Title of Project : Study of Cerebral Synapses and Circuits Using Two-photon Microscopy and Novel Optoprobes

Haruo Kasai
(The University of Tokyo, Graduate School of Medicine, Professor)

Research Project Number : 26221001　Researcher Number : 60224375
Research Area : Neuroscience
Keyword : Synapse, cerebral cortex, neural plasticity, functional imaging, neuroendocrinology

【Purpose and Background of the Research】

It is widely accepted that higher-order functions of the brain, particularly of the cerebral cortex, represent our cognition and mind. Recent functional brain imaging has revealed detailed localizations of various brain functions to specific regions in the cortex, indicating that various forms of perception, emotion, and executive functions are caused by the operation of neuronal networks in the corresponding cortical regions. Neurons extend long axons to numerous other neurons in the brain to form extensive neuronal networks, where electrical signals are transmitted via "synapses". These electrical activities, however, exist even in unawakened states of animals and hence, cannot completely account for the awake state of the brain.

We have revealed that synapses in the cerebral cortex change their shapes when their connectivity changes. Such motile synapses (spine synapses) are particularly well developed in the cerebral cortex. The motility of synapses can be induced by synchronous firing of neurons, which represent a coherent operation of neuronal circuits. The motility can be long lasting and leave traces, in a manner consistent with memory formation. A neuron in the cerebral cortex possesses thousands of spine synapses, and their motility, rather than neuronal electrical activity alone, can encode for highly diverse states.

We will develop novel methods for revealing functions of motile synapses in awake behaving animals, and in visualizing the cell-synapse assemblies responsible for various cognitive functions.

【Research Methods】

Cortical synapses can be visualized by two-photon microscopy both *in vitro* and *in vivo*. We have developed optoprobes to label and manipulate spine synapses, which are involved in learning and memory. Thus, we now can identify synapses that are involved in memory, and delineate the circuits involved in cell-synapse assembly in the cortex. We will also visualize the motilities of synapses in awake mice, and study their dependence on vigilance, stimulus selectivity, and so on. The consequences of synapse motility will be also analyzed for the presence of non-classical interactions of synapses.

【Expected Research Achievements and Scientific Significance】

Our study will clarify whether neuronal motilities, in addition to neuronal electrical activity, play essential roles in higher-order brain functions. Based on their influence on the neuronal network activity, we may obtain new insights into the localization of higher-order brain functions in specific regions of the brain and also into the mechanisms behind various mental disorders.

【Publications Relevant to the Project】

・Takahashi, N., Hatakeyama, H., Okado, H., Noguchi, J., Ohno, M. & Kasai, H. (2010). SNARE conformational changes that prepare vesicles for exocytosis. *Cell Metabolism* 12:19-29.
・Hayama, T., Noguchi, J., Watanabe, S., Ellis-Davies, G.C.R., Hayashi, A., Takahashi, N., Matsuzaki, M. & Kasai, H. (2013). GABA promotes the competitive selection of dendritic spines by controlling local Ca^{2+} signaling. *Nature Neurosci.* 16:1409-1416.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.bm2.m.u-tokyo.ac.jp
hkasai@m.u-tokyo.ac.jp

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

Title of Project : Connectomics Analysis of the Neural Networks that Regulate the Behavior of *Drosophila*

Kei Ito
(The University of Tokyo, Institute of Molecular and Cellular Biosciences, Associate Professor)

Research Project Number : 26221002　Researcher Number : 00311192
Research Area : General Neuroscience
Keyword : neural computation, connectomics, model animal, imaging, *Drosophila*

【Purpose and Background of the Research】

Output of reward- or punishment-associated learning and memory induces attractive or repulsive behaviors. However, little is understood about how signals from the learning centers are conveyed to the motor control centers to regulate such behaviors. The lack of detailed knowledge about the neural network architecture connecting these centers has made it difficult to address this problem.

The fruit fly *Drosophila melanogaster* exhibits a wide range of behaviors comparable with that of lower mammals in spite of its small brain size. Thanks to the sophisticated molecular-genetic techniques and extensive transgenic strain resources to visualize and to functionally manipulate specific neurons, it is a very powerful model system for comprehensive neural network analysis. In this project we will systematically identify neurons that connect learning and motor control centers, and reveal their roles in behavior control with sophisticated imaging and functional analyses.

【Research Methods】

Using more than 10,000 *Drosophila* transgenic expression driver strains established by us as well as by other groups, we will first identify neurons that arborize in the input/output regions of the leaning centers and dendritic regions of the motor control centers. Distributions of pre- and postsynaptic sites of these neurons will also be revealed to understand information flow. Combining these data, we will reveal direct and indirect connections from the leaning centers to motor centers.

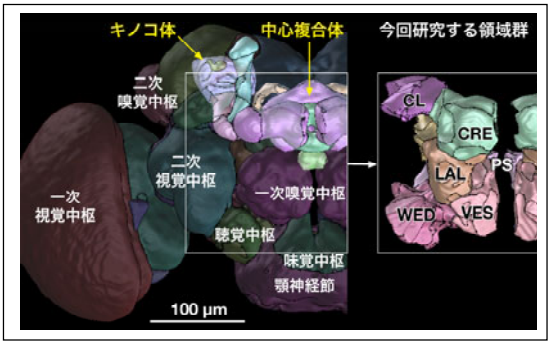


Figure 1　Components of the *Drosophila* brain

We will also investigate the activities of the identified neurons in relation to specific behaviors through imaging analysis with transgenic Calcium sensors, and reveal their roles in behavioral regulation by specifically blocking their neural activities using expression of transgenic toxins and by ectopically stimulating their activities with the expression of heat- or light-inducible ion channels.

【Expected Research Achievements and Scientific Significance】

Both in vertebrate and invertebrate brain sciences, conventional studies tend to focus on well-known brain regions with relatively simple neuronal architecture, leaving other regions uninvestigated in spite of their potential importance. This is the first attempt to shed lights on such less known, reticular parts of the brain. Systematic analysis of the neural networks and their functions should help understanding the way how associative learning centers should control animal behaviors.

【Publications Relevant to the Project】

・Ito.K., Shinomiya, K., Ito, M., Armstrong, D., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A., Keshishian. H., Restifo, L., Rössler, W., Simpson, J., Strausfeld, N. J., Strauss, R., and Vosshall, L.B; The Insect Brain Name Working Group. A systematic nomenclature for the insect brain. *Neuron*, **81**, 755-765, 2014.
・Ito, M., Masuda, N., Shinomiya, K., Endo, K., and Ito, K. Systematic analysis of neural projections reveals clonal composition of the *Drosophila* brain. *Curr. Biol*, **23**, 644–655, 2013.

【Term of Project】 FY2014-2018

【Budget Allocation】 128,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://jfly.iam.u-tokyo.ac.jp/lab/
itokei@iam.u-tokyo.ac.jp

Title of Project : Elucidating the Neural Mechanism to Generate the "Partial Awareness" by Large-scaled Neuron Network Analysis and Circuit Manipulation Techniques in Non-human Primates

Tadashi Isa
(National Institute of Natural Sciences, National Institute for Physiological Sciences, Professor)

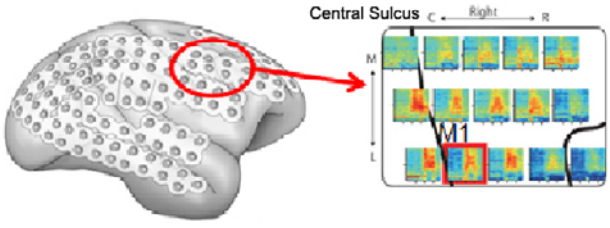
Research Project Number : 26221003　Researcher Number : 20212805
Research Area : Neuroscience
Keyword : Neural circuit, awareness, blindsight, primate, electrocorticography (ECoG)

【Purpose and Background of the Research】

It is known that some patients with damage to the primary visual cortex (V1) shows residual visuo-motor capacity to visual targets presented in the lesion-affected visual field, which is called "blindsight". To understand the neural mechanism of blindsight, we have been studying the cognitive function and neural activity of non-human primate models of blindsight with unilateral lesion of V1. The results of a series of studies have suggested that these monkeys has partial awareness of visual objects, which mimics "type II blindsight" human subjects, seen in patients who received V1 lesion at relatively young age, and in these animals, activity related to the partial awareness could be found in the midbrain superior colliculus. In this study, we aim at elucidating the neural mechanism of partial awareness by large-scaled neural recording from the blindsight monkeys.

【Research Methods】

We will place more than 100 channels of ECoG electrodes to cover the entire brain of monkeys as in the figure, and deep needle electrodes in deep brain structures such as pulvinar and superior colliculus to record local field potentials in monkeys with unilateral V1 lesion, an animal model of human blindsight. And then, we will analyze the default-mode network and visuo-motor responses during performance of visually guided saccades, and infer the circuit dynamics by analyzing the Granger causalities among signals recorded from individual channels. Based on such analysis platform, we aim at elucidating the neural mechanism of partial awareness by analyzing the difference in the state of the large-scaled network between the conditions with visual awareness and without awareness to the same visual stimuli, and change in the network dynamics induced by selective manipulation of individual components of the circuit.



(Figure) Pan-brain ECoG recording in monkey brain (adopted from Fujii and colleagues)

【Expected Research Achievements and Scientific Significance】

Recently, a large number of studies have been devoted to access the cognitive functions including attention and consciousness by applying fMRI imaging to the human brain. However, slow sampling frequency of the MRI imaging and limitation in manipulation of the human brain activity made it difficult to make in-depth analysis on the dynamics of the neural circuit. In this study, we will apply the large-scaled electrophysiological recording of brain activities combined with selective manipulation of specific component of the circuits by using optogenetics or other pharmacogenetical techniques with gene introduction with viral vectors to the brain of our blindsight model monkeys. Such studies are currently possible only in our group all over the world.

【Publications Relevant to the Project】

・Isa T, Yoshida M. (2009) Saccade control after V1 lesion revisited. Curr Opin Neurobiol, 19: 608 - 614.
・Takaura K, Yoshida M, Isa T (2011) Neural substrate of spatial memory in the superior colliculus after damage to the primary visual cortex. J Neurosci, 31: 4233-4241.
・Watanabe H, Sato M, Suzuki T, Nambu A, Nishimura Y, Kawato M, Isa T (2012) Reconstruction of movement-related intra -cortical activity from micro electrocorticogram array signals in monkey primary motor cortex. J Neural Eng, 9:036006
・Weiskrantz, L. (1986). Blindsight. A case study and implications., (Oxford: Clarendon Press).

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nips.ac.jp/hbfp/

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

Title of Project: Development of New *in vivo* Imaging Technologies by Using "Biological Optical Window"

Satoru Takahashi
(University of Tsukuba, Faculty of Medicine, Professor)

Research Project Number : 26221004 Researcher Number : 50271896
Research Area : Experimental Animal
Keyword : Research bio-resources

【Purpose and Background of the Research】

In this project, we aim to improve non-invasive *in vivo* fluorescent imaging technology by developing mice that enable monitoring of angiogenesis, tissues fibrosis and intensity of pain. We have achieved this using a novel fluorescent protein, iRFP and its derivatives, which have excitation and emission wavelengths in the "biological optical window".

【Research Methods】

1. Development of the fundamental technology required to improve the efficiency of *in vivo* imaging.
1-1. Development of a mouse expressing proteins that fluoresce in the near-infrared : We have developed a fluorescent observation method using iRFP and its derivatives.
1-2. Development of iRFP that enables repeated and stage-specific observation: We have developed *in vivo* imaging technology that enables repeated and stage-specific observation of fluorescence by developing Deg-iRFP.
1-3. Development of a custom-made melanin inhibition method: We developed technology to create albino mice by introducing a point mutation into the Tyrosinase gene using the CRISPR/Cas9 system.
1-4. Development of a custom-made body hair inhibition method: Body hair becomes an inhibitory factor when a mouse is studied by fluorescence. We have established a technique that enables introduction of the hair less (*HR^{hr}*) mutation using the CRISPR/Cas9 system.

2. Development of mice that enable the monitoring of various clinical conditions.
2-1. Development of a mouse that enables tracking of specific cells with iRFP: We expressed iRFP only in specific cell groups and in a variety of previously developed Cre-driver mice to produce a mouse that enables tracking of specific cells expressing iRFP.
2-2. Development of a mouse that enables monitoring of angiogenesis: We have developed a mouse which enables angiogenesis to be followed *in vivo* and in a stage-specific manner by inserting iRFP or Deg-iRFP into the murine Flk1 and Flt1 genes.
2-3. Development of a mouse that enables monitoring of fibrosis: We have developed a mouse that enables stage-specific *in vivo* monitoring of type 1 collagen transcription. Collagen production increases during fibrosis after tissue damage.
2-4. Development of a mouse that enables monitoring of neural activity (intensity of pain): Using the previously described Deg-iRFP, we have developed a mouse that enables us to stage-specifically monitor neural activity history.

【Expected Research Achievements and Scientific Significance】

We attempt to expand the range of applications for *in vivo* fluorescent imaging technology, is a technique widely used in life sciences research, by developing a less invasive technique that utilizes the biological optical window. Furthermore, we envisage that our techniques will lead to pain reduction in experimental animals and reduce the number of animals required to conduct statistically valid analyses.

【Publications Relevant to the Project】

- Mizuno S, Dinh TT, Kato K, Mizuno-Iijima S, Tanimoto Y, Daitoku Y, Hoshino Y, Ikawa M, Takahashi S, Sugiyama F, Yagami KI. Simple generation of albino C57BL/6J mice with G291T mutation in the tyrosinase gene by the CRISPR/Cas9 system. ***Mamm Genome.*** 2014.
- Tran TNM, Tanaka J, Hamada M, Sugiyama Y, Sakaguchi S, Nakamura M, Takahashi S, Miwa Y. *In vivo* image analysis using iRFP transgenic mouse. ***Exp Animal.*** *in press.*

【Term of Project】 FY2014-2018

【Budget Allocation】 88,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.md.tsukuba.ac.jp/basic-med/anatomy/embryology/index.html

Title of Project : Investigation of Differential Immune Status among Cancer Patients and Development of Personalized Cancer Therapy by Combining Immunomodulation

Yutaka Kawakami
(Keio University, School of Medicine, Professor)

Research Project Number : 26221005　Researcher Number : 50161287
Research Area : Biological Sciences, Oncology, Tumor therapeutics
Keyword : Cancer therapy, Personalized medicine, Immunotherapy, Immunomodulation

【Purpose and Background of the Research】

Immune status varies among cancer patients, and was reported to correlate with patients' prognosis and response to cancer therapy. However, it has not been established. The investigation of its mechanism and role in cancer treatment may lead to the understanding of cancer immunopathology and the development of new cancer therapy by combining immunomodulation.

Based on our previous researches, we are proposing the hypothesis that differential immune status is defined by balance of the anti-tumor immune induction pathway which is regulated by status of endogenous tumor antigens and immune-reactivity of patients, and the immunosuppression pathway which is regulated by oncogene and signal alterations in cancer cells.

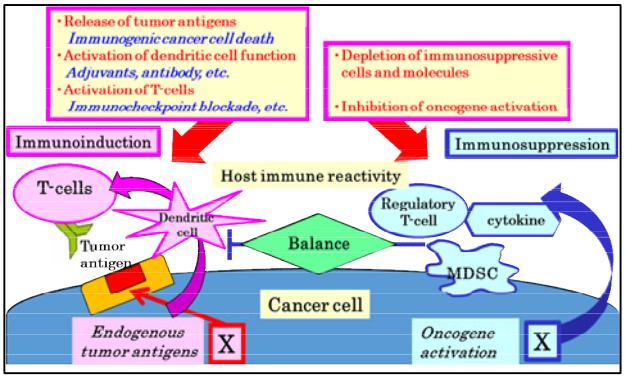
In this study, we will attempt to develop new cancer treatment strategy by modulation of immune-status through researches on immunopathology using system biological and immunological analyses of clinical samples and *in vivo* experiments using various murine models.

【Research Methods】

1. Investigation of the anti-tumor T-cell induction pathway and the development of its enhancing methods: Confirmation of the recognition of endogenous tumor antigens (e.g. mutated peptides) by tumor infiltrating T-cells. Investigation of the differential expression of immune molecules and cells, and their modulating strategies in the T-cell induction pathway.

2. Investigation of the cancer induced immunosuppression pathway and the development of its improving methods: Analyses of immunosuppressive conditions regulated by immune cells, stromal cells, and cancer cell subsets by using system biological and immunological analyses of clinical samples and *in vivo* experiments using various murine models. Development of their modulating strategies.

3. Investigation of enhancement of anti-tumor effects of various cancer therapies by immunomodulation: Evaluation of the identified immunomodulators on the augmentation of anti-tumor effects of various cancer therapies. Proposal of clinical trials after confirmation of the role of the identified immune-targets by correlation analyses between target expression and various clinicopathological factors.



Figure, Differential immune status and augmentation of cancer therapy by immunomodulation

【Expected Research Achievements and Scientific Significance】

This study's unique goal is development of personalized cancer therapy by targeting immunopathology of cancer. This study will lead to not only advance of cancer biology but also development of new diagnostic and therapeutic strategies, and will contribute to the society.

【Publications Relevant to the Project】

1. Kawakami Y, et al. Roles of signaling pathways in cancer cells and immune cells in generation of immunosuppressive tumor associated microenvironments. in "The Tumor Immuno-environment", Springer Science p307-323, 2013
2. Galon J, Kawakami Y, et al. Towards the introduction of the Immunoscore in the classification of malignant tumors. J Pathol. 232: 199-209, 2014

【Term of Project】 FY2014-2018

【Budget Allocation】 150,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://keiocancer.com/

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

Title of Project : Signal Transduction Networks Regulating Life-span and Development

Eisuke Nishida
(Kyoto University, Graduate School of Biostudies, Professor)

Research Project Number : 26221101 Researcher Number : 60143369
Research Area : Functional biochemistry
Keyword : Cell signal transduction

【Purpose and Background of the Research】

The nematode Caenorhabditis elegans (C. elegans) is a powerful model organism for studying animal life-span, because it is amenable to molecular genetic approaches, and has a short life-span. We have previously found that intermittent fasting, a dietary regimen with repeated cycles of fasting and ad libitum feeding, extends C. elegans life-span more efficiently than calorie restriction, another dietary regimen in which food intake is chronically restricted. Moreover, we have uncovered fasting-driven intracellular signaling pathways, transcription factors, and downstream longevity-related genes (Honjoh et al., Nature 457, 726-730. 2009; Uno et al., Cell Rep. 3, 79-91. 2013). The first purpose of this research project is to extend our above findings by further elucidating signal transduction networks regulating fasting (or other external stress)-induced longevity, especially focusing on related epigenetic pathways and small chemicals.

The second purpose is to elucidate signal transduction networks regulating developmental processes. We previously found that control of the duration of ERK MAP kinase activity is essential for dorsoventral patterning in Xenopus embryos (Hanafusa et al., Nature Cell Biol. 11, 106-109, 2009). We also found that the kinase SGK1, whose expression is shown to be induced by sustained ERK activation, promotes ectodermal cell survival in Xenopus embryos through a non-cell-autonomous signaling pathway (Endo et al., Sci. Signal. 4, ra2, 2011). In this research project, we will examine developmental roles of ERK signal transduction networks, especially focusing on ERK-regulated ectodermal genes. Also, we will examine developmental roles of other MAP kinases and related signaling pathways. Moreover, we will search external environment factors (such as nutrients and mechanical stress) regulating development and regeneration, and identify related intracellular signaling pathways as well as related epigenetic pathways.

【Research Methods】

We use C. elegans and Xenopus as model organisms to examine signal transduction networks regulating life-span and development. We will carry out gene expression analysis by microarray and next-generation sequencing, promoter analysis using bioinformatics (Sunadome et al., Dev. Cell 20, 192-205. 2011; Uno et al., Cell Rep. 3, 79-91. 2013), systematic RNA interference screening, systematic screening of mutants, knockdown experiments using antisense morpholino oligonucleotides, and chemical biology approaches.

【Expected Research Achievements and Scientific Significance】

Our research project will uncover novel signal transduction networks and related epigenetic pathways regulating life-span and development, as well as their interrelationships with external environment factors including nutrients and stresses. The future goal is to promote comprehensive understanding of molecular mechanisms regulating life-span and development.

【Publications Relevant to the Project】

Uno, M., Honjoh, S., Matsuda, M., Hoshikawa, H., Kishimoto, S., Yamamoto, T., Ebisuya, M., Yamamoto, T., Matsumoto, K., and Nishida, E. A fasting-responsive signaling pathway that extends life span in C. elegans. Cell Rep. 3, 79-91 (2013).
Endo, T., Kusakabe, M., Sunadome, K., Yamamoto, T., and Nishida, E. The kinase SGK1 in the endoderm and mesoderm promotes ectodermal survival by down-regulating components of the death-inducing signaling complex. Sci. Signal. 4, ra2. (2011).

【Term of Project】

FY2014-2018

【Budget Allocation】

150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.lif.kyoto-u.ac.jp/labs/signal/

Title of Project : Single-molecule Physiology by Assistance with a Soft Force

Kazuhiko Kinosita, Jr.
(Waseda University, Faculty of Science and Engineering, Professor)

Research Project Number : 26221102 Researcher Number : 30124366
Research Area : Biophysics
Keyword : Single-molecule measurements and manipulation; Structure, dynamics and functions of proteins and nucleic acids

【Purpose and Background of the Research】

A single protein molecule, a tiny entity mere millionth of a centimeter, performs a marvelous function(s) and hence is called a molecular machine. Examples include an ion channel that selects a particular species of ions and let them pass across a membrane in response to an electrical signal, and a rotary molecular motor F_1-ATPase. Mechanisms of these molecular machines can by studied by single-molecule physiology where one watches, and manipulates if needed, individual molecules at work under a microscope.

Manipulations have so far been mostly in the negative direction, obstructing or impeding the machine. Observations of natural or obstructed behaviors do not necessarily lead to an unequivocal interpretation. The best way to understand a complex system such as the lever escapement (Fig. 1) would be to "let it work" with your fingers, which also "feel" whether the move is right. We propose to do this on molecular machines. We deprive a molecular machine of its energy source, or delete its important part, and ask, instead of whether the machine fails, whether there is a way to let it work.

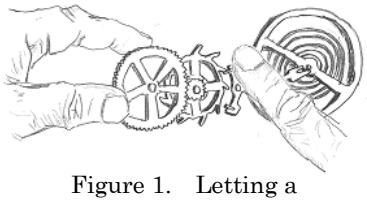


Figure 1. Letting a mechanical clock tick by

【Research Methods】

Our current goals are depicted in Fig. 2a-c.

A voltage-gated ion channel (Fig. 2a) is supposed to open when its voltage-sensor domains with multiple charges move in response to an applied voltage. Voltage, however, could exerts many effects on different parts of the channel and the membrane. We will directly pull the sensors to prove that their movement alone suffices, and inquire how much force is needed to open the channel.

F_1-ATPase, when alone, rotates by hydrolyzing ATP. In nature, it is joined to another motor F_o, which forcibly rotates F_1 in reverse, resulting in reversed hydrolysis, or ATP synthesis. To explore how, we replace F_o with magnets and apply a soft force for reverse rotation (Fig. 2b) to feel how F_1 reacts or how it may occasionally rotate on its own. We will also study reverse gyrase which winds up DNA double helix (Fig. 2c), probably for protection against thermal melting. We will twist DNA with magnets to assist, or obstruct, the enzyme.

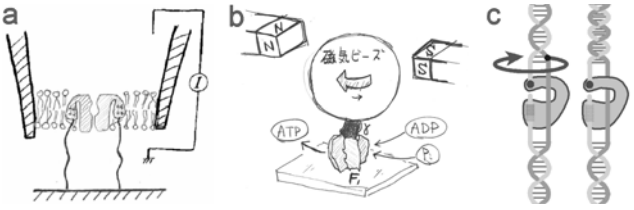


Figure 2. Letting protein machines work by a soft force.

【Expected Research Achievements and Scientific Significance】

It must be a formidable challenge to let a molecular machine work by hands. But the reward would be a decisive answer that this particular force or movement IS the causal key to the whole operation. We propose this high level of single-molecule physiology, which may also lead to the creation of new functions or new machines.

【Publications Relevant to the Project】

K. Adachi, K. Oiwa, M. Yoshida, T. Nishizaka, and K. Kinosita Jr. "Controlled rotation of the F_1-ATPase reveals differential and continuous binding changes for ATP synthesis" *Nat. Commun.* **3** (2012) 1022.

K. Yogo, T. Ogawa, M. Hayashi, Y. Harada, T. Nishizaka, and K. Kinosita Jr. "Direct observation of strand passage by DNA-topoisomerase and its limited processivity" *PLoS ONE* **7** (2012) e34920.

【Term of Project】 FY2014-2017

【Budget Allocation】 115,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.k2.phys.waseda.ac.jp

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

Title of Project : Higher-Order Functions of Stomatal Guard Cells in Plant Environmental Adaptation

Koh Iba
(Kyushu University, Faculty of Sciences, Professor)

Research Project Number : 26221103 Researcher Number : 10192501
Research Area : Plant Physiology
Keyword : stomatal responses to the environment

【Purpose and Background of the Research】

Stomata in the epidermis, formed by pairs of guard cells, serve as major gateways for gas exchange between plants and their environment, in particular the uptake of CO_2 and evaporation of water – processes vital to plant life. Guard cells integrate external environmental and intrinsic developmental signals and appropriately adjust the stomatal pore apertures to optimize growth performance.

Leaf temperature provides a convenient indicator of transpiration, and can be used to detect mutants with altered stomatal control. To identify genes that function in physiological responses in guard cells, we isolated CO_2-insensitive mutants from *Arabidopsis* through high-throughput leaf thermal imaging (**Fig. 1**). In the present study, the stoma is treated as the main organ of higher-order information processing in plants, and searches will be made for factors carrying and processing information about the external environment and internal conditions, and for factors involved in communication with other organs.

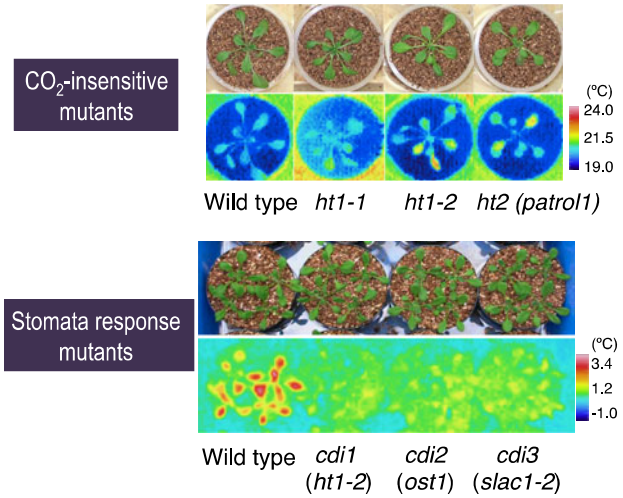


Fig. 1 Isolation of stomatal response mutants using a high-throughput thermal imaging technique.

【Research Methods】

(1) Guard cell chloroplasts are hypothesized to carry some of the higher-order functions of stomatal guard cells. We will analyze the role of guard cell chloroplasts in stomatal higher-order information processing using *gles1* mutants with non-chlorophyllous stomata.

(2) A Dof transcription factor, SCAP1, is essential for the development of functional stomata. We will analyze the formation of functional stomata by identifying factors regulating the expression of the *SCAP1* gene and of the direct target genes of SCAP1.

【Expected Research Achievements and Scientific Significance】

Stomatal higher-order information processing plays a pivotal role in the adaption and survival of plants in diverse environments, but many aspects of the underlying molecular mechanism remain unknown. Through the present study, we expect to develop a paradigm for the general characteristics of information processing in plants by demonstrating the mechanisms of the compilation and integration of higher-order information in stomata.

【Publications Relevant to the Project】

・Hashimoto-Sugimoto, M., Higaki, T., Negi, J., Hasezawa, S. and Iba, K. (2013) A Munc13-like protein in *Arabidopsis* mediates H^+-ATPase translocation that is essential for stomatal responses. ***Nature Commun.*** 4:2215 doi: 10.1038/ncomms3215.

・Negi, J., Hashimoto, M. and Iba, K. (2008) CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. ***Nature*** 452: 483-486.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://plant.biology.kyushu-u.ac.jp

Title of Project : Coordinated Regulation of Reproduction and Sexual Behavior by Peptidergic Neurons

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

Research Project Number : 26221104　Researcher Number : 70143360
Research Area : Biology: Animal Physiology and Behavior
Keyword : Neurobiology, Neurophysiology, Peptidergic neurons, GnRH, Kisspeptin

【Purpose and Background of the Research】

Animal reproduction is regulated by coordination of nervous and endocrine systems. Environmental factors such as temperature and day length are received and transmitted to the nervous and endocrine systems, and reproductive success will be achieved by this coordinated regulatory mechanism.

Here, we aim to elucidate the mechanisms of coordinated regulation of the both systems by taking advantage of the unique small fish brain model systems, which we have developed and have been utilizing to analyze the physiological functions of two kinds of peptidergic neurons, gonadotropin-releasing hormone (GnRH) and kisspeptin neurons. We will also elucidate the origin of diversity of neural functions of paralogous peptidergic systems from the view point of the evolutionary biology.

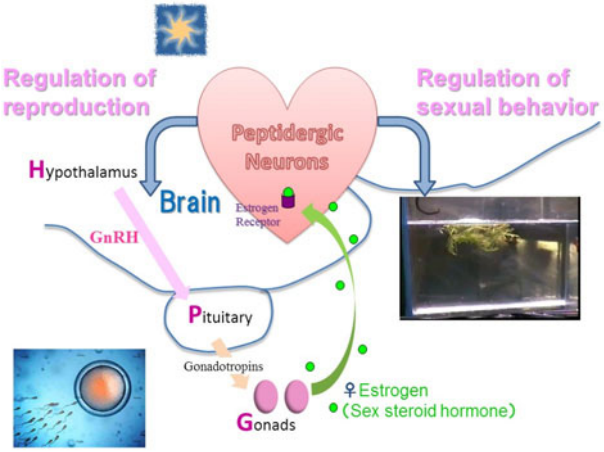


Figure 1. Coordinated regulation of reproduction and sexual behavior by peptidergic neurons

【Research Methods】

We will use various state-of-the-art multidisciplinary techniques in neurobiology such as transgenic technology, molecular biological, electrophysiological, neuroanatomical, and behavioral techniques, to name a few. We will focus on the following two topics.

1) Neural and hormonal mechanisms of hypothalamo-pituitary-gonadal axis regulation, which underlies the regulation of reproduction

2) Mechanisms underlying the coordinated regulation of reproduction and sexual behavior, which may involve RFRPs, kisspeptins (kiss1&2), and GnRH1&3 neurons.

【Expected Research Achievements and Scientific Significance】

In addition to understanding the neural and hormonal mechanisms of coordinated regulation of reproduction and sexual behavior by the above mentioned peptidergic neurons, the present research project may lead to the finding of novel types of neurons and mechanisms by analyzing the sex steroid hormone receptor-expressing GFP transgenic medaka.

【Publications Relevant to the Project】

・Karigo, T., Kanda, S., Abe, H., Okubo, K., and Oka, Y. (2012) Time-of-day dependent changes in GnRH1 neuronal activities and gonadotropin mRNA expression in a daily spawning fish, medaka. Endocrinology 153: 3394-3404.
・Kanda, S., and Oka, Y. (2012) Evolutionary insights into the steroid sensitive kiss1 and kiss2 neurons in the vertebrate brain. Frontiers in Genomic Endocrinology, 3:28. doi: 10.3389/fendo.2012.00028.
・Karigo, T., and Oka, Y. (2013) Neurobiological study of fish brains gives insights into the nature of gonadotropin-releasing hormone 1-3 neurons. Frontiers in Endocrinology", 4:177. doi: 10.3389/fendo.2013.00177.

【Term of Project】 FY2014-2018

【Budget Allocation】 77,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.biol.s.u-tokyo.ac.jp/users/naibunpi/
okay@bs.s.u-tokyo.ac.jp

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

Title of Project: Controlling Mechanism of Epigenome by Silencing and Anti-silencing

Tetsuji Kakutani
(Research Organization of Information and Systems, National Institute of Genetics, Professor)

Research Project Number : 26221105 Researcher Number : 20332174
Research Area : Genetics, chromosome dynamics
Keyword : Epigenetics, DNA methylation, Arabidopsis, chromatin

【Purpose and Background of the Research】

Epigenome dynamics is controlled by silencing and anti-silencing. However, information for the latter (anti-silencing) is limited. We are taking genetic and genomic approaches using mutants of Arabidopsis. By such approaches, we are uncovering novel anti-silencing mechanisms, which affect development and genome dynamics. In this project, we utilize these materials and solve new questions, such as control of development by heterochromatin and molecular mechanisms for the novel DNA demethylating activity.

【Research Methods】

Project1 "control of heterochromatin and its effect on development" In the mutants of a histone demethylase gene *IBM1*, heterochromatin accumulate in gene body, which is associated with diverse developmental defects (Saze et al 2008 Science; Miura 2009 EMBO J; Inagaki et al 2010 EMBO J). The heterochriomatin accumulates progressively over generations, and developmental defects become severer. We screened mutants suppressing the developmental abnormalities of the *ibm1*. Some of the suppressors have mutation in DNA methylase or histone methylase, which disrupt heterochromatin. Some of mutants suppressed the developmental defects without affecting the heterochromatin. Using them, we will try to understand control of heterochromatin and its effect on development.

Project 2 "molecular mechanisms for novel DNA demethylating activity" Through characterization of a DNA transposon, we have identified VANC protein, which has DNA demethylating activity (Fu et al 2013 EMBO J). Expression of VANC induces demethylation of a group of related transposons (Figure).

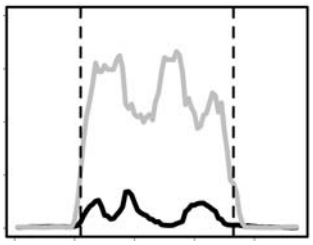


Figure – Transposon demethylation in transgenic line expressing VANC (black). Grey is a control line with methylated transposon

Demethylation induced by VANC is interesting in that entire length of transposon is affected, despite the strong specificity for the affected loci (Figure). To understand the mechanisms, we will examine distribution of VANC on chromosomes, screen proteins interacting VANC, and screen mutants affecting VANC activity.

【Expected Research Achievements and Scientific Significance】

(Project1) We will uncover mechanisms controlling heterochromatin during development. We also understand pathway between heterochromatin accumulation and developmental defects.
(Project2) We will understand mechanism for the novel DNA demethylating activity and identify host factors involved in that.

【Publications Relevant to the Project】

・ Fu Y, Kawabe A, Etcheverry M, Ito T, Toyoda A, Fujiyama A, Colot V, Tarutani Y, Kakutani T (2013) Mobilization of a plant transposon by expression of the transposon-encoded anti-silencing factor. ***EMBO J.*** 32, 2407-2417
・ Inagaki S, Miura-Kamio A, Nakamura Y, Lu F, Cui X, Cao X, Kimura H, Saze H, Kakutani T. (2010) Autocatalytic differentiation of epigenetic modifications within the Arabidopsis genome. ***EMBO J*** 29, 3496-3506.
・ Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, and Kakutani T (2009) Bursts of retrotransposition reproduced in Arabidopsis. ***Nature*** 303, 423-426.
・ Saze H, Shiraishi A, Miura A, and Kakutani T (2008) Control of Genic DNA methylation by a jmjC domain-containing protein in Arabidopsis thaliana. ***Science*** 319, 462-465

【Term of Project】

FY2014-2018

【Budget Allocation】

147,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nig.ac.jp/labs/AgrGen/home-j.html

Title of Project : Biological Synchronization in Natural Environments

Hiroshi Kudoh
(Kyoto University, Center for Ecological Research, Professor)

Research Project Number : 26221106　Researcher Number : 10291569
Research Area : Ecology
Keyword : Molecular Ecology

【Purpose and Background of the Research】

Synchronizations between individuals in biological responses are often observed under natural seasonal conditions. Synchronized flowering within a plant species is necessary for successful mating.

This project aims to understand functions of mechanisms that underlie synchronization of plant reproduction, especially in natural fluctuating environments. Based on the time-series analyses on seasonal transcriptome data, we will conduct following three studies.

1. Identification of genes that control termination of reproduction.
2. Understanding of function of gene regulatory networks under complex natural conditions.
3. Estimation of internal and external plant environments using transcriptome data.

【Research Methods】

Based on the time-series analyses on seasonal transcriptome data, we will apply following three approaches (Fig. 1).

1. Growth experiments and mutant hunting for genes that control reproductive termination.
2. Functional analyses of histone modification under complex natural conditions.
3. Modeling of internal and external plant environments from transcriptome data.

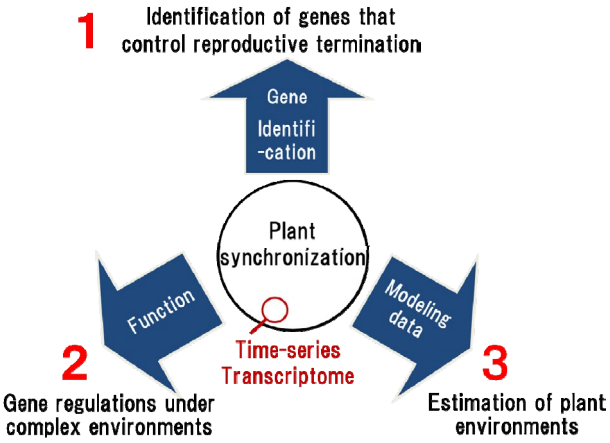


Fig. 1. Three approaches in the project.

【Expected Research Achievements and Scientific Significance】

Conventional study of reproductive timings of plants has been analyzed them as developmental events. Here, we aim to analyze reproductive timings as synchronizing events on calendar day in the natural seasonal environments (Fig. 2).

Following achievements are expected.

1. We will identify genes that determine the timing of reproductive termination.
2. Role of histone modifications as an environmental memory will be evaluated.
3. Modeling methods that estimate plant environments will be developed.

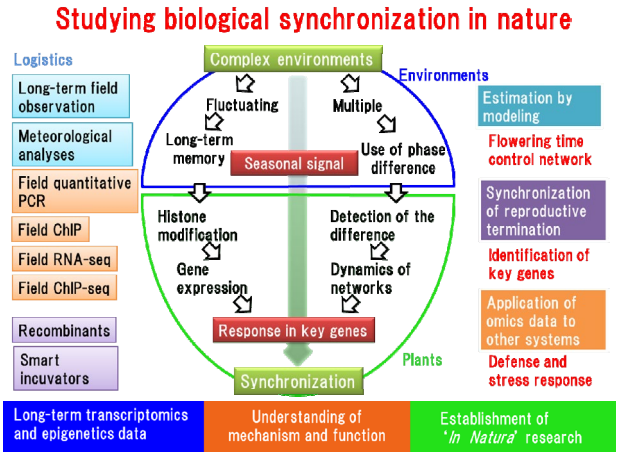


Fig. 2. A schematic diagram of the project.

【Publications Relevant to the Project】

Kudoh H, Nagano AJ (2013) Memory of temperature in the seasonal control of flowering time: an unexplored link between meteorology and molecular biology. Pontarotti P ed. *Evolutionary Biology: Exobiology and Evolutionary Mechanisms*, Springer : 195-215.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ecology.kyoto-u.ac.jp/~kudoh/index.html

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

Title of Project : Characterization of Molecular Evolution of Plant Isoquinoline Alkaloid Biosynthesis and its Application To Metabolic Engineering

Fumihiko Sato
(Kyoto University, Graduate School of Biostudies, Professor)

Research Project Number : 26221201 Researcher Number : 10127087
Research Area : Agricultural chemistry, Applied biological chemistry
Keyword : Metabolic engineering, Synthetic biology, Isoquinoline alkaloid production

【Purpose and Background of the Research】

Higher plants produce diverse low-molecular-weight chemicals such as alkaloids, terpenoids and phenylpropanoid compounds. Among these chemicals, alkaloids are particularly important in medicine due to their high biological activities. However, the low yield of metabolites, especially alkaloids, in plants limits large-scale development of the plant natural product industry. In this research, we characterize the molecular evolution of isoquinoline alkaloid (IQA) biosynthesis in higher plants and develop metabolically engineered plant cells and microbes that produce useful secondary metabolites in high yield and of high quality, using the latest techniques in metabolic engineering and synthetic biology.

【Research Methods】

Based on the molecular characterization of the genome structure of *Eschscholzia californica*, in which the IQA biosynthetic pathway has been intensively studied at the molecular level, all of the IQA biosynthetic enzyme genes and the entire enzyme network will be characterized to design more advanced metabolically engineered plant cells.

The biosynthetic pathways in other plants that produce IQAs, such as emetine in *Carapichea ipecacuanha*, galantamine in *Lycoris radiata*, aristolochic acid in *Aristolochia debilis* and so on, are characterized using RNA sequencing, metabolomic analysis and metabolic engineering.

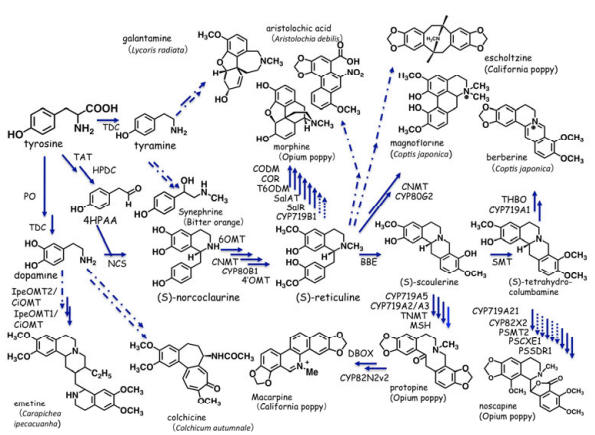


Figure 1 Biosynthetic pathway of isoquinoline alkaloids characterized in this study

With the use of this molecular information on IQA biosynthesis, IQA biosynthetic pathways are re-constructed in plant cells and/or microbial cells to produce the desired novel IQAs in high yield and with high quality. The functionalities of IQA products are also investigated using model animal cell systems.

【Expected Research Achievements and Scientific Significance】

Full characterization of isoquinoline alkaloid biosynthetic pathways at the molecular level should contribute to studies on the secondary metabolism in higher plants.

Furthermore, full information about the biosynthetic enzyme genes for IQAs should help us prepare molecular tools for re-constructing biosynthetic pathways to produce desired known and also novel IQAs.

Advanced metabolic engineering and synthetic biology should contribute to the development of plant and microbial cells that can produce useful IQAs in high yield and of high quality. These materials and the screening of biological activity performed in this study should provide useful information for the development of new medicines.

【Publications Relevant to the Project】

・Sato F, Kumagai H. (2013) Microbial Production of Isoquinoline Alkaloids as Plant Secondary Metabolites Based on Metabolic Engineering Research. **Proc. Jpn. Acad., Ser. B**, 89, 165-182.
・Nakagawa A, Minami H, Kim JS, Koyanagi T, Katayama T, Sato F, Kumagai H. (2011)□A bacterial platform for fermentative production of plant alkaloids. ・**Natre Comm.** 2, Article number: 326.

【Term of Project】 FY2014-2017

【Budget Allocation】 143,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.lif.kyoto-u.ac.jp/labs/callus/
fsato@lif.kyoto-u.ac.jp

Title of Project: Novel Preventive Strategy for Alzheimer's Disease Based on the "Toxic Conformation Theory" of Amyloid β

Kazuhiro Irie
(Kyoto University, Graduate School of Agriculture, Professor)

Research Project Number : 26221202 Researcher Number : 00168535
Research Area: Agricultural Chemistry, Bioorganic Chemistry
Keyword: chemical biology, Alzheimer's disease, amyloid β oligomer, functional food

【Purpose and Background of the Research】

Oligomers (2 or 3 x *n*-mer) of the amyloid β protein (Aβ42) play a neurotoxic role in the pathogenesis of Alzheimer's disease (AD). We previously identified a toxic conformer of Aβ42 (toxic Aβ), which had a turn at Glu22 and Asp23 to form toxic oligomers ("Toxic Conformation Theory", Figure). A monoclonal antibody (11A1) against toxic Aβ recognized intracellular Aβ (trimer). Although 11A1 is considered to be a recognition tool of trimers, there are currently no reagents that are specific for dimers. In this project, we will develop recognition tools that target dimers and trimers for the detection of toxic oligomers, and these tools will be used in the precise diagnosis of AD. We will also generate a novel mouse model of AD based on the toxic conformation theory to examine the preventive effects of antibodies and functional foods on the pathology of AD.

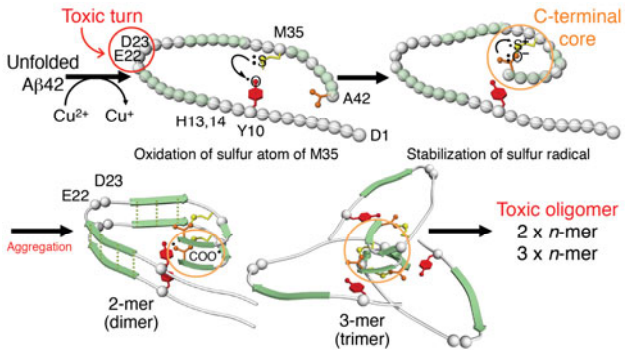


Figure 1 Proposed structure of Aβ42 oligomers with a toxic turn at E22 and D23 [Murakami, K. *et al.*, ***J. Am. Chem. Soc.***, *127*, 15168 (2005)]

【Research Methods】

1. *Development of recognition reagents against toxic Aβ as a diagnostic tool*

To develop anti-dimer reagents, the covalently-linked Aβ dimer based on the toxic conformation theory will be used as a hapten of antibodies and aptamers. The same hapten as 11A1 will be utilized as an anti-trimer reagent. Sandwich ELISA using these recognition tools will be established for diagnostic applications to the usage of biological samples.

2. *Evaluation of preventive effects of functional foods and antibodies on the pathology of AD using novel AD mice*

We will generate knock-in mice that harbor a toxic Aβ mutation as a novel AD model, and subsequently investigate the relationship between toxic Aβ and neuronal death. Using this novel model, the preventive effects of functional foods as well as the antibodies developed in the foregoing paragraph on the pathology of AD will be examined.

【Expected Research Achievements and Scientific Significance】

Since the development of therapeutic drugs is currently struggling in clinical trials, it is more essential to develop a precise diagnostic method for AD and preventive strategy by improving eating habits, both of which this project aims to realize based on the original toxic conformation theory of Aβ42.

【Publications Relevant to the Project】

・Sato, M., Murakami, K., Uno, M., Nakagawa, Y., Katayama, S., Akagi, K., Masuda, Y., Takegoshi, K., and *Irie, K.: Site-specific inhibitory mechanism for Aβ42 aggregation by catechol-type flavonoids targeting the Lys residues.
J. Biol. Chem., *288*, 23212-23224 (2013).
・Murakami, K., Horikoshi-Sakuraba, Y., Murata, N., Noda, Y., Masuda, Y., Kinoshita, N., Hatsuta, H., Murayama, S., Shirasawa, T., *Shimizu, T. and *Irie, K.: Monoclonal antibody against the turn of the 42-residue amyloid β-protein at positions 22 and 23.
ACS Chem. Neurosci., *1*, 747-756 (2010).

【Term of Project】 FY2014-2018

【Budget Allocation】 126,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.orgchem.kais.kyoto-u.ac.jp
irie@kais.kyoto-u.ac.jp

Title of Project： Identification and Application of the Bull Pheromone

Yuji Mori
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number：26221203　Researcher Number：40157871
Research Area：　Agricultural sciences
Keyword：　Pheromone, Bioactive molecules, Cattle, Veterinary science, Theriogenology

【Purpose and Background of the Research】

Improving the conception rate of dairy cows is one of the most important as well as urgent problems to be solved in the field of livestock industry. Annual cost related to the dairy reproduction problem is estimated to reach 100 billion yen in Japan.

This study is planned to contribute to overcome this problem by developing a new method, namely utilization of pheromones. In goats and sheep, small ruminant species closely related to cattle, the “male effect” has been studied and the mechanism underlying this powerful progonadal effect is now being elucidated. In contrast, little is known about cattle pheromone.

The present study, therefore, aims at identification of bull pheromone for innovating novel methods of treating as well as preventing reproductive problems in dairy cows.

In other words, the first object of the research is to isolate and identify the bull pheromone, which stimulates genital function of cows.

The second object is to design and create synthetic pheromones based on the information of chemical structure of natural pheromone and also to develop a wearable device system for exposing a cow to the pheromone on demand.

【Research Methods】

Core members of our research team have been collaborating over decades in the study of “male effect” in small ruminants. Along with past collaboration we will maintain similar research strategy and tactics. In addition, at the final stage of this study, a fairy large scale field study is going to be conducted (Figure 1).

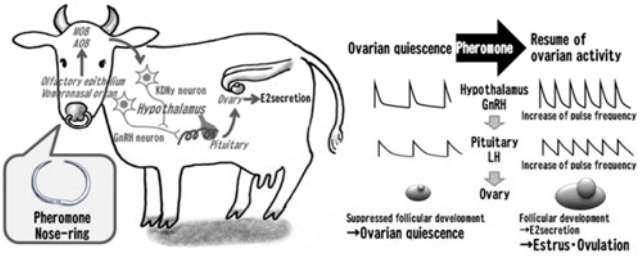


Figure 1 Conceptual illustration of the bull-pheromone usage for gonadal activation in cows

【Expected Research Achievements and Scientific Significance】

From basic scientific viewpoints including reproductive biology and neuroscience the information regarding the molecular structure of pheromone ligands and their receptors will provide insights into the evolution of chemical communication in mammals by comparing with those in other related species.

Moreover, tremendous benefit can be expected, once the pheromone is clinically applied to reproductive problems in cows such as follicular and/or luteal cysts, silent heat and delayed resumption of ovarian function postpartum. Now the conception rate at the first artificial insemination is below 50% in dairy cows, and this is a barrier against healthy management of dairy industry.

Our goal is to identify and utilize the bull-pheromone for solving these reproductive problems. As the pheromone is produced by a bull and has no worry about serious pollution or side effects, the research outcome will also be noticed internationally from the standpoint of environmental preservation as well as animal welfare.

【Publications Relevant to the Project】

Murata K., Tamogami S., Itou M., Ohkubo Y., Wakabayashi Y., Watanabe H., Okamura H., Takeuchi Y., Mori Y. (2014) Identification of an olfactory signal molecule that activates the central regulator of reproduction in goats. Current Biology 24: 681-686.

【Term of Project】

FY2014-2018

【Budget Allocation】

149,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.vm.a.u-tokyo.ac.jp/koudou/e-research.html
ve@vbm.jp (To avoid junk mails, please use "Veterinary Ethology" as the subject.)

Title of Project : Development of Novel Methods for Target Identification of Natural Products and their Application to Chemical Epigenetics

Minoru Yoshida
(RIKEN, Chemical Genetics Laboratory, Chief Scientist)

Research Project Number : 26221204　Researcher Number : 80191617
Research Area : Boundary Agriculture
Keyword : Epigenetics, Proteome, Target molecule

【Purpose and Background of the Research】

Naturally occurring bioactive small molecules (natural products) contain substances showing extremely potent and specific bioactivity. As in the case of penicillin, elucidation of the target molecules and mode of action has provided great impacts on biology. However, most of these studies were accomplished through a trial and error strategy and no rapid and systematic methodology for efficient drug target identification has been established. This project aims to develop a novel technology to detect the chemical-target interaction, thereby enabling systematic elucidation of the mode of action of target-unknown natural products and the function of the target molecules such as epigenetics. In addition, we establish a system to identify therapeutic targets based on information about genes responsible for the disease onset by using the concept of synthetic lethality.

【Research Methods】

An integrated system to identify target molecules of bioactive natural products will be developed. Specifically, we will establish barcode sequencing methods to determine chemical genomic profiles using a barcoded fission yeast gene deletion mutant collection and human cell culture infected with a pooled, barcoded shRNA library (Fig. 1) and bimolecular fluorescent complementation (BiFC) imaging to screen for protein-protein and chemical-protein interactions. By employing this target identification system integrated with affinity beads technology, the target molecules of microbial or marine natural products and their functional pathways will be systematically identified. In addition, we will find compounds with novel activities including epigenetic regulation.

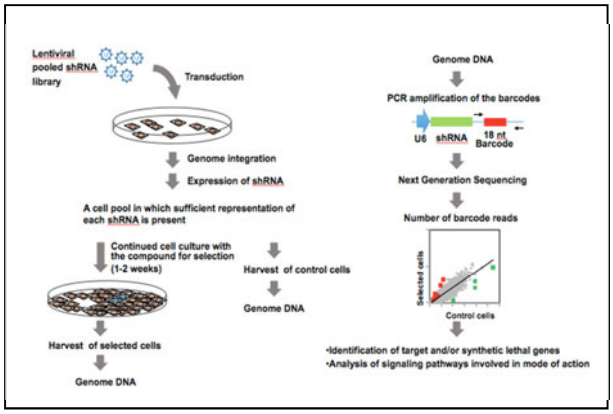


Fig. 1. Target identification by barcoded shRNA

【Expected Research Achievements and Scientific Significance】

A huge variety of microbes and plants in nature synthesize natural products with marvelous bioactivities. Their target identification has provided deep insights into drug discovery. As the process for the target identification resembles classical genetics, it is called chemical genetics, which generally uses compound-bound affinity probes. However, it is not always successful due to the unstable binding to the probes. It is therefore necessary to establish chemical genomics, an unbiased genome-wide methodology to screen for target molecules. This project will contribute to problem resolution in health, medicine, and environment, by facilitating an efficient use of bioactive substances.

【Publications Relevant to the Project】

- Nishimura, S., *et al.* Marine antifungal theonellamides targets 3β-hydroxysterol to activate Rho1 signaling. Nature Chem. Biol., 6: 519-526, 2010.
- Ito, T., *et al.* Real-time imaging of histone H4K12-specific acetylation determines the modes of action of histone deacetylase and bromodomain inhibitors. Chem. Biol., 18: 495-507, 2011.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.riken.jp/en/research/labs/chief/chem_genet/
http://www.riken.jp/en/research/labs/csrs/chem_genom/

Title of Project : Regioselective Molecular Transformation of Multifunctionalized Molecules

Takeo Kawabata
(Kyoto University, Institute for Chemical Research, Professor)

Research Project Number : 26221301 Researcher Number : 50214680
Research Area : Synthetic Organic Chemistry
Keyword : carbohydrate, peptide, supramolecule, molecular recognition, asymmetric synthesis

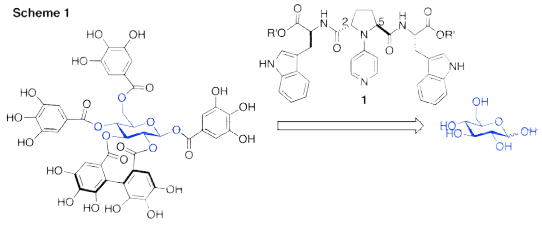
【Purpose and Background of the Research】

Regioselective manipulation of mutifunctionalized molecules such as carbohydrates has been developed via a multi-step protection-deprotection strategy based on the intrinsic reactivity of the substrate molecules. Direct regioselective transformation without protection- deprotection processes has been a long-standing object in organic synthesis. We aim to develop the methods for catalyst-controlled regioselective molecular transformation of multifunctionalized molecules, which includes those for short-step total syntheses of natural glycosides from unprotected D-glucose and regioselective cleavage of peptides under neutral conditions. Catalytic discrimination of supramolecular topological chirality will be also investigated.

【Research Methods】

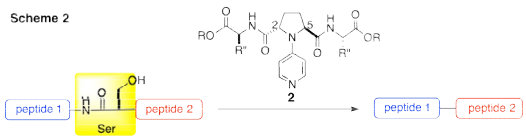
Catalyst **1** effectively promotes regioselective acylation at intrinsically less reactive C(4)-OH among four free hydroxy groups of glycopyranoses. We plan to develop a short-step total syntheses of natural glycosides staring from unprotected glucose via regioselective molecular transformation with catalyst **1** at the key step.

Scheme 1



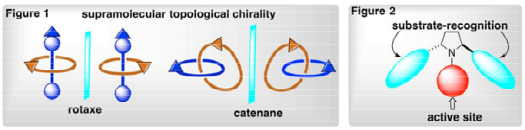
Catalyst **2** promotes regioselective peptide cleavage under neutral conditions at an ambient temperature. Scope and limitation of this reaction is examined.

Scheme 2



Dissymmetric achiral components constitute topological chirality in the formation of supramolecules such as rotaxanes and catenanes. Catalytic discrimination of this type of mobile topological chirality is investigated (Figure 1).

Regioselective molecular transformations in Schemes 1 and 2 can be achieved under fine molecular recognition by catalyst **1** and **2**. The amide side chains at C(2) and C(5) of the pyrrolidine skeleton are expected to be responsible for the molecular recognition. We plan to create new catalysts consisting of the side chains for molecular recognition and an active site toward the development of regioselective molecular transformations (Figure 2).



【Expected Research Achievements and Scientific Significance】

Unconventional approaches toward the synthesis and molecular transformation of ubiquitous molecules of biological interest such as carbohydrates and peptides will be developed. These approaches include (1) catalyst-controlled total synthesis of natural glycosides starting from D-glucose without using protective groups for glucose, and (2) regioselective peptide cleavage of serine-containing peptides. Approaches toward catalytic discrimination of topological chirality of supramolecules such as rotaxanes and catenanes will also be shown. A new catalyst design toward regioselective molecular transformations is also proposed. This project will contribute to open a new phase in organic synthesis.

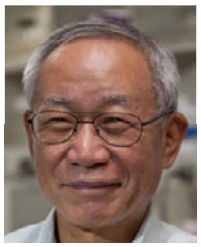
【Publications Relevant to the Project】

- Kawabata, T.; Muramatsu, W.; Nishio, T.; Shibata, T.; Schedel, H. "A Catalytic One-Step Process for the Chemo- and Regioselective Acylation of Carbohydrates", *J. Am. Chem. Soc.* **129**, 12890-12895 (2007).
- Yoshida, K.; Mishiro, Ueda, Y.; Shigeta, Furuta, T.; Kawabata, T. "Nonenzymatic Geometry-Selective Acylation of Tri- and Tetrasubstituted α,α'-Alkenediols", *Adv. Synth. Catal.* **354**, 3291-3298 (2012).

【Term of Project】 FY2014-2018

【Budget Allocation】 93, 600 Thousand Yen

【Homepage Address and Other Contact Information】 http://www.fos.kuicr.kyoto-u.ac.jp/
(kawabata@scl.kyoto-u.ac.jp)



Title of Project : Physiological Roles and Action Mechanisms of mDia-induced Actin Cytoskeleton in the Body

Shuh Narumiya
(Kyoto University, Graduate School of Medicine, Professor Emeritus)

Research Project Number : 26221302　Researcher Number : 70144350
Research Area : General medical chemistry
Keyword : Biomolecular medicine

【Purpose and Background of the Research】

Actin cytoskeleton plays critical roles in cell morphogenesis, adhesion, migration, proliferation and division. While much has been elucidated on how actin cytoskeleton is formed and functions in cultured cells, it remains largely unknown how actin cytoskeleton functions in tissue homeostasis in the body. We have generated mice deficient in each of three isoforms of mDia that catalyzes actin polymerization downstream of Rho in the cell, and revealed that the actin cytoskeleton produced by the Rho-mDia pathway functions in shaping brain structure and cytokinesis of erythroblasts. Here we combine analysis in KO mice and cultured cells, and further examine the functions of this mDia-induced actin cytoskeleton in processes such as neural plasticity in the presynapse, TCR signaling in lymphocytes, sperm morphogenesis and malignant cell transformation and cancer.

【Research Methods】

Here we examine the functions of mDia in four biological processes; 1, neural plasticity at the presynaptic terminal; 2, TCR signaling in lymphocytes; 3, sperm morphogenesis in the testis ; 4, malignant cell transformation and tumor formation in the skin. In 1, we already observed mDia 1/3-dependent contraction of the presynaptic terminal in primary culture of hippocampal neurons under tetrodotoxin treatment (Fig. 1), and examine the underlying mechanism of this phenomenon. We also examine physiological relevance of this mechanism by analyzing the stress behavior and synaptic response of mice that lack mDia1/3 specifically in nucleus accumbens. In 2, we already found impaired TCR signaling in thymocytes obtained from mDia1/3 double knockout (DKO) mice (Fig. 2). We examine how actin cytoskeleton induced by mDia/3 functions in immunological synapse formation and TCR microcluster dynamics there.

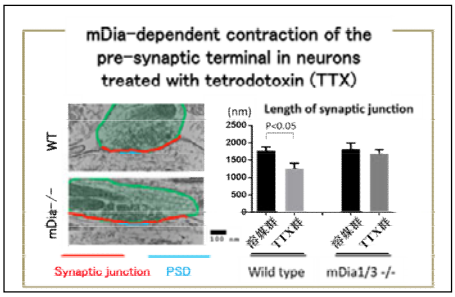


Figure 1

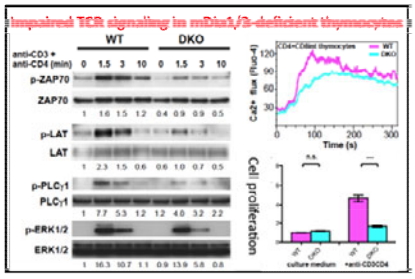


Fig. 2

In 3, we already observed impaired morphogenesis of sperm in mDia1/3 DKO testis, and this is due to the defect in Sertoli cells. We examine underlying mechanism. In 4, we combine cell transformation assay in vitro and DMBA/TPA-dependent skin carcinogenesis in vivo, and examine the role of mDia1 in cancer.

【Expected Research Achievements and Scientific Significance】

The above-mentioned experiments are expected to reveal, 1. a mechanism of neural plasticity at the presynapse and its physiological significance, 2, how mDia-induced actin is involved in TCR signaling, 3, how actin plays in interaction of Sertoli cells and sperm, and 4, how mDia-mediated actin is involved in transformation and cancer.

【Publications Relevant to the Project】

Thumkeo D, Watanabe S, Narumiya S. (2013) Physiological roles of Rho and Rho effectors in mammals. *Eur J Cell Biol.* **92**:303-315.

【Term of Project】 FY2014-2016

【Budget Allocation】 132,400 Thousand Yen

【Homepage Address and Other Contact Information】

snaru@mfour.med.kyoto-u.ac.jp

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

Biological Sciences (Medicine, Dentistry, and Pharmacy)

Title of Project : Stem Cell Regulation and Dynamics in Hair Follicle Regeneration and Aging

Emi Nishimura
(Tokyo Medical and Dental University, Medical Research Institute, Professor)

Research Project Number : 26221303　Researcher Number : 70396331
Research Area : Stem cell biology, dermatology, experimental pathology
Keywords: regeneration, aging, hair loss, tissue stem cells, self-renewal

【Purpose and Background of the Research】

In rapidly aging societies, it is urgent to address aging-associated diseases by understanding the underlying mechanisms of aging-associated tissue declines.

Hair loss and hair graying are typical aging phenotypes in mammals, but the underlying mechanisms of aging are still largely elusive in most tissues. Aging-associated somatic stem cell changes have also been reported in different tissues, but the exact mechanisms underlying the expression of aging phenotypes and whether tissue aging programs exist is still largely unknown. We have studied the mechanisms of aging-associated hair graying and hair loss by focusing on adult stem cells. We previously identified melanocyte stem cells (McSCs) within the bulge-subbulge area of mouse hair follicles. That population is cyclically activated to self-renew and to provide mature melanocytes for hair pigmentation (Nishimura EK et al. 2002). Our chronological analysis of McSCs and hair follicle stem cells (HFSCs), which function as niche cells for McSCs (Tanimura S et al. 2011), demonstrated that mouse hair follicles age through the defective renewal of McSCs. McSCs differentiate into pigment-producing melanocytes in the niche without renewing themselves under excessive genomic stress or with aging (Nishimura EK et al. 2005, Inomata K et al. 2009). As the niche plays a dominant role in McSC fate determination (Nishimura EK, 2005), aging-associated tissue changes in hair follicles may primarily originate from the aging of HFSCs.

In this study, we will characterize the underlying mechanisms of stem cell regulation and dynamics in hair follicle regeneration and aging especially by analyzing the signatures of aging HFSCs and aging-specific tissue changes in mouse and human hair follicles.

【Research Methods】

1) Analysis of HFSC aging signatures.
2) Analysis of hair follicle dynamics during aging by fate-tracing of HFSCs and other cell populations.
3) Clarification of key stem cell regulators in hair follicle regeneration and aging.
4) Clarification of the mechanisms of stem cell renewal and aging.
5) Development of methods to promote stem cell regulation and rejuvenation.

【Expected Research Achievements and Scientific Significance】

We aim to elucidate tissue aging mechanisms by focusing on stem cell aging in hair follicles. Our approach will enable us to determine whether tissue aging is programmed or not and also whether the changes originate from stem cells or other cell populations. Application of the key stem cell regulators which govern tissue aging will be beneficial for regenerative medicine and the prevention of aging-associated diseases.

【Publications Relevant to the Project】

・Tanimura S et al. Hair follicle stem cells provide a functional niche for melanocyte stem cells. **Cell Stem Cell**, 8, 177-187, **2011.**
・Inomata K et al. Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. **Cell.** 137(6):1088-99, **2009**.
・Nishimura EK et al. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche **Science.** 307(5710):720-724. **2005.**
・Nishimura EK et al. Dominant role of the niche in melanocyte stem cell rate determination. **Nature.** 416(6883):854-60, **2002.**

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.tmd.ac.jp/mri/scm/

Title of Project : Functional Analyses of Girdin Family Proteins and their Roles in Psyco-neurologic Disease and Cancer

Masahide Takahashi
(Nagoya University, Graduate School of Medicine, Professor)

Research Project Number : 26221304 Researcher Number : 40183446
Research Area : Experimental Pathology
Keyword : Animal Model, Functional Molecule

【Purpose and Background of the Research】

Cell migration is initiated in response to multiple extracellular cues and regulated by many intracellular molecules. Its dysregulation is involved in the development of various human diseases. We identified the Akt substrate Girdin which is an actin-binding protein, using a yeast two hybrid screening. Girdin is phosphorylated at the leading edge of moving cells by Akt, leading to reorganization of the actin cytoskeleton. The purpose of this study is to further analyze the functions of Girdin and its family protein Daple at the cellular and molecular levels and to elucidate their roles in the development of psyco-neurologic disease and cancer.

【Research Methods】

1. Roles of Girdin and Daple in psyco-neurologic disease and cancer: Using genetically engineered mice, we will investigate the functions of Girdin and Daple (Fig. 1) in neurogenesis and memory formation. By crossing girdin or daple knockout mice with cancer-prone mice, we will elucidate the roles of these molecules in cancer progression.

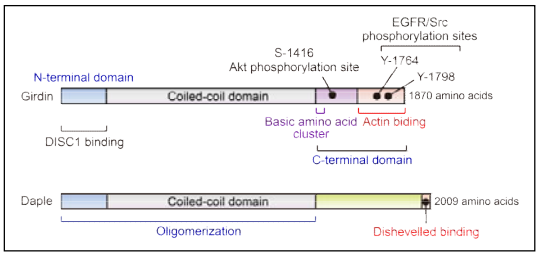


Figure 1 Structure of Girdin and Daple

2. Identification and functional analyses of Girdin or Daple-interacting proteins: To elucidate the functions of Girdin and Daple in cell migration, we will further identify proteins which specifically interact with their N- and C-terminal domains and study the functions of interacting proteins in cancer cell migration and neurogenesis.
3. Regulation of Girdin function by tyrosine phosphorylation: It turned out that Girdin is phosphorylated at tyrosine 1764 and 1798 by Src and EGF receptor. The significance of tyrosine phosphorylation of Girdin will be analyzed at cellular levels as well as in mice.

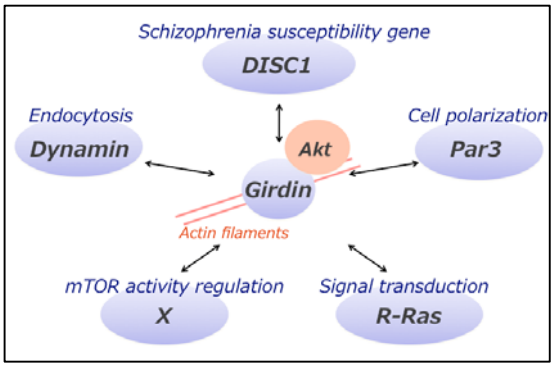


Figure 2 Identification of Girdin-interacting proteins

【Expected Research Achievements and Scientific Significance】

In this project, we will identify new Girdin and Daple interacting proteins (Fig. 2) and generate new genetically engineered mice of the girdin or daple gene. Through these analyses, we will elucidate the roles of Girdin and Daple in the development of psyco-neurologic disease and cancer. In addition, our studies will provide new insights into Girdin and Daple functions in the nervous system.

【Publications Relevant to the Project】

・Ishida-Takagishi, M., Takahashi, M. et al. The Dishevelled-associating protein Daple controls the non-canonical Wnt/Rac pathway and cell motility. **Nature Commun.** 3: 859 (2012).
・Enomoto, A., Takahashi, M. et al. Roles of Disrupted in Schizophrenia 1 interacting protein Girdin in postnatal development of the dentate gyrus. **Neuron** 63: 774-787 (2009).

【Term of Project】 FY2014-2018

【Budget Allocation】 149,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.nagoya-u.ac.jp/patho2/

【Grant-in-Aid for Scientific Research (S)】
Biological Sciences (Medicine, Dentistry, and Pharmacy)

Title of Project : Generation and Maintenance of Pathogenic Immunological Memory and its Regulation

Toshinori Nakayama
(Chiba University, Graduate School of Medicine, Professor)

Research Project Number : 26221305 Researcher Number : 50237468
Research Area : Basic Medicine, Immunology
Keyword : Immunological memory, Allergy and immune-related disorder

【Purpose and Background of the Research】

The main interest of our research is the role of transcription factors that control differentiation and maintenance of memory Th1/Th2/Th17 cells and regulation of airway inflammation (asthma). "Immunological Memory" is a crucial subject to be understood in the Immunology field. We have recently proposed a "Pathogenic Th population disease induction model" in the pathogenesis of inflammatory diseases (Fig. 1). The aims of this study are to establish this concept by analyzing the role of Polycomb and Trithorax molecules in disease, to clarify the environmental factors that control the pathogenicity of memory Th cells, and to propose new strategies in the development of treatment of inflammatory diseases.

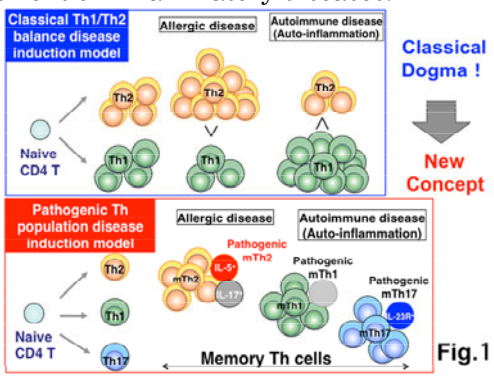


Fig.1

【Research Methods】

To identify the mechanisms regulating "Pathogenic memory Th2 cells" that induce allergic airway inflammation, we will conduct epigenetic analysis using ChIP-Seq. and RNA-Seq. We will also examine the polyps of patients with chronic sinus inflammation, while identifying the mechanism of induction of pathogenic Th2 cells in humans (e.g. by IL-33) (Fig. 2).

We will conduct epigenetic analysis on the role of EZH2 and Menin in the expression of cytokines in memory Th1, Th2, and Th17 cells.

We will analyze inducible lymphoid tissues in the lung and identify functional molecules regulating the generation and maintenance of memory Th2 cells using histological analysis with a multiphoton microscope.

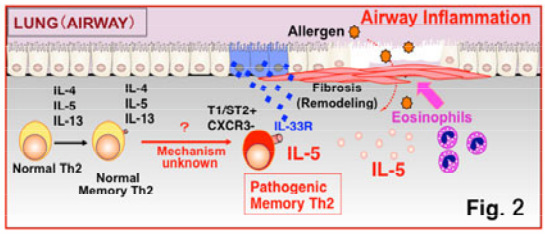


Fig. 2

【Expected Research Achievements and Scientific Significance】

Our approach to clarify the nature of "Immunological Memory" at both the molecular and chromatin levels and to prove the hypothesis that immune related disorders are induced by "Pathogenic memory Th cells" is scientifically significant. We focus on human immunology and examine inflamed tissues taken from patients. New treatment strategies in inflammatory diseases will be proposed. This study may also contribute to the development of safer and more effective vaccines. Thus, the impact of this study to society will be substantial.

【Publications Relevant to the Project】

- Endo, Y., Nakayama, T. et al., Pathogenic memory type Th2 cells in allergic inflammation. ***Trends Immunol.*** 35(2): 69-78 (2014).
- Tumes, D. J., Nakayama, T. et al., The polycomb protein Ezh2 regulates differentiation and plasticity of CD4+ T helper type 1 and type 2 cells. ***Immunity*** 39(5): 819-832 (2013).
- Kuwahara, M., Nakayama, T. et al., The transcription factor Sox4 is a downstream target of signaling by the cytokine TGF-β and suppresses TH2 differentiation. ***Nat. Immunol.*** 13:778-786 (2012).

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.m.chiba-u.ac.jp/class/meneki/english/

Title of Project : Intrinsic and Extrinsic Mechanisms of Generation and Maintenance of Memory B Cells

Tomohiro Kurosaki
(Osaka University, WPI Immunology Frontier Research Center, Professor)

Research Project Number : 26221306　Researcher Number : 50178125
Research Area : Immunology
Keyword : follicular dendritic cells, humoral immunity, memory T_{fh}, memory B, high affinity

【Purpose and Background of the Research】

The most striking feature of the adaptive immunity is the generation of immunological memory. In the case of memory B cells, they remember the previously experienced antigen and respond more quickly after re-exposure to the same antigen.

Although the phenomenon of the immunological memory has been well recognized, molecular mechanisms underlying the rapid responsiveness, high affinity antibodies for antigen, and longevity of memory B cells are not clear. Here, we focus on two questions; (1) how do memory B cells generate high-affinity IgG antibodies upon secondary exposure? (2) how do memory B cells have long life span?

【Research Methods】

IgM-type and IgG1-type memory B cells are generated from naïve IgM B cells through their interaction with T_{fh} type T cells and follicular dendritic cells (FDCs) *in vivo*. Therefore, in order to clarify the molecular mechanisms underlying generation of the high affinity IgG1 antibodies and longevity of memory B cells, it is a prerequisite to understand which type of cells (IgM-type memory B cells, IgG1-type memory B cells, T_{fh} T cells, and FDCs) are majorly responsible for. To address these issues, we will first establish the mice strain which can induce the depletion of the above mentioned cell types specifically. Then, we will analyze the effects of such depletion on generation of high affinity IgG1 antibodies and longevity of memory B cells.

Then, by comparing RNA sequence data of the responsive cells at naïve, effector, versus memory states, we will pick up the candidate molecules to explain the unique characters at the memory state. Then by using functional assays, we will determine the key molecules for exerting such uniqueness.

As specific subjects

(1) Establishment of mice strain which specifically deplete IgM- and IgG1-type memory B cells
(2) Determining the functional differences between IgM- and IgG1-type memory B cells
(3) Searching the molecular mechanisms underlying the above differences
(4) Analyzing the effects of ablation of T_{fh} T cells and FDC on longevity of memory B cells

【Expected Research Achievements and Scientific Significance】

Vaccination is a typical way to utilize the immune memory system; particularly, in the case of life-threating influenza and HIV virus, the humoral memory system plays a dominant role for their protection. However, we have not yet succeeded in development of good vaccination ways. To do this, basic understanding of which types of cells are critical for generation of effective IgG antibodies as well as for long term effectiveness is essential for new development for vaccination.

Thus, new evidence generated by this project will contribute not only to expansion of our knowledge about humoral memory at cellular and molecular levels, but to development of a new type of vaccination.

【Publications Relevant to the Project】

・Kometani K, et al. Repression of the Transcription Factor Bach2 Contributes to Predisposition of IgG1 Memory B Cells toward Plasma Cell Differentiation. Immunity 39, 136-147, 2013
・Ise W, Kometani K, Kurosaki T. Memory B cells. Nat. Rev. Immunol (in press)

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://lymph.ifrec.osaka-u.ac.jp/index_e.html

Title of Project : Designing and Developing Innovative Use of Newly Discovered Colonic Epithelial Culture Method Applicable to Clinical Medicine

Mamoru Watanabe
(Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, Professor)

Research Project Number : 26221307　Researcher Number : 10175127
Research Area : Medical Science
Keyword : Gastrointestinal Disease, Colonic Disease

【Purpose and Background of the Research】

The failure of intestinal environment due to the malfunction of the intestinal epithelial cells is suggested with an intractable cause for the inflammatory bowel disease (IBD). In this study, we thought that the improvement of intestinal environment in IBD by controlling the fate of intestinal epithelial stem cells could reset the homeostasis, resulting in the fundamental healing of IBD. We therefore aimed to develop primary culture method of intestinal epithelial stem cells and the stem cell transplantation model for the elucidation of the relationship between intestinal environment and whole-body homeostasis. Moreover, we propose a new concept to be able to control not only the intestinal diseases but also lifestyle-related diseases due to the intestinal epithelial stem cells.

【Research Methods】

Using primary culture system of the intestinal epithelial stem cells originally established, visible and diachronic evaluation system would be constructed for the stem cell functions such as the cell division, lifespan and differentiation. Furthermore, we would build an in vitro intestinal model including stem cells and enterobacterial flora to assess the mutual relations with the intestinal environment including hormone secretion and mucosal immunity. Specifically, we perform the following analysis.

1) Construction of the in vitro intestinal model by the primary culture organoids.

a) The establishment of the co-culture system between intestinal organoid with dendritic cell (DC) or intraepithelial lymphocyte (IEL).

b) The establishment of the enterobacterial flora model by the injection of the bacteria into the organoid lumen.

c) Construction of high fat diet model in organoid

2) Establishment of the functional analysis for intestinal epithelial stem cells.

a) The analysis of stem cell dynamics by single stem cell visualization.

b) Signal analysis in the intestinal epithelial stem cells.

c) The regulation for the determination of stem cell fate.

3) The analysis for the abnormality of intestinal epithelial stem cell in whole body diseases.

a) Construction of the high efficiency epithelium cell transplantation model.

b) Dysfunction of the intestinal epithelial stem cell in the chronic disease model mouse.

c) Intestinal epithelial stem cell culture in the patients with lifestyle-related diseases.

【Expected Research Achievements and Scientific Significance】

In late years, it has been suggested that the pathogenesis of lifestyle-related diseases as well as a chronic gastrointestinal disease, is due to the intestinal epithelial function including mucosal immunity, intestinal hormone and the enterobacterial flora. We generate the ex vivo model of the intestine with complicated environment, which can exhibit the mutual relations of various factors in the intestinal tract. Finally, we hope that resetting intestinal environment by controlling the fate of an intestinal epithelial stem cell could cut off the malignant circulation in the chronic disease.

【Publications Relevant to the Project】

1) Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, Ichinose S, Nagaishi T, Okamoto R, Tsuchiya K, Clevers H, Watanabe M: Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. *Nature Medicine*18: 618-623, 2012.

2) Fordham RP, Yui S, Hannan NRF, Madgwick A, Vallier L, Pedersen RA, Nakamura T, Watanabe M, Jensen KB: Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. *Cell Stem Cell.* 13:734-744,2013.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,100 Thousand Yen

【Homepage Address and Other Contact Information】

Title of Project : Exploring Genetic Basis of Myelodysplastic Syndromes (MDS)

Seishi Ogawa
(Kyoto University, Graduate School of Medicine, Professor)

Research Project Number : 26221308 Researcher Number : 60292900
Research Area : Medicine, dentistry, and pharmacy
Keyword : Myelodysplastic syndromes, RNA splicing, Clonal evolution

【Purpose and Background of the Research】

Myelodysplastic syndromes (MDS) and related disorders are a highly heterogeneous group of chronic myeloid neoplasms, characterized by varying degrees of cytopenias and/or abnormal myeloproliferation with bone marrow dysplasia, as well as a high propensity of acute myeloid leukemia (AML). As for their pathogenesis, substantial advances have been made in our understanding of MDS pathogenesis during the past decade through identification of a number of gene mutations frequently found in MDS. Especially, the discovery of frequent mutations in RNA splicing factors by our and other groups, provide a novel clue to understand MDS pathogenesis.

The purpose of the current study is to extend these findings of recent years, obtaining a better understanding of the molecular pathogenesis of MDS. Specifically we will elucidate clonal architecture and its chronological behavior during course of MDS in terms of gene mutations especially of RNA splicing factors and understand the functional/molecular basis of these relevant mutations during the development of MDS. We will also try to identify lead compounds amenable for targeting RNA splicing factor mutations.

【Research Methods】

Combining the state-of-the-art genomics /genetics and functional studies using mice models, as well as high throughput screening of chemical compounds, we will be extensively analyzed chronological behavior of MDS clones using deep whole exome sequencing of carefully collected/fractionated MDS samples over time to reveal fine structure of MDS clones, and thereafter, we will directly tested/translated the obtained knowledge in mice model. Clonal evolution in AA patients after immunosuppressive therapies and normal population in elderly, to obtain an insight into the origin of MDS clones. Finally, we will try to screen those chemical compounds that selectively kills MDS cells having splicing factor mutations. I believe these studies will certainly contribute to substantially expand our understanding of MDS, based on which we could finally contrive better diagnostics and therapeutics for MDS patients.

【Expected Research Achievements and Scientific Significance】

Through the project, the molecular pathogenesis of MDS in terms of clonal evolution and its difference from related myeloid neoplasms will be clarified. Especially the role of RNA splicing factor mutations will be delineated. The obtained knowledge will be applied to the improvement of the management of patients with MDS.

【Publications Relevant to the Project】

・Yoshida K, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. ***Nature*** 478(7367):64-69. 2011
・ Kon A, et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. ***Nature genetics*** 45(10):1232-1237. 2013
・ Makishima H, et.al, Maciejewski JP. Somatic SETBP1 mutations in myeloid malignancies. ***Nature genetics*** 45(8):942-946. 2013
・ Haferlach T, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. ***Leukemia*** 28(2):241-247. 2014

【Term of Project】 FY2014-2018

【Budget Allocation】 149,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.genome.umin.jp
sogawa-tky@umin.ac.jp

【Grant-in-Aid for Scientific Research (S)】
Biological Sciences (Medicine, Dentistry, and Pharmacy)

Title of Project : Homeostasis of Hematopoietic Stem Cell Maintenance

Toshio Suda
(Kumamoto University, Priority Organization for Innovation and Excellence, Visiting Professor)

Research Project Number : 26221309 Researcher Number : 60118453
Research Area : Medicine, dentistry, and pharmacy
Keyword : hematopoietic stem cells, niche, radical oxygen, hypoxia

【Purpose and Background of the Research】

Stem cells have a capacity for differentiating to multilineage and sustaining the undifferentiate state. Proliferation and differentiation of stem cells are determined not only by intrinsic program but by their microenvironment (niche) such as niche cells and niche factors. To develop the technology for the regulation of stem cells, it is critical to understand how niche is involved in the determination of stem cell fate. In this project, we will re-analyze the histological structure for hematopoietic stem cell (HSC) niche in the bone marrow and clarify which molecules control the stem cell behavior in the niche.

We will analyze how stem cells block the cell division and sustain the quiescence in the hypoxic niche.

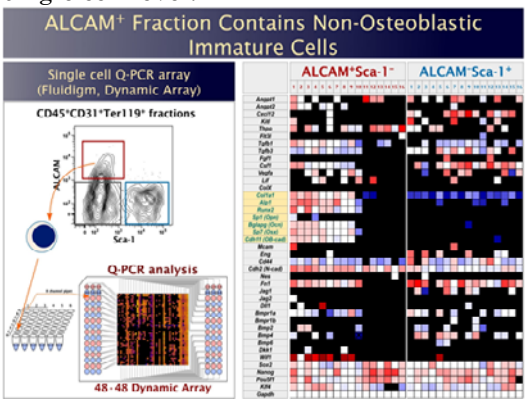
【Research Methods】

A) Analyses for HSC niche

We analyze the structure for HSCs niche in the bone marrow by immunohistochemistry and electronmicroscopy. Especially we focus our study on perivascular cells and osteoblast-osteoclast interaction and identify the niche factors acting on HSCs. We analyze the metabolic state of HSCs which are located in the hypoxic niche in order to clarify the mechanism how HSCs sustain the undifferentiated state.

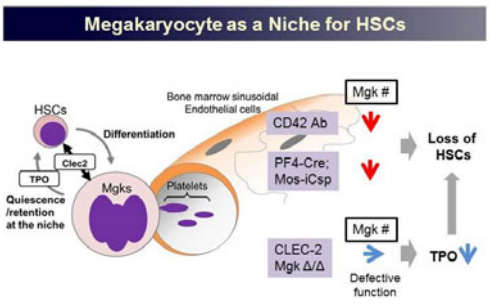
B) Niche reconstruction and regulation

Based on the studies described above, we try to control the cell cycle of HSCs and enhance the efficiency of bone marrow transplantation. We will study the self-renewal events of stem cells at the single cell level.



【Expected Research Achievements and Scientific Significance】

Based on physiological research, we will also clarify whether stem cell aging is related to the pathogenesis of hematological malignancies. We will analyze the aging process in HSCs and their niche and clarify the accumulation of DNA damage. We will focus on the telomere dysfunction in HSCs from the Shelterin function and clarify the pathogenesis of CLL and MDS, which are known to be niche cell-derived and age-dependent. These studies may help to elucidate the pathophysiology of diseases and provide critical clues to develop novel treatment and preemptive measures for the prevention of these diseases.



(Nakamura-Ishizu & Takubo et al. *under revision*)

【Publications Relevant to the Project】

Suda T, Takubo K, Semenza GL: Metabolic regulation of hematopoietic stem cells in the hypoxic niche. ***Cell Stem Cell*** 9: 298-310, 2011

Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T: Tie2/Angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. ***Cell***, 118: 149-161, 2004

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://web.sc.itc.keio.ac.jp/celldiff/index.html
sudato@z3.keio.jp

Title of Project : Elucidation of the Transcriptional Regulation of Runx2 and Development of the Drugs for Osteoporosis and Osteoarthritis

Toshihisa Komori
(Nagasaki University, Graduate School of Biomedical Sciences, Professor)

Research Project Number : 26221310 Researcher Number : 00252677
Research Area : Morphological basic dentistry
Keyword : transcriptional regulation, development and differentiation, drug discovery, cell and tissue, gene

【Purpose and Background of the Research】

We have clarified that Runx2 is an essential transcription factor for skeletal development, that Runx2 is essential for osteoblast differentiation and chondrocyte maturation, and that Runx2 is a responsible molecule for osteoarthritis (Fig. 1).

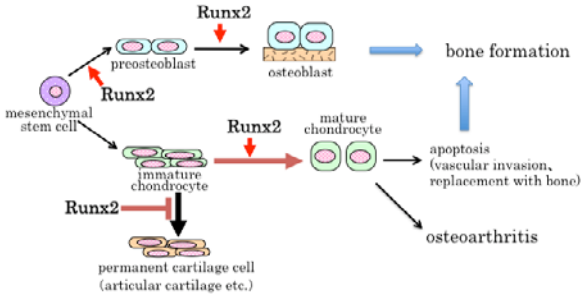


Fig. 1 The functions of Runx2

Therefore, Runx2 exerts positive effects on bone but negative effects on cartilage. The elucidation of the transcriptional regulation of Runx2 in osteoblasts and chondrocytes makes a great advance in the understanding of the molecular mechanism of skeletal development and maintenance. Further, it makes possible to regulate Runx2 in osteoblasts and chondrocytes separately, which allows us to develop the drugs for osteoporosis and osteoarthritis. In this study, we elucidate the transcriptional regulation of Runx2, and develop the drugs for osteoporosis and osteoarthritis based on the molecular mechanisms of the transcriptional regulation.

【Research Methods】

We generate reporter mice driven by genome DNA of Runx2 locus and identify the enhancers for Runx2 expression in osteoblasts and chondrocytes. Next, the molecular mechanisms of the activation of the enhancers are elucidated. The chemical compounds, which enhance Runx2 expression in osteoblast precursors, are candidates of the drugs for osteoporosis, and the chemical compounds, which inhibit Runx2 expression in chondrocytes, are candidates of the drugs for osteoarthritis (Fig. 2). Therefore, we identify the chemical compounds, which enhance osteoblast-specific enhancer or inhibit chondrocyte-specific enhancer, through high throughput screening, and examine the effects using animal models.

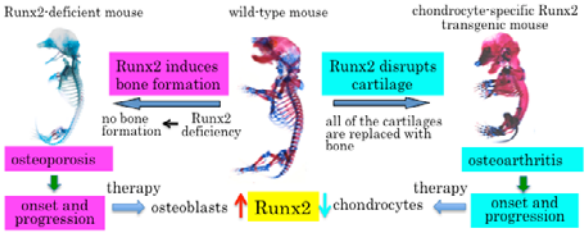


Fig. 2 The development of the drugs for osteoporosis and osteoarthritis by regulating Runx2 expression

【Expected Research Achievements and Scientific Significance】

As Runx2 plays a central role in skeletal development, the elucidation of the transcriptional regulation of Runx2 makes a great advance in the understanding of skeletal development. This study also contributes to the treatment of osteoporosis and osteoarthritis by developing the drugs.

【Publications Relevant to the Project】

・Kawane T, Komori H, (10 authors), and Komori T. Dlx5 and Mef2 Regulate a Novel Runx2 Enhancer for Osteoblast-Specific Expression. J Bone Miner Res. 2014 Apr 1. doi: 10.1002/jbmr.2240. [Epub ahead of print]
・Komori T. Signaling networks in RUNX2-dependent bone development. J Cell Biochem. 112 (3): 750-755, 2011.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.de.nagasaki-u.ac.jp/dokuji/kaibou-2/index.html

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

Biological Sciences (Medicine, Dentistry, and Pharmacy)

Title of Project : Development of a Novel Strategy for Bone Regeneration with 'Small Molecule-mediated Osteo-reprogramming' and Understanding of Genomic Mechanisms Underlying the Process

Tsuyoshi Takato
(The University of Tokyo Hospital, Professor)

Research Project Number : 26221311　Researcher Number : 90171454
Research Area : Oral and maxillofacial surgery
Keyword : Oral surgery, bone regenerative medicine

【Purpose and Background of the Research】

We have been working on the direct reprogramming of somatic cells into osteoblasts, the identification of osteogenic small molecules, and the development of osteoconductive scaffolds. In this study, we aim to develop a novel platform for bone regeneration by the direct reprogramming toward osteoblasts with small molecules, which we call hereafter 'small molecule-mediated osteo-reprogramming.' Besides the osteo-reprogramming protocol, we will optimize scaffolds to appropriately apply the reprogrammed cells to living bodies (Figure 1). We also hope to gain epigenetic insights into the osteoblast differentiation program by exploring reprogramming mechanisms through genome-wide approaches.

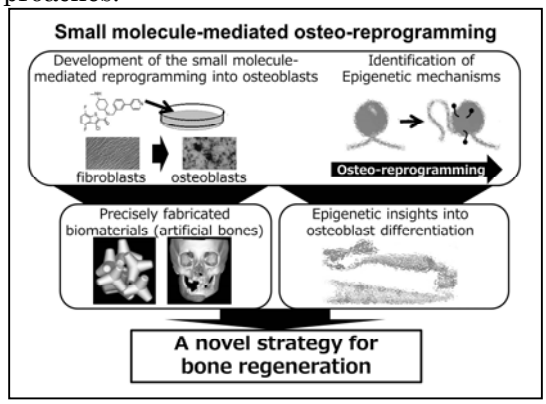


Figure 1 General flowchart of this study

【Research Methods】

1. Development of protocols for the small molecule-mediated osteo-reprogramming

Protocols for the small molecule-mediated osteo-reprogramming of mouse dermal fibroblasts are optimized. Optimized protocols are then verified in human dermal fibroblasts.

2. Investigation of epigenetic mechanisms underlying the osteo-reprogramming

We investigate the dynamics of the epigenetic status and gene expression profile during the osteo-reprogramming of human dermal fibroblasts, aiming to identify characteristics of reprogrammed cells and to obtain molecular basis of the reprogramming process in terms of epigenetics.

3. Evaluation of bone regeneration by the small molecule-mediated osteo-reprogramming

We assess bone regeneration by the fibroblast-derived osteoblasts in several animal models. Cellular mechanisms during the regeneration process are also examined.

【Expected Research Achievements and Scientific Significance】

There is no report available on the development of a platform for bone regeneration with small molecule-mediated direct reprogramming, or the creation of three-dimensional bone tissues based on the direct reprogramming. Therefore, this study will be a milestone in the field of skeletal regenerative medicine, contributing to the bone biology by clarifying epigenetic mechanisms during the reprogramming process. Given that basic and applied researches are cooperatively carried out in this study, one can expect that this is an important first step for generating novel research fields as well as therapeutic strategies that have never been established.

【Publications Relevant to the Project】

- Kanke K, Takato T et al. Stepwise differentiation of pluripotent stem cells into osteoblasts using four small molecules under serum-free and feeder-free conditions. *Stem Cell Rep* 2:751, 2014
- Saijo H, Takato T et al. A novel method for designing and fabricating custom-made artificial bones. *Int J Oral Maxillofac Surg* 40:955, 2011
- Ohba S, Takato T et al. Identification of a potent combination of osteogenic genes for bone regeneration using embryonic stem (ES) cell-based sensor. *FASEB J* 21(8):1777, 2007

【Term of Project】

FY2014-2018

【Budget Allocation】

136,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://plaza.umin.ac.jp/~oralsurg/