482**, 369-374 (2012).
・"Structure and function of a membrane component SecDF that enhances protein export" T. Tsukazaki（8 authors） K. Ito and O. Nureki ***Nature* 474**, 235-238 (2011).
・"Conformational transition of Sec machinery inferred from bacterial SecYE structures" T. Tsukazaki（8 authors） K. Ito and O. Nureki ***Nature* 455**, 988-991 (2008).

【Term of Project】 FY2012-2016

【Budget Allocation】 167,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nurekilab.net/

Title of Project : Opening up New Structural Biology by High-speed Atomic Force Microscopy

Toshio Ando
(Kanazawa University, School of Mathematics and Physics, Professor)

Research Area : Biophysics
Keyword : Structure, dynamics and functions of proteins and nucleic acids; Bioimaging

【Purpose and Background of the Research】

Since the function of proteins is tightly related to their structure, the detailed structure of proteins has been extensively studied but structures obtained are limited to static snapshots. The dynamic behavior of protein molecules in action has been studied by single-molecule techniques but the entities (protein molecules) are invisible. Thus, the simultaneous assessment of structure and dynamics has long been infeasible, meaning that we have to infer how proteins operate to function from gleaned data with significant resolution gaps. Therefore, directly visualizing functioning protein molecules at high spatial and temporal resolution has long been a "holy grail" for biological science. To overcome this longstanding problem, Ando has been developing high-speed atomic force microscopy (HS-AFM) since 1993, which has now reached its maturity. In fact, recent application studies conducted by Ando's group have continuously demonstrated that high-speed AFM is a powerful new approach to providing unique and deep insights into the functional mechanism of proteins (Fig.1). Moreover, it was recently demonstrated that even *in situ* dynamic visualization of protein molecules moving on live bacterial cell surfaces is also possible. Based on this innovative development of technology, this project aims at further expanding application studies and developing the next generation of microscopy techniques.

【Research Methods】

This project will perform the following studies. **(a)** HS-AFM imaging studies on various proteins will be performed through extensive collaborations with biologists: motor proteins, AAA proteins, DNA-related proteins, intrinsically disordered proteins, membrane transport proteins. **(b)** *In situ* video imaging will be carried out for outer surfaces of bacteria and isolated intracellular organelles (nuclei and mitochondria) to reveal dynamic molecular processes occurring thereon. **(c)** A non-contact type of high-speed scanning probe microscopy (SPM) will be developed to make it possible to visualize the surface structure of live eukaryotic cells. Moreover, developing new microscopy techniques will be attempted to make it possible to observe the interior of live cells at high spatiotemporal resolution.

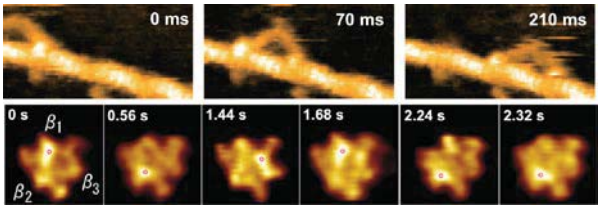

Figure 1 Proteins in action captured by HS-AFM. Upper panel: walking myosin V, Lower panel: rotary propagation of conformational change of rotorless F_1-ATPase.

【Expected Research Achievements and Scientific Significance】

Extensive successful demonstration of dynamic imaging of proteins in action will be achieved, which will innovate on the present condition of structural biology to open up "dynamic structural biology". The *in situ* imaging, together with new high-speed SPM techniques, will also bring a great impact to cell biology.

【Publications Relevant to the Project】

N. Kodera et al., "Video imaging of walking myosin V by high-speed atomic force microscopy", *Nature* **468**, 72-76 (2010).
T. Uchihashi et al., "High-speed atomic force microscopy reveals rotary catalysis of rotorless F_1-ATPase", *Science* **333**, 755-758 (2011).

【Term of Project】

FY2012-2016

【Budget Allocation】

165,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.s.kanazawa-u.ac.jp/phys/biophys/index.htm
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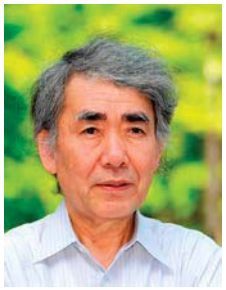

Title of Project : Mechanism of the maintenance of ER homeostasis by redox regulation

Kazuhiro Nagata
(Kyoto Sangyo University, Faculty of Life Sciences, Professor)

Research Area : Biology・Biological Science・Cell Biology
Keyword : Protein degradation, ER

【Purpose and Background of the Research】

Membrane and secretory proteins misfolded in the endoplasmic reticulum (ER) are degraded by so-called ER-associated degradation (ERAD) by ubiquitin-proteasome system after retrogradely translocated from the ER to the cytosol. We have found two critical factors involved in the ERAD, EDEM and ERdj5. ERdj5 reduces and cleaves the disulfide bonds in misfolded proteins to facilitate the retrotranslocation. Mammalian ER contains more than 20 oxidoreductases that are important not only for productive folding of nascent proteins but also for their degradation. In the ER, the maintenance of protein homeostasis, redox homeostasis and calcium homeostasis are indispensable for cell survival. In this study, we reveal the mechanism of the maintenance of ER homeostasis by redox regulation and analyze the crosstalk between the homeostasis at the molecular level.

【Research Methods】

(1) Maintenance of protein homeostasis by quality control (ERAD) in the ER

We have revealed that ERdj5 reduces and cleaves disulfide bonds in misfolded proteins in the ER to facilitate the retrotranslocation for degradation. However, it remains to be addressed how ERdj5 obtain reducing power in the oxidative conditions in the ER. Even if disulfide bonds are reduced, they would be expected to be oxidized rapidly. We will focus how reduced forms of cysteins are maintained until they reach the retrotranslocation channel by exploring the binding or escort proteins for ERdj5.

(2) Maintenance of redox homeostasis in the ER

As the ER is the major organelle for protein synthesis, more than 20 oxidoreductases exist in the mammalian ER in addition to the molecular chaperones and folding enzymes. However, little is known on how so many oxidoreductases are necessary in mammalian cells, and how they exert oxidative or reductive forces for the client proteins through their networks or cascades. We focus our effort on two representative oxidases, Ero1a and PDI, both of which make a regulatory hub complex. We reveal the entity of cascade of oxidative reaction by this hub complex on other oxidoreductases in the ER, and also reveal the intramolecular and intermolecular electron transfer pathways by biochemical approaches.

(3) Maintenance of calcium homeostasis in the ER

ER is known as the main organelle for calcium storage as well as protein synthesis. Calcium homeostasis is regulated by two calcium pumps, IP3 receptor and SERCA2 for influx and efflux, respectively. Both of which are known to be regulated by the redox conditions in the ER. Thus, we will identify which oxidoreductases are involved in the regulation of these calcium pumps.

【Expected Research Achievements and Scientific Significance】

The maintenance of three major homeostasis in the ER, including protein, redox and calcium homeostasis, are requisite for cell survival. Among them, regulation of calcium in the ER is would be the central issue of this project. Our study will reveal the molecular mechanism of the crosstalk between the homeostasis and would shed light on the indispensible role of the ER.

【Publications Relevant to the Project】

Usioda, R. et al., ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. ***Science*** **321**; 569-572 (2008)
Hagiwara, M. et al., Structural bases of an ERAD pathway mediated by the ER-resident protein disulfide reductase ERdj5. ***Mol. Cell*** **41**; 432-444 (2011)

【Term of Project】 FY2012-2016

【Budget Allocation】 167,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.cc.kyoto-su.ac.jp/~nagata/index-e.html

【Grant-in-Aid for Scientific Research(S)】

Biological Sciences (Agricultural sciences)

Title of Project: Elucidation of mechanisms of biomaterial conversion mediated by amino group-modifying carrier protein and application

Makoto Nishiyama
(The University of Tokyo, Biotechnology Research Center,
Professor)

Research Area : Applied Microbiology, Applied Biochemistry
Keyword : Microbial Metabolism, Enzyme Chemistry

【Purpose and Background of the Research】

Carrier proteins bound to carboxyl group are often found in biosynthesis of fatty acids and polyketides for efficient reaction; however, a carrier protein bound to amino group had not been found. We found a novel amino group=bound carrier protein, LysW, in lysine biosynthesis of thermophile. We are also finding the presence of LysW homologs that are involved in the secondary metabolism in *Streptomyces* as well as biosynthesis of amino acid other than lysine. These observations suggest that enzymatic systems using LysW-related carrier proteins play crucial roles in cellular bioconversions.

In this research, using available forefront technologies in structural biology, genetics, natural chemistry, bioinformatics, and so on, we will analyze the primary and secondary metabolisms in which LysW homologs are involved and will try to elucidate recognition of LysW by metabolic enzymes, whole metabolic systems containing LysW homologs, and their regulation. We also try to establish basis for production of useful materials, based on the accumulated information.

【Research Methods】

Most interesting points in this study are how LysW is recognized by enzymes in lysine biosynthesis and how enzymes of lysine and their counterpart of arginine discriminate substrates; however, these remain unsolved. In this research, we will elucidate at molecular and atomic levels how LysW homologs are incorporated into amino acid biosynthetic machineries to contribute to the primary metabolisms, by using techniques including structural biology.

Recently we also find that homologs of LysW and related enzymes are involved in the secondary metabolite biosynthesis in *Streptomyces*. We will analyze the LysW homologs and elucidate what are synthesized by the system. From these studies, we can figure out the biological systems in which amino group-modifying LysW functions. Furthermore, we will try to apply the basic knowledge on recognition mechanisms of LysW homolog and their derivatives at molecular and atomic levels to production of useful materials such as amino acid and secondary metabolites.

【Expected Research Achievements and Scientific Significance】

LysW is unique in that it is served not only as a protecting group, but also as a carrier protein for biosynthetic intermediates. Our research with high originality is expected to produce new basic finding. In addition, because LysW homologs are suggested to be involved in arginine biosynthesis and secondary metabolite production, research on biosynthetic systems containing LysW homologs will be assessed to provide unprecedented biosynthetic system and explore new research area. We had discovered LysW-mediated biosynthesis, and our activity is leading in the field. Promotion of the activity will assure our research of the international initiative. This study will have a ripple effect on both basic science and application, because amino acids and biologically active substance are applicable materials.

【Publications Relevant to the Project】

・T. Okada, T. Tomita, A.P. Wulandari, T. Kuzuyama, and M. Nishiyama. Mechanism of substrate recognition and insight into feedback inhibition of homocitrate synthase from *Thermus thermophilus*. ***J Biol Chem***, **285,** 4195-4205 (2010)

・A. Horie, T. Tomita, A. Saiki, H. Kono, H. Taka, R. Mineki, T. Fujimura, C. Nishiyama, T. Kuzuyama, and M. Nishiyama. Discovery of proteinaceous N-modification in lysine biosynthesis of *Thermus thermophilus*. ***Nat. Chem. Biol.***, **5,** 673-679 (2009)

【Term of Project】 FY2012-2016

【Budget Allocation】 159,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/biotec-res-ctr/saibo ukinou/

Title of Project： The molecular mechanism of ER stress response and the pathophysiology of ER stress disorders

Kenji Kohno
(Nara Institute of Science and Technology, Graduate School of Biological Sciences, Professor)

Research Area：Applied Biochemistry
Keyword：Cell response, Signal transduction

【Purpose and Background of the Research】

The endoplasmic reticulum (ER) is protein synthesis machineries in the cell. The newly synthesized proteins are correctly folded and assembled by the assistance of ER molecular chaperones, and finally gain of protein function. When cells are exposed to various environmental or intracellular stresses, unfolded or misfolded proteins accumulate in the ER. This state is called ER stress. Upon ER stress, cells activate unfolded protein response (UPR) pathway to upregulate the genes encoding ER chaperones and the components of ERAD (ER associated degradation), leading to alleviate the ER stress. UPR has a unique system, which contains unconventional splicing of *XBP1* mRNA by ER stress sensor IRE1 and the regulated intramembrane proteolysis of ATF6. Further ER stress has been related to neurodegenerative disease, some diabetes, and inflammatory bowel disease, but their precise molecular mechanisms have remained unclear.

In this research, we focus on physiological ER stresses observed in pancreatic islet or caused by parasitic infection, and would elucidate the role of ER stress response to maintain cellular homeostasis under physiological ER stress condition by using ER stress sensor-KO mice.

【Research Methods】

We are interested in physiological ER stress observed in islet or caused by parasitic infection. In this analysis, we will use ER stress sensor-deficient mice (IRE1α, ATF6α, IRE1α/ATF6α, and IRE1β KO mice, respectively). To analyze the symptom of diabetes mellitus, serum glucose, insulin synthesis, and glucose tolerance test are performed. In parasitic infection, we perform histochemical and electron microscopic analyses of goblet cells in small intestine after parasitic infection or IL33 administration, and also analyze the characteristics of mucin (Muc2) by immunofluorescence and SDS-PAGE. In addition to analysis of whole body level, synthesis and secretion of insulin or mucin are studied in pancreatic islet β cells and goblet cells, respectively, using biochemical and genetic engineering techniques.

【Expected Research Achievements and Scientific Significance】

Since ER stress response pathway is constitutively activated in pancreatic islet, we speculate that activation of ER stress response is quite important for insulin production and/or the survival of β cells. To address this question, we would like to demonstrate whether the disruption of IRE1α pathway would develop diabetes. Further, we would like to know the role of IRE1α in insulin synthesis, maturation, secretion or β-cell survival. Another ER stress sensor IRE1β would play an important role in proliferation and maturation of goblet cells, which secrete mucin and other factors to protect intestinal epithelial cells from various invaders. From these studies, we would provide the direct evidence that ER stress response is quite important for protecting and maintaining specific cells and tissues from physiological ER stress.

【Publications Relevant to the Project】

・Iwawaki, T., Akai, R., Yamanaka, S., & Kohno, K. Function of IRE1alpha in the placenta is essential for placental development and embryonic viability. Proc Natl Acad Sci USA, 106, 16657-16662 (2009)
・Yanagitani, K., Kimata, Y., Kadokura, H., & Kohno, K. Translational pausing ensures membrane targeting and cytoplasmic splicing of *XBP1u* mRNA. Science, 331, 387-399 (2011)

【Term of Project】

FY2012-2016

【Budget Allocation】

159,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://bsw3.naist.jp/kouno/kouno.html

【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Agricultural sciences)

Title of Project : "Speaking Cell Approach" by on-site/real-time cellular and molecular measurements

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

Research Area : Agricultural Engineering
Keyword : Cell and Tissue, Plants, Mass Spectrometry

【Purpose and Background of the Research】

In FY2011, a plant growth factory complex was built in the Faculty of Agriculture, Ehime University. After the Great East Japan Earthquake and Tsunami (2011), the Science Council of Japan published a recommendation, which encouraged the building of plant growth factories in order to secure the nation's food supply and food security. This project is related to the development of a new control system to operate plant growth factories more efficiently in the future.

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 known as the "speaking cell approach" (SCA). In the present study, methods for on-site/real time cellular and molecular measurement techniques will be developed by using a cell pressure probe and probe electrospray ionization mass spectrometry for SCA.

【Research Methods】

A cell pressure probe measures the 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. 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 SCA in plant factories (i.e., Nano-Precision Agriculture).

【Expected Research Achievements and Scientific Significance】

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 SCA in plant growth factories, resulting in high efficiency of energy usage and high quality production.

【Publications Relevant to the Project】

・Nonami, H. (2001) Water Relations in Plant Physiology (Japanese). Yokendo Ltd., Tokyo, pp.263.
・Yu, Z., Chen, L.C., Erra-Balsells, R., Nonami, H., Hiraoka, K. (2010) Real-time reaction monitoring by probe electrospray ionization mass spectrometry. Rapid Communications in Mass Spectrometry 24 (11), pp. 1507-1513.
・Yu, Z., Chen, L.C., Suzuki, H., Ariyada, O., Erra-Balsells, R., Nonami, H., Hiraoka, K. (2009) Direct profiling of phytochemicals in tulip tissues and in vivo monitoring of the change of carbohydrate content in tulip bulbs by probe electrospray ionization mass spectrometry. Journal of the American Society for Mass Spectrometry 20 (12), pp. 2304-2311.
・Yousef Gholipour, Hiroshi Nonami, and Rosa Erra-Balsells (2008) Application of pressure probe and UV-MALDI-TOF MS for direct analysis of plant underivatized carbohydrates in subpicoliter single-cell cytoplasm extract. Journal of the American Society for Mass Spectrometry 19: 1841–1848.

【Term of Project】 FY2012-2016
【Budget Allocation】 152,600 Thousand Yen
【Homepage Address and Other Contact Information】

http://web.agr.ehime-u.ac.jp/~pbb/
nonami@agr.ehime-u.ac.jp

Title of Project : Morphogenesis, regeneration and congenital disease in gallbladder and bile duct system in mammals

Yoshiakira Kanai
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Associate Professor)

Research Area : Agricultural sciences
Keyword : Developmental Biology, Pathological condition

【Purpose and Background of the Research】

In mice and humans, the biliary duct system, consisting of gallbladder, cystic duct, intra- and extra-hepatic bile duct and common bile duct, is responsible for transporting bile from the liver to the duodenum. Congenital biliary diseases can lead to the accumulation of bile in the liver, preventing the excretion of detoxification products, which ultimately results in liver injury. It was shown that, in mouse embryogenesis, a cell-autonomous *Sox17* activity is required for the specification/differentiation of gallbladder/bile duct progenitors during ventral foregut morphogenesis. *Sox17* expression is continuously maintained in a certain population of the bile duct progenitor throughout fetal and perinatal periods. However, there remains unclear when, where and how these SOX17-positive progenitor cells contribute to the development and maintenance of the biliary duct system during late organogenic stages and to the congenital biliary disorder in mammals.

【Research Methods】

In this research project, we analyzed the following four aspects of SOX17 function in the morphogenesis, maturation and regeneration of the mouse biliary duct system by using the *Sox17*-mutant embryos and chimeric embryos of *Sox17*-null ES cells in combination with two novel culture systems of the gallbladder primordium and whole-mount anterior trunk including foregut and heart primordia: 1) Dynamics of *Sox17*-null, heterozygotous and wildtype gallbladder progenitor cells during normal development; 2) Pathogenic mechanisms of the embryonic hepatitis in *Sox17* heterozygotous livers, 3) Identification of SOX17-target genes in the gallbladder progenitor cells; and 4) Potential contribution of SOX17-positive progenitor cells into the liver primodium during regeneration of the fetal hepatocytes damaged by TRECK (Toxin Receptor Cell-Kockout) method.

【Expected Research Achievements and Scientific Significance】

This study aims to explore biological significance of SOX17-positive gallbladder progenitor cells in epithelial maturation, maintenance and regeneration of the biliary duct system at the mid- and late-organogenic stages of mammalian embryos. The underlying mechanisms for the dynamics of gallbladder progenitor cells during morphogenesis, maintenance and regeneration will be also clarified at cellular and molecular levels, which will provide cellular and molecular basis of congenital bile-duct dysgenesis and biliary atresia. Moreover, these findings will contribute to develop new insights for prevention and cure of congenital biliary diseases and perinatal hepatitis in newborn infants.

【Publications Relevant to the Project】

・Saund RS et al., Gut endoderm is involved in transfer of left right asymmetry from the node to the lateral plate mesoderm in the mouse embryo. Development, 139(13):2426-2435, 2012.
・Uemura M et al., Expression and function of mouse Sox17 gene in the specification of gallbladder/bile-duct progenitors during early foregut morphogenesis. Biochem Biophys Res Commun. 391(1):357-363, 2010.
・Hara K et al., Evidence for crucial role of hindgut expansion in directing proper migration of primordial germ cells in mouse early embryogenesis. Dev Biol. 330(2):427-439, 2009.
・Matsui T et al., Redundant roles of Sox17 and Sox18 in postnatal angiogenesis in mice. J Cell Sci. 119(17):3513-3526, 2006.
・Kanai-Azuma M et al., Depletion of definitive gut endoderm in Sox17-null mutant mice. Development. 129(10):2367-2379, 2002.

【Term of Project】 FY2012-2016
【Budget Allocation】 157,200 Thousand Yen
【Homepage Address】
http://www.vm.a.u-tokyo.ac.jp/kaibo/index.html

【Grant-in-Aid for Scientific Research(S)】

Biological Sciences (Agricultural sciences)

Title of Project : Study on agricultural systems adapting fluctuating climates using agro-ecological resources management model in tropical Asia and Africa

Shinya Funakawa
(Kyoto University, Graduate School of Global Environmental Studies, Professor)

Research Area : soil science, environmental agronomy
Keyword : balance between resources and environment, fluctuating climates, management of agro-ecological resources, tropical agriculture

【Purpose and Background of the Research】

Global warming is now recognized as one of major threats for human beings. It is required to construct socio-economic systems to adapt such a change, as well as to decrease the impact itself. Although stable food supply is one of the major concerns under the situation, it is still hard to design agricultural production systems that adapt to fluctuating climates especially in Asian and African countries in tropics where socio-economic conditions are still fragile.

The present study is aiming at firstly solving individual ecosystem processes that bring weakness in tropical agriculture, and then integrating these analyses into technical solutions and sustainability management under fluctuating climates, which are further extended to regional or national scales using GIS techniques. By using these methodologies, we try to propose a technological package of agricultural production systems that could be applied in reality to tropical countries where socio-economic infrastructures are still insufficiently developed.

【Research Methods】

In the present study, field- and laboratory-based experiments will be conducted for achieving the objectives presenting above. Research sites will be installed in Northeast Thailand, West Sumatra province in Indonesia, Morogoro province in Tanzania and East province of Cameroon. The main research subjects are: 1) comprehensive management of C, N and mineral nutrients fluxes in agricultural ecosystems, 2) strategic utilization of soil microbial biomass through providing temporal ephemeral niches, 3) establishing countermeasures against soil erosion with special reference to mineralogical properties, 4) breeding for crop varieties adapting low-fertilizer application, 5) establishing the management model for sustainable use of agro-ecological resources and 6) optimization of cropping techniques and establishing flexible crop management system (Figure 1).

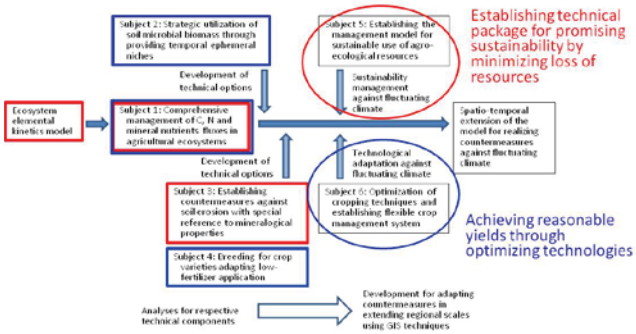

Figure 1 Respective subjects in the study

【Expected Research Achievements and Scientific Significance】

- Ecological alterations after reclaiming natural ecosystems for agricultural uses, under different climatic and geological conditions, could be analyzed and evaluated comparatively using the ecosystem elemental kinetics model.
- Analyses for individual ecosystem processes involved in the subjects 1 through 4 could be integrated into technical solutions and sustainability management under fluctuating climates, which could be further extended to regional or national scales using GIS techniques. These analyses would allow us to establish actual countermeasures against fluctuating climates in reality.

【Publications Relevant to the Project】

- Funakawa S, Watanabe T, Kadono A, Nakao A, Fujii K, Kosaki T 2011: 4. Soil resources and human adaptation in forest and agricultural ecosystems in humid Asia. *In* World Soil Resources and Food Security. Eds. R. Lal and B.A. Stewart. p.53–167, CRC Press, Taylor & Francis Group, Boca Raton, London, New York.
- Funakawa S, Watanabe T, Nakao A, Fujii K, Kosaki T 2011: 5. Pedogenetic acidification in upland soils under different bioclimatic conditions in humid Asia. ibid, p.169–269.

【Term of Project】 FY2012-2016

【Budget Allocation】 155,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://rafale.kais.kyoto-u.ac.jp

Title of Project： Molecular elucidation of plant immune systems

Ken Shirasu
(RIKEN, Plant Science Center, Group Director)

Research Area：Agricultural sciences
Keyword：Molecular interactions

【Purpose and Background of the Research】

In this project, we aim to understand how plants defend themselves and how pathogens overcome the defence system. Although a number of plant immunity components and pathogen effectors have been isolated, full elucidation of molecular mechanisms at protein level is not yet achieved. By using genomics, proteomics, and chemical genomics tools established in our laboratory, we will identify important proteins essential for plant immunity and pathogen virulence. Through structural and functional analyses of the proteins and their associated components, we aim to provide a unified view of plant immune systems. Especially, we will thoroughly characterize targets of newly identified plant immune inhibitors to set a new paradigm in the field.

【Research Methods】

We will identify and characterize targets of the plant immune inhibitors, immune sensor complexes, chaperone complexes (Fig 1), and ubiquitin ligase complexes by using a highly sensitive mass spectrometry and structural analysis tools. We also identify novel components in plant immunity by using genetics and chemical genomics. Furthermore, we will elucidate suppressor function of pathogen derived effectors by structural analysis (Fig.2).

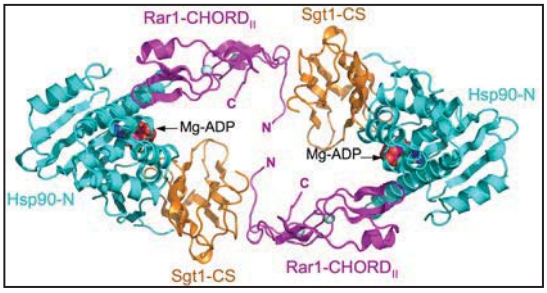

Fig 1. Immune Chaperone complex (Mol Cell 2010)

【Expected Research Achievements and Scientific Significance】

The study trend of plant immunity research is shifting from classic genetical analysis to genome-based systematic reverse genetics.

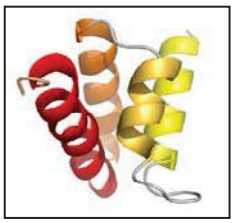

Fig.2 Structure of potato blight effector (PNAS)

However, there are only few good examples for protein structure-based characterization of mechanisms. Similarly, successful use of chemical genomics has been very limited. In this project we will utilize chemical genomics tools we established in our laboratory to identify targets of the novel plant immune inhibitors. Structural and functional analyses of the target proteins will be conducted. In addition, we will study important immune-related proteins whose functions are not clear. The novel insights we learn from our study on immune mechanisms including identification of new receptors and structural analyses will contribute to development of better breeding methods and agrochemicals with a distinct mode of action.

【Publications Relevant to the Project】

・Yaeno, T., Li, H., Chaparro-Garcia, A., Schornack, S., Koshiba, S., Watanabe, S., Kigawa, T., Kamoun, S., and Shirasu, K., Phosphatidylinositol monophosphate-binding interface in the oomycete RXLR effector AVR3a is required for its stability in host cells to modulate plant immunity. Pro Natl Acad Sci USA, (2011) 108: 14682-14687.
・Zhang, M., Kadota, Y., Prodromou, C., Shirasu, K*., and Pearl, L.H*., Structural basis for assembly of Hsp90-Sgt1-CHORD protein complexes: implications for chaperoning of NLR innate immunity receptors. Mol Cell, (2010) 39: 269-281. *co-corresponding authors

【Term of Project】 FY2012-2016

【Budget Allocation】 124,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://ksg.psc.riken.jp/index.en.html

【Grant-in-Aid for Scientific Research (S)】
Biological Sciences (Medicine, dentistry, and pharmacy Ⅰ)

Title of Project : Towards Next-Generation Aromatic Chemistry: Development of Synthetic Methods, Theory and Novel Functionalities

Masanobu Uchiyama
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Area : Organic Synthesis, Elements Chemistry, Theoretical Chemistry
Keywords : Aromaticity, Molecular Transformation, Material Science, Spectroscopy

【Purpose and Background of the Research】

Sophisticated aromatic compounds that interact with light are required for various advanced technologies, including storage media, organic semiconductors, laser printers, photodynamic therapy of cancer, nonlinear optics, deodorants, and molecular imaging. This project aims to develop new synthetic methods, to extend theoretical principles, and to obtain aromatic molecules with unique functionalities suitable for next-generation technological applications.

【Research Methods】

Interdisciplinary research from various viewpoints, including organic chemistry, physical chemistry, and theoretical chemistry, is needed to develop novel functionalized aromatic molecules for many future applications. We intend to focus on the following four areas:

① Development of breakthrough synthetic processes to construct aromatic rings and to provide new tools for chemo-, regio- and stereo-selective introduction of various functional groups into aromatic molecules
② Systematically extending our understanding of the principles of aromaticity (theoretical investigation of the origin of homo-, Mobius-, anti-, and non-aromaticities)
③ Construction of novel metal-containing (organic-inorganic hybrid-type) aromatic compounds, aiming at the emergence of new features that have not been observed in usual organic aromatics
④ Creation of stable near-infrared light-emitting aromatic molecules having a low HOMO level, with potential applications for organic solar cells, photodynamic therapy of cancer, and near-infrared imaging

【Expected Research Achievements and Scientific Significance】

A potential application for the unusual light-capturing ability of next-generation aromatic compounds could be in tandem solar cells. Such compounds could also be useful for cancer treatment by photodynamic therapy with light-absorbing compounds. By specifically illuminating the tumor, only cancerous cells are heated and consequently killed. As infrared light penetrates well through human tissue, photodynamic compounds that absorb strongly in this wavelength region are particularly desirable for this purpose.

【Publications Relevant to the Project】

1. A. Muranaka, S. Yasuike, C-Y. Liu, J. Kurita, N. Kakusawa, T. Tsuchiya, M. Okuda, N. Kobayashi, Y. Matsumoto, K. Yoshida, D. Hashizume, M. Uchiyama, "Effect of Periodic Replacement of the Heteroatom on the Spectroscopic Properties of Indole and Benzofuran Derivatives", *J. Phys. Chem. A*, **2009**, *113*, 464-473.
2. A. Muranaka, M. Yonehara, M. Uchiyama, "Azulenocyanine: A New Family of Phthalocyanines with Intense Near-IR Absorption", *J. Am. Chem. Soc.,* **2010**, *132,* 7844-7845.
3. Y. Yamamoto, Y. Hirata, M. Kodama, T. Yamaguchi, S. Matsukawa, K-Y Akiba, D. Hashizume, F. Iwasaki, A. Muranaka, M. Uchiyama, P. Chen, K. Kadish, N. Kobayashi, "Synthesis, Reactions, and Electronic Properties of 16 π-electron Octaisobutyltetraphenylporphyrin (OiBTPP)", *J. Am. Chem. Soc.,* **2010**, *132,* 12627-12638.

【Term of Project】

FY2012-2016

【Budget Allocation】

167,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.f.u-tokyo.ac.jp/~kisoyuki/

Title of Project : Development of quantitative prediction methods for alteration in pharmacokinetics caused by interindividual variability in transporter function and transporter-mediated drug-drug interaction

Yuichi Sugiyama
(RIKEN, Innovation Center, Head of Sugiyama Laboratory)

Research Area : Pharmacokinetics and drug metabolism
Keyword : Transporter, Drug-drug interaction, Polymorphism, PBPK modeling

【Purpose and Background of the Research】

Transporters are expressed in liver, kidney, intestine and blood-brain barrier and known to mediate transports of various drugs and endogenous molecules. It has been reported that transporter functions are altered by polymorphisms, hepatic/renal disorders and drug-drug-interactions (DDI) in human, which may affect pharmacokinetics (PK) and main/adverse effects of clinically-used drugs. The purpose of this study is to establish methods for the quantitative prediction of PK variation and alteration based on mechanisms.

【Research Methods】

In order to accomplish our purpose, we should perform 5 studies: (1) Developing transporter-specific probes (substrates) and inhibitors which are useful for the evaluation of transporter functions in human. (2) Constructing methods for the quantitative prediction of transporter polymorphism effects on PK (Fig. 1). (3) Analyzing alteration in transporter functions in patients with hepatic/renal disorders, and constructing prediction methods for PK alteration. (4) Establishing quantitative prediction methods of DDI based on PBPK modeling and simulation (Fig. 2), performing several clinical studies. (5) Developing PET/SPECT probes which are useful for real-time and non-invasive analyses of drug distribution. Predicting effects at the target organs and toxicity more precisely using transporter probes.

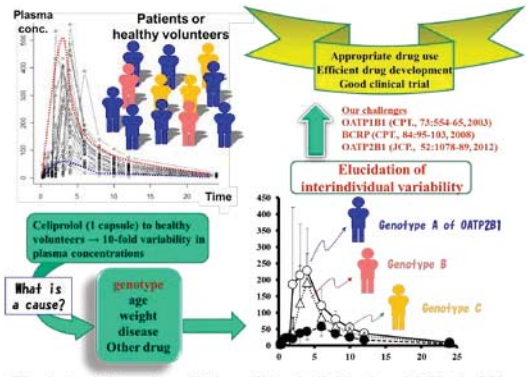

Fig. 1 Analysis and prediction of interindividual variability in PK

【Expected Research Achievements and Scientific Significance】

Our achievements will contribute to understanding variations in PK parameters and DDI mechanisms in drug development, and also contribute to improving efficacy and safety of clinical drug uses.

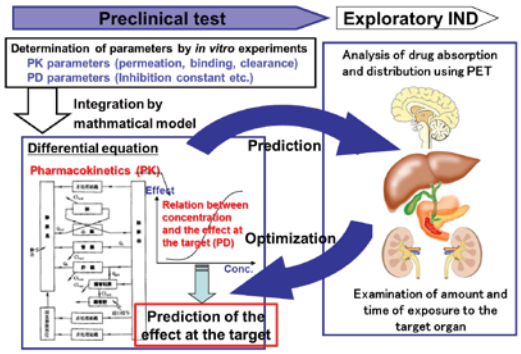

Fig. 2 Prediction of PK and drug effects based on PBPK modeling and simulation

【Publications Relevant to the Project】

Ito S, Sugiyama Y et al. Competitive inhibition of the luminal efflux by multidrug and toxin extrusions, but not basolateral uptake by organic cation transporter 2, is the likely mechanism underlying the pharmacokinetic drug-drug interactions caused by cimetidine in the kidney. J Pharmacol Exp Ther 340:393-403, 2012.

Maeda K, Sugiyama Y et al. Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. Clin Pharmacol Ther 90:575-581, 2011.

Kusuhara H, Maeda K and Sugiyama Y. Impact of drug transporters in the pharmacological and adverse reactions of drugs. In New Horizons in Predictive Toxicology. Current Status and Application, ed. AlanG.E. Wilson, pp 563-588, RSC Publishing, 2012.

【Term of Project】 FY2012-2016
【Budget Allocation】 148,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.riken.jp/r-world/research/lab/ippm/index.html

Title of Project : Biology of diversity and asymmetry of membrane lipids

Takao Shimizu
(The University of Tokyo, Graduate School of Medicine, Project Professor)

Research Area : Medicine
Keyword : Lipidomics, metabolomics, phospholipids

【Purpose and Background of the Research】

Glycerophospholipids are a major constituent of biological membrane using their amphipathic properties (soluble both in water and lipids).

The glycerophospholipids have diverse and asymmetrical characters; *sn*-1 position of glycerol backbone contains only saturated or mono-unsaturated fatty acids, while *sn*-2 position attaches polyunsaturated fatty acids such as arachidonic acid or eicosapentaenoic acid. However, the molecular mechanisms and biological consequence of the diversity remains to be clarified. We and other group recently identified a gene family of phospholipase A2 and lysophospholipid acyltransferases.

In this research grant, it is our goal to reveal the biological significance of lipid diversity, by the combination of cell and molecular biology and lipid biochemistry (LC-MS).

【Research Methods】

Heterologous overexpression and /or knockdown of phospholipase A2s and acyltransferases in cultured cells. We identify the detail composition of glycerophospholipids by LC-MS (lipidomics approach) and analyze phenotypes of thus treated cells. We will establish genetic engineered mice which either overexpress or lack these genes involved in phospholipid metabolism. Analyses of membrane phospholipids by LC-MS and various phenotypes of the mice will be done. We will develop and improve lipidomics studies with high sensitivity, accuracy and highthroughput.

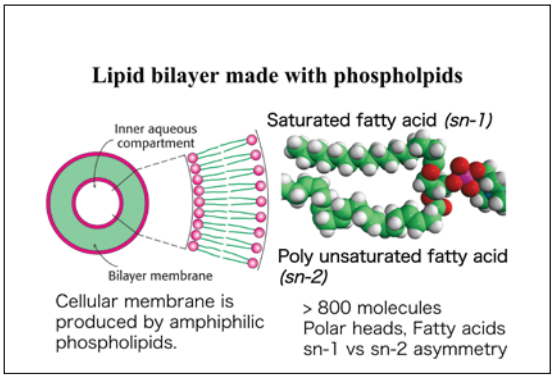

Figure 1

【Expected Research Achievements and Scientific Significance】

We will expect following future outcome with the present research project. (1)Identification of molecular mechanism and biological output of diversity and asymmetry of membrane phospholipids. (2)Development of drug screening systems and potential lead compounds which affects membrane biogenesis and diversity. (3)Development of lipidomics procedures and LC-MS instruments. (4) The nurture of young scientists with high quality background of lipid biochemistry and MS analyses.

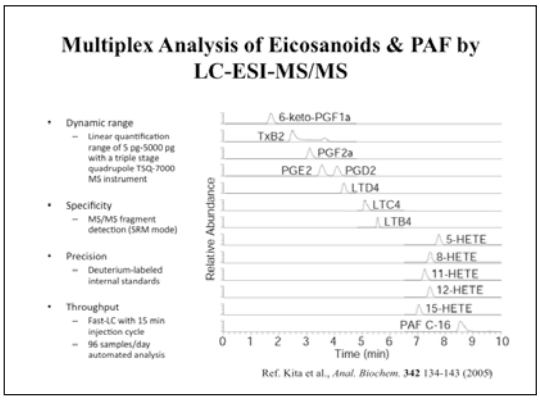

Figure 2

【Publications Relevant to the Project】

- Shimizu, T. (2009) Lipid mediators in health and disease. *Ann. Rev. Pharmacol. Toxicol. 49, 123-150.*
- Jonson, F, Marcardi, DA.Kita, Y. et al. Mouse and human neutrophils induce anaphylaxis. *J. Clin. Invest.* 121, 1484-1496

【Term of Project】 FY2012-2016
【Budget Allocation】 167,800 Thousand Yen
【Homepage Address and Other Contact Information】

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tshimizu@m.u-tokyo.ac.jp

Title of Project : Spatiotemporal and structural analysis of the regulation of T cell activation

Takashi Saito
(RIKEN, Research Center for Allergy and Immunology, Group Director)

Research Area : Immunology
Keyword : Lymphocyte, Antigen recognition, Adaptive immunigy

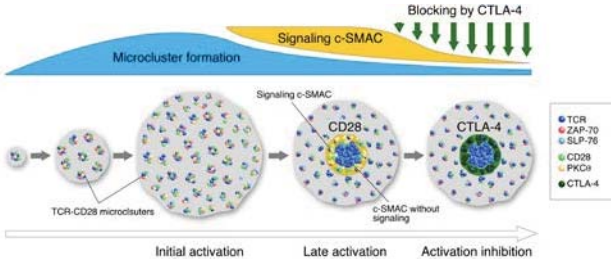
【Purpose and Background of the Research】

T cells play central roles in regulation of immune responses, but also induce autoimmune and allergic diseases upon excess activation. Therefore, elucidation of the mechanism of T cell activation and its regulation is a bridgehead to immune regulation. This project aims to clarify a full picture of the mechanisms of antigen recognition and activation of T cells and its spatiotemporal regulation by imaging and structural analyses.

On the basis of our finding that TCR microcluster is responsible for antigen recognition and T cell signaling, we will clarify the signal transduction pathways through TCR microclusters and various system for regulation, and the activation and regulation of autoreactive T cells. For this purpose, ① Molecular basis of antigen recognition and activation through structural analysis of the full-length of the TCR complex ② Intracellular spatial regulation and in vivo analysis of T cell activation signals through TCR microclusters. ③ induction mechanism of "signal memory" from cell contact to lead activation. ④ Regulation of cell movement by activation signals. ⑤regulation of T cell activation by co-stimulation and innate signals. ⑥activation regulation of self-reactive T cells. We aim comprehensive analysis of T cell activation regulation through TCR microclusters.

【Research Methods】

1. Establishment of the structural basis of T cell activation by analyzing the whole structure of the complex of TCR-CD3 and pMHC. We apply the recent developed techniques of crystlization of transmembrane-containing proteins to TCR complex.
2. Intracellular spatial signaling and degradation regulation of TCR by TCR microclusters will be clarified. Activation regulation by co-stimulation (such as ICOS, PD-1)and innate signaling.. These are performed using planar bilayer system containing GPI-anchored MHC/ICAM/CD80 and T cells expressing various fluorescent-tagged molecules.
3. Analysis of in vivo synapse formation and accumulation of "signal memory" by analyzing Ca signals.
4. Semi-activation stages of self-reactive T cells are analyzed by T-DC interaction..



【Expected Research Achievements and Scientific Significance】

We will clarify two issues. One is to clarfy how to pre-activated T cells under steady-state condition and how and where they are fully activated. Second, spatiotempral signal transduction of T cell activation will be clarified. On the basis of these analysis, not only simple inhibitors of kinases but also new generation of immune-modulators with the concept by taking consideration of spatiotemporal regulation. Elucidation of activation mechanism of self-reactive T cells may contribute for regulation of autoimmune and allergic diseases.

【Publications Relevant to the Project】

・Yokosuka, T., Kobayashi, W., Sakata-Sogawa, K., Saito, T.: Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters through protein kinase C θ translocation. ***Immunity.*** 29: 589-601, 2008.
・Hashimoto-Tane, A., Yokosuka, T., Sakata-Sogawa, K., Saito, T.: Dynein-driven transport of T cell receptor microclusters regulates immune synapse and T cell activation. ***Immunity.*** 34:919-931, 2011.

【Term of Project】 FY2012-2016
【Budget Allocation】 167,700 Thousand Yen
【Homepage Address and Other Contact Information】
http://www.rcai.riken.go.jp/group/signaling/index.html

Title of Project : Global analysis of genetic and epigenetic alterations during inflammation-associated gastrointestinal cancer development, and elucidation of its mechanism

Tsutomu Chiba
(Kyoto University, Graduate School of Medicine, Professor)

Research Area : Medicine, dentistry, and pharmacy , Clinical internal medicine
Keyword : Gastroenterology (Upper gastroenterology, Lower gastroenterology, Hepatology)

【Purpose and Background of the Research】

Cancers of the digestive organs (GI cancers) are the most significant cause of cancer death. Development of many GI cancers is underlain by inflammation with or without infection, and cancer development is characterized by stepwise accumulation of various gene rearrangements, including gene mutation, deletion and translocation. Interestingly, non-cancerous inflammatory tissues already possess considerable levels of gene mutations. These data suggest that inflammation may accelerate various gene mutations and aberrations.

Recently, we have been focusing on the role of activation-induced cytidine deaminase (AID), in the induction of gene mutation and aberration during inflammation-associated carcinogenesis. We observed that 1) ectopic expression of AID in various cancer development, 2) epithelial cells in the inflammatory tissues are associated with ectopic AID expression together with accumulation of various gene mutations and 3) AID expression is induced in epithelial cells by *H.pylori* or HCV infection, and also by various cytokines. These data suggested that AID plays an important role in the development of inflammation-associated cancers by inducing gene mutations. However, a whole picture of gene mutations and aberrations in inflammation-associated cancers, and precise molecular mechanisms of how those genetic alterations are induced during inflammation are not fully clarified. In this study, therefore, we try to 1) investigate a whole picture of genetic and epigenetic changes induced by inflammation during carcinogenesis, and to 2) elucidate mechanisms of how those genetic and epigenetic changes are induced during inflammation by focusing on the role of AID.

【Research Methods】

By utilizing ultra-deep sequencer, we will perform genome wide analysis of genetic changes induced during inflammation-associated cancer development. For this purpose, we will examine various organs of AID transgenic mice, and various human inflammatory tissues. For genome analysis, we will use whole exome capture system that covers whole exons, and extract all the genetic changes. For epigenetic analysis, DNA will be extracted from all the clinical as well as mouse samples, methylated DNA fragments recovered by binding to methyl DNA binding protein, and applied to ultra-deep sequencer. These genetic and epigenetic changes will be dissected along with expression of AID.

We will establish mouse models in which stem cells are labeled, and by combining with inflammation-associated cancer models, we will observe the relationship between AID expression and genetic changes in tissue stem cells, and elucidate importance of AID expression in stem cells in the development of inflammation-associated cancer.

【Expected Research Achievements and Scientific Significance】

It has been believed for long time that genetic alterations in cancer tissues are introduced mainly by extrinsic mutators. In this study, by focusing on roles of AID, it is expected that the mechanisms for induction of genetic and epigenetic alterations by an intrinsic mutator during inflammation-associated cancer development will be elucidated. In addition, by using second generation genome analyzer, we will be able to clarify the mechanistic relationship between genetic and epigenetic changes in inflammation-associated cancer development.

【Publications Relevant to the Project】

・Takai A, Marusawa H, Watanabe T, Chiba T, et al.: Targeting activation-induced cytidine deaminase prevents colon cancer development despite persistent colonic inflammation. Oncogene 31:1733-1742:2012.
・Matsumoto Y, Marusawa H, Chiba T, et al.: Helicobacter pylori infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nature Medicine 13:470-476:2007.

【Term of Project】 FY2012-2014
【Budget Allocation】 132,100 Thousand Yen
【Homepage Address and Other Contact Information】
http://www.med.kyoto-u.ac.jp/E/grad_school/introduction/1304/

Title of Project : Study on the relationship between multiple sclerosis and gut microbiome

Takashi Yamamura
(National Center of Neurology and Psychiatry, National Institute of Neuroscience, Head of Immunology Department)

Research Area : Medicine, dentistry, and pharmacy
Keyword : Neuroimmunopathology

【Purpose and Background of the Research】

The purpose of this study is to test the hypothesis that multiple sclerosis (MS) pathogenesis may involve alteration of gut microbiome due to the change of life style. In Japan there was only 1000 registered patients with MS 30 years ago. But since then the patient number has greatly increased, and now reaches over 15,000. In Japan, an increase of inflammatory bowel diseases is also apparent, which is suspected to result from dysbiosis of gut flora. We have reported that altering gut flora significantly reduces the severity of experimental autoimmune encephalomyelitis (EAE), a rodent model for MS, in parallel with reducing Th17 cells (Yokote et al. Am J Pathol 2008).

【Research Methods】

We here examine the microbiome in the feces of MS and healthy subjects by using the gene sequence-based analysis of 16S rRNA and bacterial metagenome. In parallel, we analyze clinical profiles of the patients

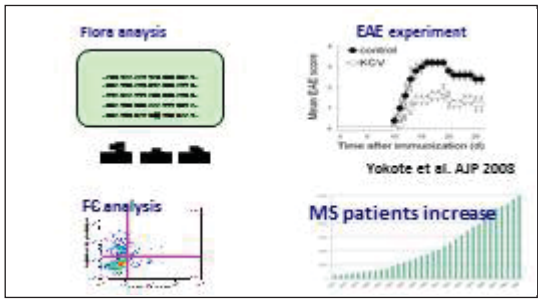


Figure 1

and immunological parameters by using flow cytometry. After we identify bacterial species that are greatly reduced or increased in the feces of MS, we will transfer the bacteria in germ-free mice and ask whether the gnotobiotic mice may represent immunological abnormalities that are relevant to the prevention or augmentation of MS.

【Expected Research Achievements and Scientific Significance】

Recent works have firmly established that gut flora is critically involved in the development of autoimmune diseases in rodents. However, it is not clear if the observations have relevance to understanding much more complex human pathogenesis. This project is primarily planned for identifying alterations of gut microbiome in MS. Owing to the power of gene-sequencing analysis, we are assured that our primary aim will be fulfilled and we may be able to identify bacterium, which plays a key role in MS or in healthy status.

Our results should also have implications for understanding the mutual relationship between human immune system and commensal flora.

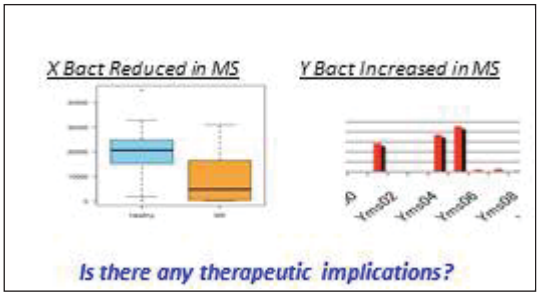


Figure 2

【Publications Relevant to the Project】

Yokote H et al.: NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. ***Am J Pathol*** 173: 1714-1723, 2008

Miyazaki Y et al. : Mucosal-associated invariant T cells regulate T helper type 1 response in multiple sclerosis. ***Int. Immunol.*** 23:332-337, 2011

【Term of Project】 FY2012-2015

【Budget Allocation】 112,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ncnp.go.jp/nin/guide/r_men/index.html

【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Medicine, dentistry, and pharmacy Ⅱ)

Title of Project : Elucidation of pancreatic β-cell function by metabolomics and its clinical application

Susumu Seino
(Kobe University, Graduate School of Medicine, Professor)

Research Area : Diabetes
Keyword : Insulin secretion, Metabolome

【Purpose and Background of the Research】

Glucose and lipid metabolism in pancreatic β-cells play an important role in β-cell function. However, metabolic signals derived from glucose and lipid metabolism involved in insulin secretion, β-cell differentiation and regeneration, and pathogenesis and pathophysiology of diabetes remain largely unknown.

In this study, we aim to elucidate by metabolomics-based analyses:

(1) metabolic signals involved in insulin secretion
(2) metabolic signals involved in β-cell differentiation and regeneration
(3) metabolic signal-derived biomarkers for diabetes

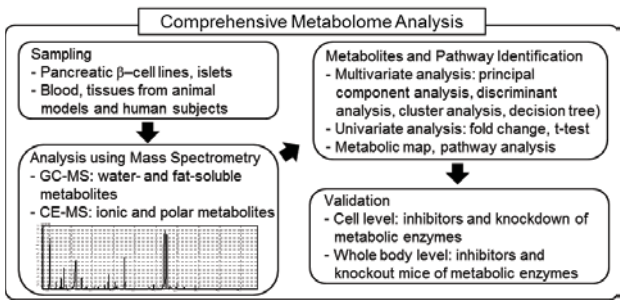
【Research Methods】

(1) Elucidation of metabolic signals involved in insulin secretion

We will identify novel metabolic signals involved in insulin secretion by comprehensive metabolome analysis using pancreatic β-cell lines. We will clarify the roles of metabolic signals at cell, islet, and whole-body levels.

(2) Elucidation of metabolic signals involved in β-cell differentiation and regeneration

We will perform comprehensive metabolome analysis on the mouse model enabling β-cell tracing to clarify the role of metabolic signals in β-cell differentiation and regeneration.



(3) Identification of metabolic signal-derived biomarkers for diabetes

We will perform comprehensive metabolome analyses of human samples (diabetes, impaired glucose tolerance, and normal controls) as well as animal models, and identify novel biomarkers for diabetes derived from metabolic signals.

【Expected Research Achievements and Scientific Significance】

In specific aim (1), novel metabolic signals regulating insulin secretion and interaction among the signals identified will be clarified, which will greatly enhance our understanding of mechanisms of insulin secretion and identify new therapeutic targets of diabetes. In specific aim (2), metabolic signals involved in β-cell differentiation and regeneration will be identified, which will contribute to establishment of the basis for β-cell replacement therapy for diabetes as well as clarification of the role of cell metabolism in β-cell differentiation and regeneration. In specific aim (3), novel biomarkers for impaired glucose intolerance (IGT) and diabetes before the blood glucose level rises will be identified, which will establish prediction markers of the development of diabetes and contribute to primary intervention for the disease. The present study will provide great insight into biological science and clinical practice.

【Publications Relevant to the Project】

・Seino S, Shibasaki T, Minami K. Dynamics of insulin secretion and the clinical implications for obesity and diabetes. **J Clin Invest** 121:2118-2125, 2011
・Zhang CL, Katoh M, Shibasaki T, Minami K, Sunaga Y, Takahashi H, Yokoi N, Iwasaki M, Miki T, Seino S. The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. **Science** 325:607-610, 2009

【Term of Project】 FY2012-2016

【Budget Allocation】 167,600 Thousand Yen

【Homepage Address and Other Contact Information】

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Title of Project： Species Difference in Pharmacokinetics of Widely Used Drugs: a PET Microdosing Study

Jun Hatazawa
(Osaka University, Graduate School of Medicine, Professor)

Research Area：Nuclear Medicine, Radiological Science
Keyword：Positron Emission Tomography, Pharmacokinetics, Species Difference

【Purpose and Background of the Research】

Drugs are essential for treatment of diseases. Drug has been developed based on the absorption, distribution, metabolism, and excretion in animals. Some compounds were not effective in men, or other compounds induced unexpected side effects. These discrepancies can be reduced if we know human pharmacokinetics during very early stage of drug development. The PET microdosing study has been designed to estimate tracer-dose pharmacokinetics in humans for candidate compounds of drugs.

In the present study, we study species similarity or difference in pharmacokinetics of drugs now used in the clinical setting.

【Research Methods】

By means of Positron Emission Tomography (PET), we preliminarily investigated phramacokinetics of acetylcholine esterase inhibitor Donepezil chloride, which is used in patients with Alzheimer's disease, in rats and humans. In rats, high accumulation of C-11 Donepezil was found in liver immediately after venous administration. The target organ brain showed only 2% uptake of total administered dose. Radioactivity of metabolized drug was found in the intestine and urinary tract 30 min after injection (Figure 1)..

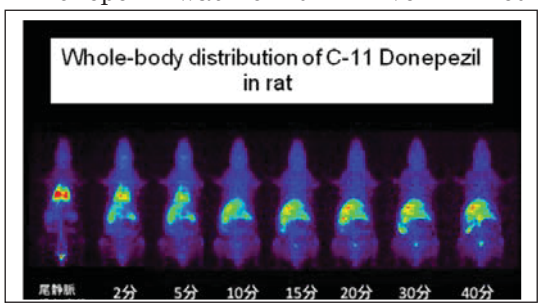

Figure 1　rats

In humans, high accumulation was found not only in liver but pancreas and myocardium. The target organ brain showed similar uptake (2%) (Fig. 2).

Based on this preliminary study, we revealed that 1) Only small amount of Donepezil was uptaken by the target organ although the drug is already proved to be effective to slow down the progression of symptoms, 2) Donepezil administered was immediately excreted through hepato-biliary and urinary tracts after administration, and 3) there was a species difference in non-target-organ accumulation.

【Expected Research Achievements and Scientific Significance】

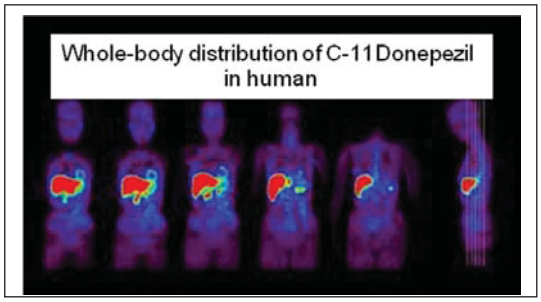

Figure 2　human

We extend our preliminary analysis on the species difference in pharmacokinetics of drugs commonly used in the clinical setting. We reveal the pharmacokinetic requirement of candidate compounds in the early stage of new drug development and when it is used in the clinical setting.

【Publications Relevant to the Project】

Hasegawa Y, Kanai Y, Hasegawa S, Okamoto T, Matsui T, Shimosegawa E, Kurachi Y, Hatazawa J. Evaluation of brain and whole-body pharmaco kinetics of 11C-labeled diphenylhydantoin in rats by means of planar positron imaging system. **Ann Nucl Med**. 2008 May;22(4):301-7.

【Term of Project】 FY2012-2016

【Budget Allocation】 121,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.osaka-u.ac.jp/pub/tracer/index- jp.htm
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Title of Project : Analysis of the functional interaction among bone, gut and energy metabolism with a special reference to gender difference

Masato Hirata
(Kyushu University, Graduate School of Dental Science, Professor)

Research Area : Oral Bioscience, Biochemistry, Pharmacology
Keyword : osteocalcin, incretin, insulin, energy metabolism, bone property

【Purpose and Background of the Research】

Globalization of markets has brought Japanese a westernized dietary habits and lifestyle, increasing the rate of obesity and metabolic syndrome. Because obesity and metabolic syndromes are caused by energy flux disruptions, elucidation of the regulatory mechanism of energy metabolism raises the possibility for new targets of therapy for the diseases.

Recently, it has been reported that bone is an active endocrine organ that secretes hormones. Especially, bone derived osteocalcin (OC) is a hormone that promotes insulin production, enhancing glucose utilization and energy expenditure.

On the other hand, incretins are gut-derived hormones secreted in response to the food intake. Incretins have numerous physiological functions including potentiation of glucose-stimulated insulin secretion. Therefore, we assume that OC might be involved in secretion of incretins. We then term this "bone-gut-metabolism flow, BGM Flow".

We found that female, but not male, mice lacking PRIP (phospholipase C-related but catalytically inactive protein), showed increased bone formation with high serum OC level. Therefore, in this study, we are going to challenge to clarify the mechanisms involved in BGM Flow, with a special reference to the roles of PRIP and gender difference.

【Research Methods】

Using wild-type and PRIP-KO mice, and when needed mice generated by mating PRIP-KO mice with metabolism-relating genes deficient mice, *in vivo* experiments are performed to analyze the effect of OC application on body weight, serum levels of incretins and insulin, body temperature, respiratory ratio, GTT and ITT etc. *In vitro* experiments are also performed using the organs and cells isolated from the above-mentioned mice, and cultured cell line regarding incretin and insulin signaling

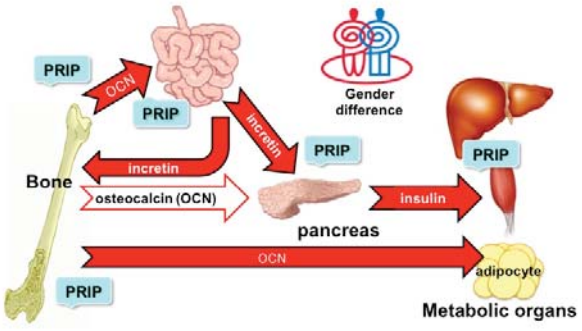

Figure 1 BGM Flow (Bone-Gut-Metabolism)

【Expected Research Achievements and Scientific Significance】

In the machinery of glucose/energy metabolism regulation by bone-derived OC, we would originally confirm the presence of Gprc6a, a putative uncarboxylated-OC receptor, in the epithelial cells of mouse small intestine, and OC induces incretin secretion. In addition to the direct effect of OC on insulin secretion from pancreas, it is for the first time to show that the effect also through the incretin secreted from the gut. Furthermore, the establishment of "BGM Flow" would be a reasonable extension of our PRIP-related works. The outcome would provide a new insight into obesity/energy metabolism studies and their therapies.

【Publications Relevant to the Project】

・ Tsutsumi, K., Matsuda, M., Kotani, M., Mizokami, A., Murakami, A., Takahashi, I., Terada, Y., Kanematsu, T., Fukami, K., Takenawa, T., Jimi, E. and Hirata, M.: Involvement of PRIP, phospholipase C-related but catalytically inactive protein, in bone formation. *J. Biol. Chem.* 286:31032-31042, 2011.

・ Gao, J., Takeuchi, H., Zhang, Z., Fukuda, M. and Hirata, M.: Phospholipase C-related but catalytically inactive protein (PRIP) modulates synaptosomal-associated protein 25 (SNAP-25) phosphorylation and exocytosis. *J. Biol. Chem.* 287:10565-10578, 2012.

【Term of Project】 FY2012-2016
【Budget Allocation】 167,700 Thousand Yen

【Homepage Address and Other Contact Information】

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