Title of Project : Decision Making in the Mouse Olfactory System

Hitoshi Sakano
(University of Fukui, School of Medical Sciences, Adjunct Professor)

Research Project Number : 17H06160 Researcher Number : 90262154
Research Area : Neuroscience, Molecular Biology
Keyword : neural circuit, olfactory, transgenic mice, optogenetics

【Purpose and Background of the Research】

The mammalian olfactory system recognizes a diverse repertoire of chemical information that induces distinct behavioral responses based on the odor qualities (Fig.1). In rodents, odor ligands are detected by olfactory sensory neurons (OSNs) in the olfactory epithelium (OE). Since OSNs expressing the same type of receptor send their axons to a specific target site, glomerulus, odor signals detected in the OE are converted into a topographic map of activated glomeruli. Odor information encoded in the olfactory bulb (OB) is then conveyed by projection neurons, mitral/tufted (M/T) cells, to various areas in the olfactory cortex (OC) to elicit odor responses. In this project, we plan to study how the neural circuits are formed for innate olfactory decisions. In adults, quality decisions of sensory inputs are made not only by the hard-wired circuits, but also by the memory-based learned circuits. However, these two decisions may differ: For example, the aversive odor quality of fermented foods could be converted to attractive one, once we experienced their good tastes. In this project, we will also study how the conflicted decisions, innate vs. learned, are balanced and modulated for behavioral responses.

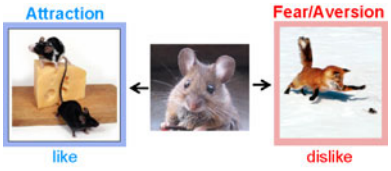


Fig.1 Olfactory perception

【Research Methods】

We have recently shown that photo-activation of a single glomerulus in the fear domain of dorsal OB induces immobility (freezing) in the channel-rhodopsin knock-in (KI) mouse. Similar mice will be generated for various innate responses, which will allow us to map functional domains in the OB. Using this photo-activation system, responsive OC regions will be identified. We will then study how the M/T cells connect the functional domains in the OB and particular OC regions to induce specific olfactory responses. We will identify specific sets of axon-guidance molecules for different subsets of M/T cells that convey particular odor information to responding OC regions. It has been reported that the aversive odor quality is processed by the cortical amygdala (CoA) to induce stress reactions, whereas the attractive odor quality is given in the anterior MeA (Fig.2). We will study how the two qualities are balanced between the CoA and MeA for decision making, when the innate quality of a particular odorant is aversive but the learned quality is attractive.

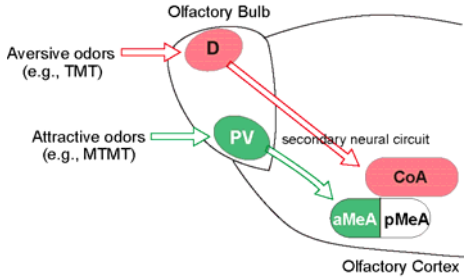


Fig.2 Hard-wired olfactory neural circuit

【Expected Research Achievements and Scientific Significance】

This research will reveal the domain organization of the olfactory map in the OB, circuit connections between the OB and OC, and functional sub-regions in the OC. This research will also give us a new insight into our understanding of decision making for sensory inputs to induce various behavioral responses.

【Publications Relevant to the Project】

Saito, H., *et al.*: Immobility responses are induced by photoactivation of single glomerular species responsive to fox odor TMT. *Nat. Comm.* 8, 16011 doi: 10.1038/ncomms16011 (2017)
Inokuchi, K., *et al.*: *Nrp2* is sufficient to instruct circuit formation of mitral cells to mediate odor-induced attractive social responses. *Nat. Comm.* 8, 15977 doi: 10.1038/ncomms15977 (2017)

【Term of Project】 FY2017-2021

【Budget Allocation】 158,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://t-profile.ad.u-fukui.ac.jp/profile/ja.3c1e4d8f29d84458520e17560c007669.html

Title of Project : Elucidation of cortical neural circuits for meta-memory: Optogenetic manipulation of retrospection

Yasushi Miyashita
(Juntendo University, Graduate School of Medicine, Professor)

Research Project Number : 17H06161 Researcher Number : 40114673
Research Area : Biological Sciences
Keyword : Cognitive neuroscience

【Purpose and Background of the Research】

Elucidation of high-level cognitive functions is an important goal in neuroscience. Studies on the "memory systems in the narrow sense" (which executes memory encoding, storage and recollection) have dramatically progressed in the past ten years. However, to elucidate the neural basis of the "continuity of the individual conscious experience," clarification of the "meta-memory system" that introspectively monitors memory processing is essential. In this study, we aim to investigate the introspective aspects of memory that have been previously studied only in humans; we will introduce a non-human primate model, and will accomplish it by psychophysically controlling the animal's behavior and applying electrophysiological, optogenetic and magnetic resonance imaging (MRI) methods that have been developed in the previous studies on the "memory system in the narrow sense".

【Research Methods】

(1) Establishment of a meta-memory task using monkeys and identification of cortical meta-memory circuit

We adopt a meta-memory task, which combines a Yes/No-type recognition memory test (Memory stage) with a confidence judgment test using the post-decision wagering paradigm (Bet stage) (Fig.1). We will identify the "meta-memory-related brain areas" using functional MRI (fMRI) on monkeys, while they perform the meta-memory task. For identification of meta-memory-related brain areas, we will use two types of cognitive subtraction approaches. One approach relies on the calculation of fMRI-signal differences between the "High-Bet" condition and the "Low-Bet" condition. The other relies on calculation of the correlation between the meta-memory behavioral index (meta-d' index or Φ index) and the fMRI signal.

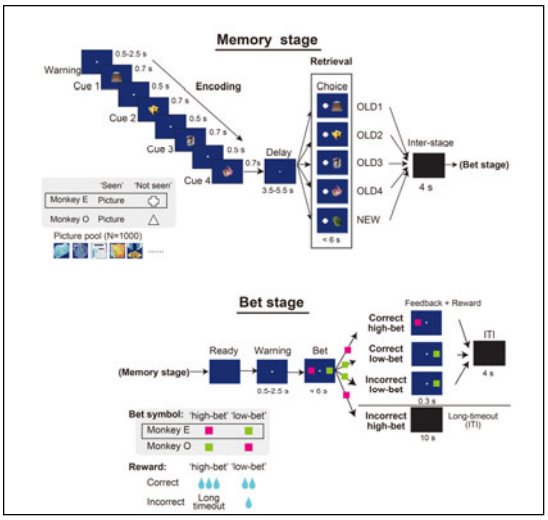


Fig.1 Meta-memory task

(2) Development of optogenetic methods in monkey cortex

AAV5.CAMKII.hChR2.GFP.WPRE.SV40 will be injected into the monkey temporal cortex. In a recognition memory task, we will measure the psychometric function related to Old/New judgment, by changing the Old/New valence of the stimuli. Effects caused by the illumination of 473 nm laser light (and 594 nm laser light as control) will be examined to assess whether the psychometric function of Old/New judgment shifts significantly or not.

(3) Optogenetic intervention of monkey cortical network during meta-memory performance

In the meta-memory-related brain area identified in (1), excitatory or inhibitory optogenetic vector constructs will be injected and the causal behavioral impact of the intervention will be psychophysically measured according to the procedure that was developed in (2).

【Expected Research Achievements and Scientific Significance】

This study aims to elucidate the introspective aspects of memory in an animal model, by utilizing invasive biological procedures. It will pave the way for the study of neuronal mechanisms for introspection and self-reflection of one's own mind.

【Publications Relevant to the Project】

・Takeuchi, D., Hirabayashi, T., Tamura, K. and Miyashita, Y. : Reversal of interlaminar signal between sensory and memory processing in monkey temporal cortex. ***Science*** 331, 1443-1447, 2011.
・Hirabayashi, T., Takeuchi, D., Tamura, K., and Miyashita, Y. : Microcircuits for Hierarchical Elaboration of Object Coding Across Primate Temporal Areas. ***Science*** 341, 191-195, 2013.

【Term of Project】 FY2017-2021

【Budget Allocation】 161,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.physiol.m.u-tokyo.ac.jp/

Title of Project : Association of immune responses with racial differences of cancer development

Hiroyoshi Nishikawa
(Nagoya University, Graduate School of Medicine, Professor)

Research Project Number : 17H06162 Researcher Number : 10444431
Research Area : Biological Science, Oncology, Tumor biology
Keyword : Cancer development, Cancer immunity

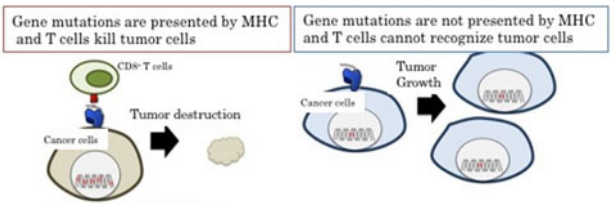
【Purpose and Background of the Research】

Genome instability induces high frequencies of mutations in oncogenes and tumor suppressor genes (so-called cancer driver genes), resulting in transformation of cells leading to cancer development. While Asians and Caucasians are similarly exposed to carcinogens such as UV, radiation and carcinogenic chemical substances, the frequency of cancer development depending on driver gene mutations often exhibits racial differences, indicating that host defense mechanism(s) against cancer development play critical roles for the differences.

In this study, we focus on the racial differences of HLA allele because our preliminary data reveal that racial differences of cancer development can be attributed to HLA differences. The association between lung cancer development by driver genes and HLA allele is investigated. The differences of HLA allele represent the distinct antigen presentation, resulting in different immune responses against the abnormal gene products. HLA alleles resistant/sensitive to driver gene-induced cancers elicit different immune responses against mutated driver gene products that can induce tumor rejection in humans with resistant HLA allele (immune surveillance), leading to the elucidation of essential anti-tumor immune responses.

【Research Methods】

1) The association between lung cancer development by driver genes such as EGFR and KRAS and HLA allele, is investigated and HLA allele sensitive to the cancer development is elucidated.
2) Based on the data in 1), immune responses against abnormal gene products are predicted for antigen presentation and then confirmed by inducing T cells in humans with/without sensitive HLA allele. The T-cell responses are functionally and phenotypically investigated in depth.
3) Whether the immune responses are faithfully associated with cancer immune surveillance is evaluated with mouse models with HLA allele.



【Expected Research Achievements and Scientific Significance】

Clarifying racial differences of cancer development uncovers people susceptible to develop cancers associated with driver gene mutations, leading to preventive medicine.

The immune responses responsible for cancer immune surveillance are closely associated with anti-tumor immune responses inducing tumor regression, leading to the development of effective cancer immunotherapy.

【Publications Relevant to the Project】

・Saito T, Nishikawa H, et al; Two FOXP3+CD4+ T cell subpopulations distinctly control the prognosis of colorectal cancers. **Nat Med**. 22(6):679-84 2016.
・Maeda Y, Nishikawa H, et al. Detection of self-reactive $CD8^+$ T cells with an anergic phenotype in healthy individuals. **Science**. 346(6216):1536-40 2014.

【Term of Project】 FY2017-2021

【Budget Allocation】 161,700 Thousand Yen

【Homepage Address and Other Contact Information】

https://www.med.nagoya-u.ac.jp/medical_J/laboratory/basic-med/micro-immunology/immunology/
http://epoc.ncc.go.jp/division/immunology/

Title of Project : The roles of membrane lipids for intracellular signaling platform

Hiroyuki Arai
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number : 17H06164 Researcher Number : 40167987
Research Area : biological sciences
Keyword : lipid biology

【Purpose and Background of the Research】

The cytoplasm of eukaryotic cell is elaborately subdivided into discrete, specialized, membrane-bound structures called intracellular organelles. Each organelle has been shown to have characteristic lipids such as phosphoinositides. However, the roles of these membrane lipids of organelles have remained poorly understood.

Our previous studies revealed that phosphatidylserine, a relatively minor constituent of biological membranes, was concentrated on the cytosolic leaflet of recycling endosomes and was required for the membrane traffic through recycling endosomes (PNAS 2011, EMBO 2015). These studies also revealed that the pleckstrin homology (PH) domain of evectin-2 bound specifically to phosphatidylserine.

In this project, we will identify the proteins proximal to specific phospholipids using several lipid-binding domains such as the PH domain of evectin-2 and comprehensively understand the biological functions of organelle membranes as a device for effective and accurate cellular signaling.

【Research Methods】

We have recently developed methods to identify proteins proximal to phosphatidylserine in cytoplasmic leaflet of recycling endosomes. In this project, we will apply these methods to other phospholipids present in other organelles.

【Expected Research Achievements and Scientific Significance】

Identification of proteins proximal to organelle phospholipid will address important but so far unresolved questions in membrane biology: "How and why does each organelle have characteristic phospholipids?" Our studies will also discover new drug targets in the inflammatory diseases and cancer.

【Publications Relevant to the Project】

- M Uchida, Y., Hasegawa, J., Chinnapen, D., Inoue, T., Okazaki, S., Kato, R., Wakatsuki, S., Misaki, R., Koike, M., Uchiyama, Y., Iemura, S., Natsume, T., Kuwahara, R., Nakagawa, T., Nishikawa, K., Mukai, K., Miyoshi, E., Taniguchi, N., Sheff, D., Lencer, W. I., Taguchi, T., and Arai, H. (2011). Intracellular phosphatidylserine is essential for retrograde membrane traffic through endosomes. **Proc Natl Acad Sci U S A** 108, 15846-15851.
- Lee, S., Uchida, Y., Wang, J., Matsudaira, T., Nakagawa, T., Kishimoto, T., Mukai, K., Inaba, T., Kobayashi, T., Molday, R. S., Taguchi, T., and Arai, H. (2015). Transport through recycling endosomes requires EHD1 recruitment by a phosphatidylserine translocase. **EMBO J** 34, 669-688.
- Mukai, K., Konno, H., Akiba, T., Uemura, T., Waguri, S., Kobayashi, T., Barber, G. N., Arai, H., and Taguchi, T. (2016). Activation of STING requires palmitoylation at the Golgi. **Nat Commun** 7, 11932.

【Term of Project】

FY2017-2021

【Budget Allocation】

156,700 Thousand Yen

【Homepage Address and Other Contact Information】

https://sites.google.com/site/eiseikagaku/

Title of Project : An Integrated Multi-scale Approach for Studying Cyanobacterial Circadian Clock System

Shuji Akiyama
(Institute for Molecular Science, Research Center of Integrative Molecular Systems, Professor)

Research Project Number : 17H06165 Researcher Number : 50391842
Research Area : Biophysics
Keyword : Biological Clock, Clock Protein, Cyanobacteria, KaiC

【Purpose and Background of the Research】

Circadian rhythms are self-sustained oscillations with a period of approximately 24 h, enabling organisms to adapt to daily alterations in the environment. So far, many studies have investigated the time-measuring mechanism in the circadian clocks from bacteria to mammals. However, it remains unknown how the period is implemented in clock oscillators and kept unaffected against temperature changes (temperature compensation). In this research project, we will study cyanobacterial circadian clock as a model system and address these questions using a multidisciplinary approach including, biophysics, structural biology, chronobiology, molecular biology, and control engineering.

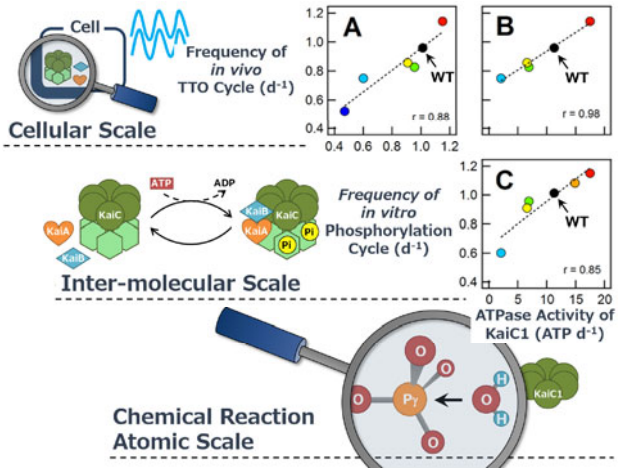


Figure 1 Cyanobacterial Circadian Clock System

【Research Methods】

Both the frequency (reciprocal of the period) and the temperature compensation of cellular rhythms are under strong influences of KaiC, a core clock protein in cyanobacteria. For example, the more *in vitro* ATPace activity of KaiC increases, the higher the frequency of the cellular rhythm becomes (Figure 1, panel B). Taking advantage of this trans-hierarchical correspondence, we will study the mechanisms of the circadian periodicity and temperature compensation both encoded in KaiC in accordance with the following 5 points.

<1> Large-scale screening of KaiC mutants.
<2> Mapping a frequency-to-structure correspondence in KaiC.
<3> Neutron crystallographic study on the active site of KaiC ATPase.
<4> X-ray crystallographic study on KaiC.
<5> Imaging and spectroscopic characterizations of the solution structure and dynamics of KaiC.

【Expected Research Achievements and Scientific Significance】

Results from our research project would provide an answer to the fundamental yet long-standing question in chronobiology: what determines the temperature-compensated 24 h period?

【Publications Relevant to the Project】

・Abe, J., Hiyama, T. B., Mukaiyama, A., Son, S., Mori, T., Saito, S., Osako, M., Wolanin, J., Yamashita, E., Kondo, T., and Akiyama, S. Atomic-scale Origins of Slowness in the Cyanobacterial Circadian Clock. *Science* 349, 312-316 (2015).
・Akiyama, S. Structural and dynamic aspects of protein clocks: How can they be so slow and stable? *Cellular and Molecular Life Sciences* 69, 2147-2160 (2012).
・Murayama, Y., Mukaiyama, A., Imai, K., Onoue, Y., Tsunoda, A., Nohara, A., Ishida, T., Maéda, Y., Terauchi, K., Kondo, T., and Akiyama, S. Tracking and visualizing the circadian ticking of the cyanobacterial clock protein KaiC in solution. *The EMBO Journal* 30, 68-78 (2011).

【Term of Project】

FY2017-2021

【Budget Allocation】

157,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://bms.ims.ac.jp/AkiyamaG/index.html

Title of Project : Mechanism of sex differentiation of germ cells

Yumiko Saga
(National Institute of Genetics, Genetic Strains Research Center, Professor)

Research Project Number : 17H06166 Researcher Number : 50221271
Research Area : Developmental Biology
Keyword : Germ cells

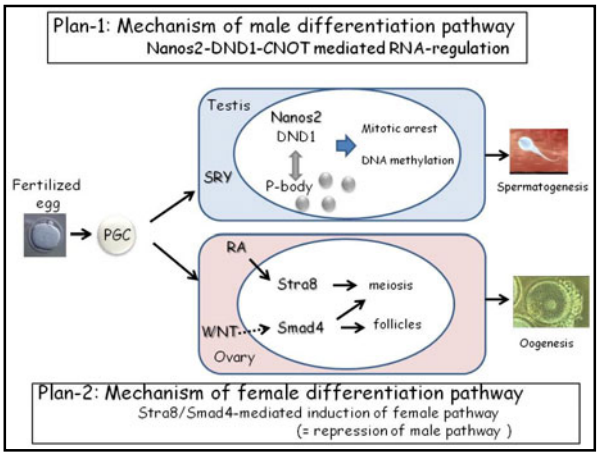
【Purpose and Background of the Research】

The differential programming of sperm and eggs in gonads is a fundamental topic in reproductive biology. Although the sexual fate of germ cells is believed to be determined by signaling factors from sexually differentiated somatic cells in fetal gonads, the molecular mechanism that determines germ cell fate is poorly understood.

We aim to elucidate the molecular mechanism of sex differentiation of germ cells. With regard to male differentiation pathway, we will clarify the mechanism how Nanos2-DND1-CNOT recognizes and suppresses target RNAs. We try to reconstruct the germ cell system using somatic cells. Regarding the feminization mechanism, we clarify the targets of Smad4 and Stra8 which are determinants for feminization, and clarify the molecular mechanism of feminization mediated by these genes by performing in vivo function analysis.

【Research Methods】

Research plan 1: Analysis of RNA regulatory mechanism involved in male pathway. We focus on Nanos2-DND1-CNOT1 complex localized in the P-body, the center of RNA metabolism. I will perform the following experiment. 1) Identify the necessity of P-body for Nanos2 function.
2) Identify factors required for reconstruct Nanos 2-DND1-CNOT function even in the somatic cells to clarify target RNA recognition mechanism.
Research plan 2: We aim to elucidate feminization mechanism of germ cells by focusing two factors Smad4 and Stra8. We will identify targets of Smad4 and conduct function assay to ask whether the deficiency induces sexual fate change from female to male in the absence of Stra8.



【Expected Research Achievements and Scientific Significance】

Nanos2-mediated germ cell specific RNA machinery is required for masculinization of mammalian germ cells. If you can elucidate the RNA regulatory mechanism and reproduce the RNA machinery in somatic cells, it will be useful to identify and verify the target RNAs. It may help to make germ cell from somatic cells via RNA regulation. It also contributes to the innovation of RNA manipulation technology.

Clarification of the feminization mechanism may lead to the understanding of sex determination of germ cells. We recently showed that germ cells could take male pathway even in the ovary if two genes were knocked out. This suggests that induction of feminization is essential for sex determination, and elucidation of its molecular mechanism greatly contribute to understanding of germ cell sex determination mechanism.

【Publications Relevant to the Project】

・Suzuki A, Niimi Y, Shinmyozu K, Zhou Z, Kiso M, Saga Y. Dead end1 is an essential partner of NANOS2 for selective binding of target RNAs in male germ cell development. ***EMBO Rep.*** 17(1):37-46 (2016).
・Wu Q, Fukuda K, Kato Y, Zhou Z, Deng C-X, Saga Y. Sexual Fate Change of XX Germ Cells Caused by the Deletion of SMAD4 and STRA8 Independent of Somatic Sex Reprogramming. ***PLOS Biol.*** 14(9):e1002553 (2016)

【Term of Project】

FY2017-2021

【Budget Allocation】

156,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nig.ac.jp/labs/MamDev/home-j.html>.3

Title of Project：Molecular mechanisms for centromere formation

Tatsuo Fukagawa
(Osaka University, Graduate School of Frontier Biosciences, Professor)

Research Project Number：17H06167　Researcher Number：60321600
Research Area：Chromosome Dynamics
Keyword：Centromere, Chromosome segregation, Chromosome function, Epigenetics

【Purpose and Background of the Research】

Chromosome segregation during mitosis is critical to transfer genetic information to daughter cells in all organisms. If errors of chromosome segregation occurred, chromosome instability would be caused. Therefore, it is one of the most important topics in genetics to understand mechanisms how chromosomes are faithfully segregated.

In this project, we focus on centromere, which is a critical genome region for chromosome segregation, and aim to define the molecular mechanisms how centromeres are formed.

【Research Methods】

I) Protein interaction network during progression of cell cycle

We have previously found that centromere proteins dynamically interact each other during progression of cell cycle. It is critical to address how such dynamic changes for protein-protein interaction in centromeres occur. In this project, we focus on phosphorylation of centromere proteins and try to clarify how the phosphorylation regulates the dynamic changes of protein-protein interaction in centromeres.

In addition to this, as shown in Fig.1, we proposed that the Ndc80 complex is recruited to centromeres by two pathways. We also clarify how and why these two pathways exist.

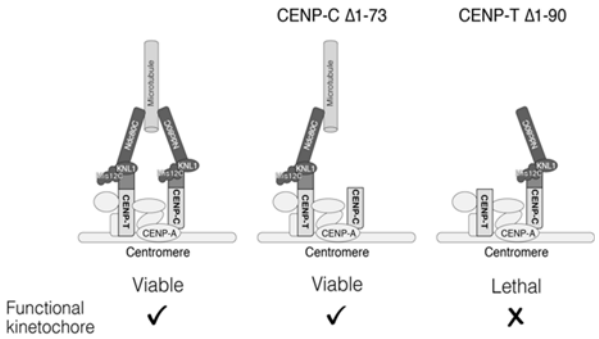


Fig 1. Model of kinetochore assembly. The Ndc80 complex is recruited by both CENP-T- and CENP-C-pathway (left). CENP-C Δ 1-73 cells, in which only CENP-T pathway is active, create kinetochore (middle), but CENP-T Δ 1-90 cells, in which only CENP-C pathway is active, do not form kinetochore (right).

II) Genome organization of centromeres

We have identified centromere-specific histone modifications utilizing non-repetitive centromeres. In this project, we will try to identify additional histone modifications in centromeres and clarify biological significance of such modifications for centromere specification and assembly.

III) Structural analysis of centromere protein complexes using Cryo-EM

Addition to functional analyses of centromere proteins, it is important to solve high-resolution structure of centromere protein complexes. In this project, we try to solve some of centromere protein complexes using Cryo-EM. This information would be useful to understand centromere architecture.

【Expected Research Achievements and Scientific Significance】

We propose this project based our achievements in centromere study. Combined our previous knowledge with results in this project, we expect to clarify mechanism how centromeres are formed. As we can perform various experiments using our own original assay, we are standing a good position to lead the research-field of centromere biology.

【Publications Relevant to the Project】

・Fukagawa T et al., The centromere: chromatin foundation for the kinetochore machinery. Dev. Cell, 30, 496-508 (2014).
・Nishino T et al., CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. Cell, 148, 487-501 (2012).

【Term of Project】

FY2017-2021

【Budget Allocation】

157,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.fbs.osaka-u.ac.jp/labs/fukagawa/
tfukagawa@fbs.osaka-u.ac.jp

Title of Project : Studies on mechanisms of biosynthesis of biomolecules via amino-group carrier protein and expansion of structural diversity of secondary metabolites

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

Research Project Number : 17H06168 Researcher Number : 00208240
Research Area : Applied Microbiology, Applied Biochemistry
Keyword : Microbial Metabolism, Enzyme Chemistry

【Purpose and Background of the Research】

Carrier proteins that bind a carboxyl group of a substrate are known in biosynthesis of fatty acids and polyketides. We found a novel-type carrier protein, amino-group carrier protein (AmCP), which binds the amino group of a substrate, in lysine and arginine biosynthesis of thermophiles. We also showed involvement of AmCP in secondary metabolisms of *Streptomyces*. These observations suggest that metabolic system using AmCP plays crucial roles in cellular processes.

In this study, we will determine the crystal structures of lysine/arginine biosynthetic enzymes, and analyze secondary metabolite biosynthesis using AmCP in *Streptomyces.* Through a series of the studies, mechanisms to create structural diversities of chemical compounds, which will expand chemical library, will be elucidated.

【Research Methods】

We will analyze functions of AmCP-mediated systems in lysine/arginine biosynthesis and secondary metabolisms at molecular and atomic levels. Structural biology is mainly conducted for enzymes for lysine and arginine biosynthesis from thermophiles, which will reveal mechanisms by which enzymes had acquired high substrate specificity. Especially, enzyme-AmCP complexes

Fig. 1. *amcp*-containing clusters for secondary metabolite biosynthesis. Red ovals show *nrps* genes.

are main targets of this analysis. Because most reactions in secondary metabolisms are unknown, we will analyze and unveil them by forefront technologies. This analysis will be conducted especially for enzymes that are involved in novel reactions and/or novel chemical architecture formation.

【Expected Research Achievements and Scientific Significance】

We discovered that AmCP is utilized in both primary and secondary metabolisms. These discoveries with high originality are highly evaluated in the related fields. This study analyzes enzymes that use AmCP globally at atomic levels, and determines chemical structures of their products. Our study that may discover unprecedented chemistry generated by unknown enzymes is highly creative and pioneering, leading to generation of new research fields, and therefore will contribute to keeping our international initiative in the fields. We will analyze biosynthesis of useful biomolecules, and, therefore, will provide valuable information as applied science.

【Publications Relevant to the Project】

T. Ouchi, T. Tomita, A. Horie, A. Yoshida, T. Kuzuyama, M. Nishiyama et al. (2013) Lysine and arginine biosyntheses mediated by a common carrier protein in *Sulfolobus*. *Nat Chem Biol*, **9,** 277-283.

F. Hasebe, K. Matsuda, T. Shiraishi, T. Tomita, T. Kuzuyama, M. Nishiyama et al. (2016) Amino group carrier protein-mediated secondary metabolite biosynthesis in *Streptomyces*. *Nat Chem Biol,* **12**, 967-972.

【Term of Project】 FY2017-2021

【Budget Allocation】 160,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/biotec-res-ctr/saibou kinou/

Title of Project : Development of soluble expression technology and utilization of enzymes from plants and animals

Yasuhisa Asano
(Toyama Prefectural University, Faculty of Engineering, Professor)

Research Project Number : 17H06169　Researcher Number : 00222589
Research Area : Enzyme Engineering
Keyword : Gene Expression, Enzyme, Soluble Expression,

【Purpose and Background of the Research】

We named the microbial metabolic pathway of aldoxime and nitriles as the "aldoxime-nitrile pathway", and further proving its occurrence in plants and an animal, by clarifying the structure and functions of various enzymes involved in the pathway. Furthermore, we characterized a new hydroxynitrile lyase (HNL) from an arthropod, millipede. The results of the transcriptomic analyses indicated that the millipede is full of proteins with new structures.

On the other hand, followed by our discovery of a mutation-directed soluble expression in *Escherichia coli* of the gene for a plant HNL, we are discovering that the solubilities are caused by structural alterations found in specific positions of the proteins.

In this research, we will solve two major inter-related problems in enzyme engineering. One is the improvement of the heterologous expression in the soluble form of enzymes of plant and animal origins by mutation of their genes, according to the "α-helix rule" and by a program "INTMSAlign-HiSol". Another is discovery and assignment of the enzymes responsible for cyanide metabolism in the millipede by the genome analyses.

【Research Methods】

By random mutagenesis and the statistical analyses of the mutation sites of the resulting soluble mutations in proteins, we will discover rules among the mutation sites causing the soluble expression. We will then predict how to mutate the enzyme for soluble expression according to the "α -helix rule" and with an aid of a program "INTMSAlign- HiSol". With these methods, it will become possible for many proteins from plants and animals to be expressed in soluble forms in *E. coli*. These technologies will be utilized in the research for the metabolism and enzymology of higher organisms.

We will next search for enzymes participating in the aldoxime-nitrile pathway of the millipede, by its transcriptomic analyses. The reaction mechanism of the millipede HNL will be clarified by studying the relationship between the activity and the structures of the mutants by X-ray crystallography.

【Expected Research Achievements and Scientific Significance】

This research is challenging because there has not been a systematic analyses of the heterologous expression of proteins in soluble forms, by discovering the relationship between the primary structures and how they are expressed.

We will clarify the millipede genes for cyanide and the primary metabolism. It is also challenging to assign the genes of the millipede enzymes by our soluble expression technology.

【Publications Relevant to the Project】

・Y. Asano, M. Dadashipour, M. Yamazaki, N. Doi, and H. Komeda, Functional expression of a plant hydroxynitrile lyase in *Escherichia coli* by directed evolution: Creation and characterization of highly *in vivo* soluble mutants, *Protein Engineering Design and Selection*, **24 (8)**, 607-616 (2011).
・S. Nakano, and Y. Asano, Protein evolution analysis of *S*-hydroxynitrile lyase by complete sequence design utilizing the INTMSAlign software, *Scientific Reports*, **5**, 8193 (2015).

【Term of Project】 FY2017-2021

【Budget Allocation】 157,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.pu-toyama.ac.jp/BR/asano/homepage.html
http://www.jst.go.jp/erato/asano/index.html

Title of Project : Life science basis of short-lived reactive species originated from foods

Koji Uchida
(The University of Tokyo, Graduate School of Agriculture and Life Sciences, Professor)

Research Project Number : 17H06170 Researcher Number : 40203533
Research Area : Agricultural Chemistry, Food Sciences
Keyword : Short-lived molecules, innate immunity, polysulphides, protein folding

【Purpose and Background of the Research】

Diets rich in vegetables are associated with a reduced risk of several major diseases, including cancers, diabetes, hypertension, and heart disease. It has been shown that non-nutritive plant chemicals, including polyphenols and sulfer compounds, in plant vegetables play a critical role in their beneficial effects. These functional ingredients produce various intermediates via metabolism that are extremely unstable and highly reactive toward biological components, such as proteins. Modification of proteins by these reactive species is suggested to be closely associated with the regulation of protein functions, showing their beneficial effects on human health. Thus, it can be speculated that plant-derived food molecules show their intrinsic health-related function via production of these reactive intermediates followed by interaction with proteins.

The aim of this project is to characterize unstable short-lived reactive species originated from foods and establish novel gain-of-function mechanism of proteins through covalent interaction with these molecules, thereby contributing to biological events (Fig. 1).

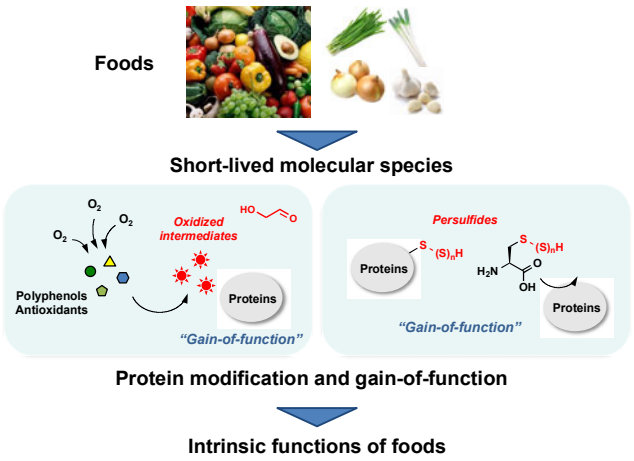


Fig.1. Function of food-derived short-lived reactive species in health.

【Research Methods】

Following items regarding the short-lived reactive species, including antioxidant metabolites and persulfides, will be specifically focused.

1. Identification and detection of short-lived reactive species derived from antioxidants
2. Gain of new function of protein by short-lived antioxidant intermediates
3. Protein persulfidation by persulfide molecules
4. Regulation of intracellular protein function by short-lived reactive species.

【Expected Research Achievements and Scientific Significance】

It is expected that some of the intrinsic functions of antioxidants, such as polyphenols, are proved to be due to their unstable short-lived reactive species. It is also expected that persulfides, a novel active sulfur species, are established as a new signal molecule derived from foods. A research area on the basis of the production of these reactive species originated from foods will be launched.

【Publications Relevant to the Project】

・ Hatasa *et al.* (2016) Oxidative deamination of serum albumins by (-)-epigallocatechin-3-*O*-gallate: A potential mechanism for the formation of innate antigens by antioxidants. ***PLoS ONE*** 11(4):e0153002.
・ Miyashita *et al.* (2014) Lysine pyrrolation is a naturally-occurring covalent modification involved in the production of DNA mimic proteins. ***Sci. Rep.*** 4:5343.

【Term of Project】 FY2017-2021

【Budget Allocation】 157,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/foodchem/index.html
E-mail: a-uchida@mail.ecc.u-tokyo.ac.jp

Title of Project : Establishment of "Minimum-loss" agriculture

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

Research Project Number : 17H06171 Researcher Number : 20244577
Research Area : Environmental agronomy
Keyword : Environmentally friendly agriculture, ecosystems, traditional agriculture, soil microorganisms

【Purpose and Background of the Research】

The main objective of the present study is to establish a "minimum-loss" agricultural system to mitigate the risks of long-term sustainability of agricultural production as well as to cope with environmental problems derived from human agricultural activities, both of which have risen during the development of modern agriculture. The "minimum-loss" agriculture system involves techniques to minimize: 1) the leaching loss of nutritional elements from the bottom of soils, 2) the emission loss of gaseous components from soil surface and 3) erosion of soil particles and organic materials from the soil surface. We aim to study ecosystem processes and agro-technical management of natural and traditional agricultural ecosystems, respectively, that have sustained for at least certain period in the past, by adopting the approach and methodology of ecology and/or regional studies (farming technology theory), and identifying several techniques available in the context of modern agriculture.

【Research Methods】

In order to establish "minimum-loss" agriculture, we set up research phases in three stages. In order to analyze the ecological processes in individual ecosystems (stage 1), the following sub-themes were developed both in the natural and traditional agricultural ecosystems in Asia and Africa, 1) to examine the process of establishing a symbiotic relationship between plants and soil microbes in the rhizosphere and determining factors of nitrogen flux in the context of resource (N and P) acquisition strategy of these ecosystems, 2) to understand the energy transformation and biochemical reactions in plant-microbial symbiosis, 3) to analyze nutritional-requirements of traditional crops, 4) to evaluate land and water management practices in traditional agriculture by monitoring soil properties, rainfall and water movement in agricultural land, and 5) to re-evaluate multiple cropping systems for traditional agriculture. These ecological processes analyzed above are interpreted by application of the farming technology theory (stage 2), followed by reconstruction of probable technical components in the context of "minimum-loss" agriculture (stage 3) .

【Expected Research Achievements and Scientific Significance】

The following four points are expected to be direct and indirect achievements:

1) Local agriculture is understood in the context of biogeochemical and ecological adaptation.
2) Establishment of "minimum-loss" agriculture based on ecological insights.
3) These research achievements would enable a "paradigm shift" from agricultural technologies that emphasize high yield and increased benefits to that based on sustainability and environmental rationale.
4) It creates an opportunity to recognize local agriculture again amidst globalization.

【Publications Relevant to the Project】

・Funakawa S (Ed) 2017: Soils, Ecosystem Processes, and Agricultural Development: Tropical Asia and Sub-Saharan Africa. Springer, pp.392.
・Funakawa S, Watanabe T, et al. 2011: 4 Soil resources and human adaptation in forest and agricultural ecosystems in humid Asia and 5 Pedogenetic acidification in upland soils under different bioclimatic conditions in humid Asia. In World Soil Resources and Food Security. Eds. R. Lal and B.A. Stewart. p.53–269, CRC Press, Taylor & Francis Group, Boca Raton, London, New York.

【Term of Project】 FY2017-2021

【Budget Allocation】 148,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.soils.kais.kyoto-u.ac.jp/
funakawa@kais.kyoto-u.ac.jp

Title of Project : Molecular elucidation of plant-pathogen interactions

Ken Shirasu
(RIKEN Center for Sustainable Resource Science, Group Director)

Research Project Number : 17H06172 Researcher Number : 20425630
Research Area : Agricultural science, Boundary agriculture, Applied molecular and cellular biology
Keyword : Molecular interactions, Biological interactions

【Purpose and Background of the Research】

The plant immunity system is shaped by millions of years of coevolution between plants and their pathogens such as viruses, bacteria, fungi, and nematodes, resulting in enormous complexity at the molecular level. The invading pathogens thus need special weapons to conquer the established immune system of their corresponding host, as the host would defend itself like a fortified castle. These weapons are known as effectors, which modulate plant immunity and enable parasitic infection. For example, some effectors attack the first layer of the plant immune system conferred by cell surface pattern-recognition receptors. To detect such effectors, plants have evolved other types of receptors called Resistance proteins, which recognize the presence of pathogen effectors directly or indirectly and launch strong counter attack, known as effector-triggered immunity. However, the functions of many plant immunity signaling proteins and most pathogen effectors remain elusive.

In this study, we aim to identify plant and pathogen proteins that are important for plant-pathogen interactions and isolate their complex to provide a unified view of how plants defend themselves against pathogens.

【Research Methods】

Main focus will be on the reactive oxygen species (ROS) producing enzyme and sensor complexes, kinase complexes, superoxide complexes, and ubiquitin ligase complexes that have been isolated in our laboratory. Using highly sensitive mass-spectroscopy, we aim to identify their interacting proteins. We also aim to isolate plant immunity related proteins downstream of such protein complexes. We will perform genetic and biochemical analyses, as well as various omics approaches to analyze function of novel proteins involved in plant immunity. In addition, to understand how pathogens overcome the immunity, we will determine de novo genome sequences of various pathogens and their expression profiles. This approach will provide a strong research base for studying pathogens at the molecular level.

【Expected Research Achievements and Scientific Significance】

A number of immune receptors and sensors against various pathogens have been identified by mainly genetic analyses. However, the diversity of pathogens is extremely high and plants should have much more receptors and sensors to fight against such enemies. Immune signals generated by such receptors and sensors should merge in the cell and go out the cell but the exact mechanism remains highly elusive. In our study, we aim to understand how the immune signals merge, especially by elucidating the key ROS-mediated pathway. We compare plant and animal innate immune systems to find commonalities and differences. Our data may reveal a novel signaling mechanism and its biological significance.

【Publications Relevant to the Project】

- Gan, P., et al., Genus-wide comparative genome analyses of Colletotrichum species reveal specific gene family losses and gains during adaptation to specific infection lifestyles. (2016) Genome Biology and Evolution. 8: 1467-1481.
- Kadota, Y., et al., Direct regulation of the NADPH oxidase RBOHD by the PRR associated kinase BIK1 is required for ROS burst and plant immunity. (2014) Mol. Cell 54: 43-55.

【Term of Project】 FY2017-2021

【Budget Allocation】 156,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://plantimmunity.riken.jp

Title of Project : New Molecular Technologies to Open Up Multiple Applications of Light in Life Science and Materials Science

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

Research Project Number : 17H06173 Researcher Number : 00271916
Research Area : Organic Synthesis, Elements Chemistry, Theoretical Chemistry
Keyword : Molecular Transformation, Material Science, Spectroscopy

【Purpose and Background of the Research】

Complex π-electron compounds that interact with light have long attracted interest, and have become increasingly important in recent years for potential applications in life science and materials science. However, there are still severe limitations in rational design, synthesis, and derivatization of π-electron organic molecules, as well as in their functional modification (absorption/emission properties, quantum yield, thermo-stability, *etc.*), and these issues have hampered their practical application in advanced technologies, including storage media, organic semiconductors, laser printers, photodynamic therapy of cancer, nonlinear optics, deodorants, and molecular imaging. This project aims to develop new synthetic methods, to extend theoretical principles, and to synthesize novel π-electron molecules with unique functionalities suitable for next-generation technological applications.

【Research Methods】

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

① Breakthrough synthetic chemistry to construct π-electron molecules and to provide new tools for chemo-, regio- and stereo-selective introduction of various functional groups into π-electron molecules
② Molecular science for the utilization of light
③ Theoretical chemistry to underpin the utilization of light
④ Molecular technology for the utilization of light

【Expected Research Achievements and Scientific Significance】

Besides providing new molecules, tools, and theoretical principles, one of the specific aims of this project is to create near-infrared light-capturing π-electron compounds. Such compounds could be useful for cancer treatment by photodynamic therapy, *in-vivo* 3D imaging, and many other purposes. As near-infrared light penetrates well through human tissue, compounds that absorb strongly in this wavelength region are particularly desirable for photodynamic therapy, since specific illumination of the tumor would allow only cancerous cells to be heated/damaged and consequently killed.

【Publications Relevant to the Project】

・N. Toriumi, A. Muranaka, E. Kayahara, S. Yamago, M. Uchiyama "In-plane Aromaticity in Cycloparaphenylene Dications: A Magnetic Circular Dichroism and Theoretical Study" *J. Am. Chem. Soc.,* **137**, 82-85 (2015)
・D.-Y. Wang, M. Kawahata, Z.-K. Yang, K. Miyamoto, K. Yamaguchi, C. Wang, M. Uchiyama, "Stille Coupling *via* C–N Bond Cleavage" *Nature. Commun.*, **7**, 12937 (2016)
・N. Tezuka, K. Shimojo, K. Hirano, C. Wang, T. Saito, R. Takita, M. Uchiyama, "Direct Hydroxylation and Amination of Arenes via Deprotonative Cupration" *J. Am. Chem. Soc.*, **138**, 9166–9171 (2016)
・M. Tanioka, S. Kamino, A. Muranaka, Y. Ooyama, H. Ota, Y. Shirasaki, J. Horigome, M. Ueda, M. Uchiyama, D. Sawada, S. Enomoto, "Reversible Near-Infrared/Blue Mechano-fluorochromism of Aminobenzopyranoxanthene" *J. Am. Chem. Soc.,* **137**, 6436-6439 (2015)

【Term of Project】

FY2017-2021

【Budget Allocation】

163,300 Thousand Yen

【Homepage Address and Other Contact Information】

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

Title of Project : Extensive analyses of the LUBAC ubiquitin ligase

Kazuhiro Iwai
(Kyoto University, Graduate School of Medicine, Professor)

Research Project Number : 17H06174 Researcher Number : 60252459
Research Area : General Medical Chemistry
Keyword : ubiquitin, regulation of inflammation, B cell lymphomas, LUBAC ligase, myopathy

【Purpose and Background of the Research】

The ubiquitin conjugation system has been identified as a part of protein degradation system. Then, the ubiquitin research has been developed in tight relationship with proteolysis. However, linear ubiquitin chains and the LUBAC ligase (composed of three subunits) generating the chain specifically, which we discovered, are now world-widely recognized as a reversible post-translational system that is involved in signaling leading to NF-κB activation and protection from cell death.
Moreover, aberrant activation or impairment of the LUBAC ligase activity is shown to be involved in some form of B cell lymphomas or immunodeficiency and autoinflammation, respectively. Thus, LUBAC and the linear ubiquitin chain also attract attentions of clinicians. The leader of this research project discovered LUBAC and the linear ubiquitin chain and we have already generated transgenic and conditional knockout mice of the subunits of LUBAC. In this research project, we intend to develop LUBAC research further and to build the basis for the translational research to cure lymphomas and autoinflammatory diseases by manipulating LUBAC.

【Research Methods】

Using multidisciplinary techniques including structural biology, biochemistry and mouse genetics, we intend to perform research from the following four points.

1. Structural and functional analyses of regulation and activation mechanisms of the LUBAC ubiquitin ligase
2. Dissection of the roles LUBAC and the linear ubiquitin chains played in inflammation and immune-regulation
3. Dissection of new pathophysiological function of LUBAC using genetically engineered mice
4. Dissection of the roles of LUBAC in lymphomagenesis and development of LUBAC inhibitors

【Expected Research Achievements and Scientific Significance】

The following achievement will be expected.

1. Elucidation of regulatory mechanism of the LUBAC ligase
2. Elucidation of pathogenesis mechanisms of LUBAC-related disorders
3. Elucidation of activation mechanism of inflammasomes in which LUBAC and autophagy are involved
4. Roles of LUBAC played in immune-regulation mediated by regulatory T cells

Our research project might lead to the development of the followings.

1. Development of drug modulating LUBAC activity as an anti-cancer drug
2. Provide new aspects in the research of autoimmune and autoinflammatory diseases

【Publications Relevant to the Project】

1. Iwai, K., Fujita, H., and Sasaki, Y. Linear ubiquitin chains: NF-κB signalling, cell death, and beyond. **Nature Rev. Mol. Cell Biol.** 15(8):503-508, 2014.
2. Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sataka, S.-I., Tanaka, K., Nakano, H., and Iwai, K. SHARPIN is a component of the NF-κB activating linear ubiquitin chain assembly complex. **Nature** 471:633-636, 2011.

【Term of Project】 FY2017-2021

【Budget Allocation】 157,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://mcp.med.kyoto-u.ac.jp/
kiwai@mcp.med.kyoto-u.ac.jp

Title of Project : Immune systems involved in the resolution of inflammation and tissue repair

Akihiko Yoshimura
(Keio University, School of Medicine, Professor)

Research Project Number : 17H06175 Researcher Number : 90182815
Research Area : Basic Medicine Immunology
Keyword : Inflammation, Immune Signaling, Cytokine, Tolerance and Autoimmunity

【Purpose and Background of the Research】

Our group has investigated the mechanisms of promotion of inflammation by innate immune response within 7 days after onset of mouse experimental cerebral ischemia (cerebral infarction) model (Fig. 1). We discovered that infiltrating macrophages are activated by DAMPs (danger associate molecular patterns) such as peroxiredoxin derived from dead cells, and then release inflammatory cytokines during the acute phase within one day of onset of stroke. This contributes to expansion of the infarct lesion and deterioration of neurological symptoms. Then γδ T cells infiltrate 2 or 3 days after onset of stroke, and produce IL-17 that promotes deterioration of the disease state. On the other hand, we have also elucidated the mechanism of resolving inflammation. We identified the scavenger receptors for the clearance of DAMPs, and also macrophages which are involved in the termination of inflammation. Furthermore, we found that at the chronic phase of the stroke, a large amount of T cells accumulate in the brain. In this study, we aim to elucidate the significance of repairing macrophages and brain infiltrated T cells for resolution of inflammation and tissue repair and to identify factors that define these processes.

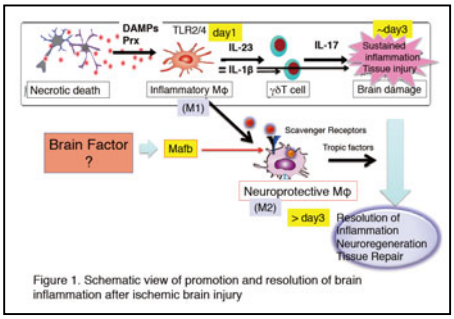


Figure 1. Schematic view of promotion and resolution of brain inflammation after ischemic brain injury

【Research Methods】

(1)Identification of the brain factor that induces repairing macrophages; It is considered that macrophages have an activity to induce expression of Igf1 and Msr1 in the brain. We will purify the factors from brain extract and examine whether this factor can induce repairing macrophages. We also search a master regulator for the generation of reparing macrophages by the RNA-seq technology.
(2) Elucidation of the mechanism of amplification and infiltration of T cells at the chronic phase of cerebral infarction; Most of CD4 positive T cells infiltrated into the brain of cerebral infarcted mice were Th1 and regulatory T (Treg) cells. In order to clarify the significance of each cell, activation of astrocytes, glial scar formation, and improvement of neurologic symptoms will be examined using IFNγ-deficient mice and Treg-depleted mice. We also analyze the chemokine-chemokine receptor and T cell receptor of infiltrated T cells and clarify the mechanism of infiltration and amplification of T cells into the brain.
(3) Elucidation of the relationship between T cells and microglia, astrocytes; We establish a co-culture system of T cells, microglia and astrocytes *in vitro* and elucidate the molecular mechanism by which T cells control these cells.
(4) Elucidation of the significance of T cells in other tissue injuries; We will investigate whether the repair mechanism in T cell tissue damage revealed in this study also applies to other chronic inflammatory systems such as myocardial infarction model and Alzheimer model.

【Expected Research Achievements and Scientific Significance】

Although inflammation has been often considered in relation to tissue injury, however, inflammation is also important for the initiation of tissue repair. In particular, there is in a dynamic equilibrium state including the acquired immune system during chronic stage of inflammation. The inflammatory processes leading from "removal of damaged cells" to "tissue repair" will be clarified by our research. Furthermore, new cells and soluble factors which are involved in these processes will be identified. Our goal is the development of new therapies for tissue injuries at the chronic phase including brain stroke.

【Publications Relevant to the Project】

Shichita T, Yoshimura A et al. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain ***Nature Medicine*** 2012 Jun;18(6):911-917.

Shichita T, Ito M, Yoshimura A.et al.
MAFB prevents excess inflammation after ischemic stroke by accelerating clearance of damage signals through MSR1.
Nat Med. 2017 Jun;23(6):723-732

【Term of Project】

FY2017-2021

【Budget Allocation】

158,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://new2.immunoreg.jp

Title of Project : Gaining Integrative Understanding of Gastrointestinal Disease Phenotypes through Establishment of an Organoid Library

Toshiro Sato
(Keio University, School of Medicine, Associate Professor)

Research Project Number : 17H06176 Researcher Number : 70365245
Research Area : Gastroenterology
Keyword : Lower gastroenterology (small intestine, colon)

【Purpose and Background of the Research】

Our gastrointestinal epithelium accumulates genetic mutations along with age, which eventually lead to tumorigenesis. The advance in molecular biology has enabled to identify a number of oncogenic mutations. Recent development of next generation sequencing further deepened our understanding of genetic contribution to the cancer. Nevertheless, we still have little understanding of life-threatening malignant cellular behaviors, such as tumor invasion and metastasis. The long-standing challenge prompted us to shift our focus on conventional genome analysis using “dead cells” to the cell biological analysis using “living cells” from patients (Figure 1).

We recently succeeded in establishing organoid culture technology that enabled growth of stereotypic structures from human gastrointestinal tissue in vitro. By combining with genome editing technology, the organoid technology has revealed genotype-phenotype correlations during human tumorigenesis and has become a valuable a biological platform.

In this study, using the latest version of organoid technology, we establish a gastrointestinal organoid library with each organoid characterized by comprehensive molecular analyses and drug screening tests. The established organoids will be provided to research community, in order to gain deeper biological insights into malignant transformation of gastrointestinal cancer.

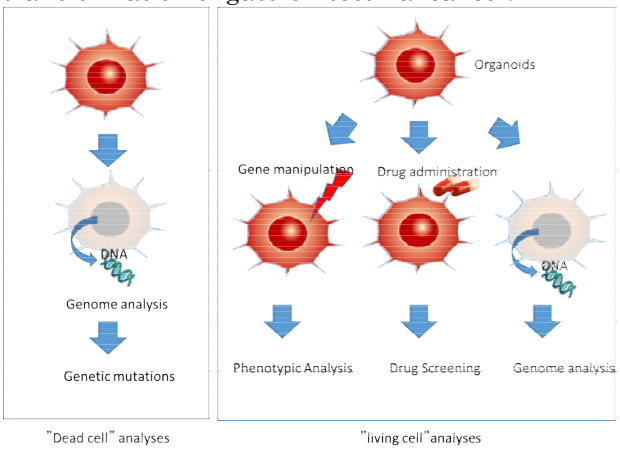


Figure 1. Organod technology realizes “Living Cell” analyses

【Research Methods】

To efficiently propagate patient-derived diseased tissues, we refine the organoid culture protocol and establish an organoid library. The established organoids are subjected to comprehensive molecular analyses encompassing whole exome analysis, gene expression analysis, epigenetic analysis and high throughput drug screening tests. These data sets are systemically analyzed to reveal relationships between genotypes and phenotypes. We also reconstruct genetic mutations into organoids in order to understand the oncogenic contribution of ill-defined genetic mutations that are highlighted by above analyses. We streamline the system for organoid biobanking to distribute the established organoids to research community.

【Expected Research Achievements and Scientific Significance】

This study can provide novel cell biological insights into malignant cell behavior of cancer cells, which may not be achieved by conventional genomic analyses. Furthermore, distribution of organoids to research community will promote multidisciplinary research.

【Publications Relevant to the Project】

・Matano M, Date S, Shimokawa M, Takano A, Fujii M, Ohta Y, Watanabe T, Kanai T, Sato T*. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nature Medicine. 2015;21:256-62.
・Fujii M, Shimokawa M, Date S, Takano A, Matano M, Ohta Y, Nanki K, Kawasaki K, Nakazato Y, Uraoka T, Watanabe T, Kanai T, Sato T*. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements. Cell Stem Cell 2016:18:827-38.

【Term of Project】 FY2017-2021

【Budget Allocation】 159,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.keio-med.jp/gastro/index.html
t.sato@keio.jp

Title of Project : Kidney reconstitution and disease modeling based on nephron induction methods in vitro

Ryuichi Nishinakamura
(Kumamoto University, Institute of Molecular Embryology and Genetics, Professor)

Research Project Number : 17H06177 Researcher Number : 70291309
Research Area : Nephrology, Developmental Biology
Keyword : Kidney development, iPS cell, nephron progenitor

【Purpose and Background of the Research】

The kidney develops through the interactions of three precursor tissues: nephron progenitors, ureteric buds, and stromal progenitors. We have previously reported the induction method of nephron progenitors from multipotent stem cells. One of the aims of this project is to induce the ureteric bud in addition to nephron progenitors, and utilize these methods to reveal mechanisms underlying the hereditary kidney diseases. The other aim is to induce the stromal progenitors and combine them with nephron progenitors and the ureteric bud to generate the genuine three-dimensional kidney structures.

【Research Methods】

We will establish methods to induce the ureteric bud and stromal progenitors from mouse ES cells, and subsequently from human iPS cells. We then apply the methods to the iPS cells derived from hereditary kidney diseases, to model the phenotypes and reveal mechanisms underlying the diseases. We will also establish a method to combine the three progenitors to reconstitute the genuine three-dimensional kidney structures from mouse ES cells, and eventually from human iPS cells.

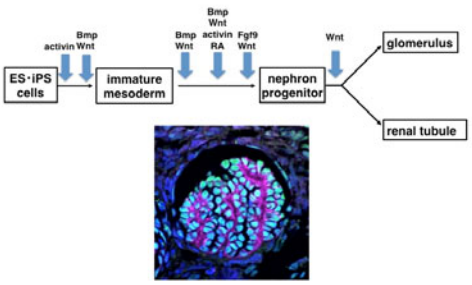


Figure 1 Renal glomerulus generated in vitro

【Expected Research Achievements and Scientific Significance】

Easy and frequent access to human kidney tissues will accelerate the human developmental nephrology and reveal the species differences from other animal models including mice. In addition, elucidation of the mechanisms of the hereditary kidney diseases will serve as basis for drug screening aiming at the treatment. Furthermore, generation of the three-dimensional kidney structures will advance the regenerative medicine toward the renal transplantation of the induced organs in the future.

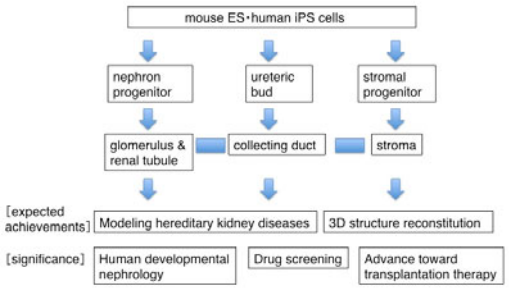


Figure 2 Expected achievements and significance

【Publications Relevant to the Project】

・Sharmin S, Taguchi A, Kaku Y, Yoshimura Y, Ohmori T, Sakuma T, Mukoyama M, Yamamoto T, Kurihara H, and Nishinakamura R. Human induced pluripotent stem cell-derived podocytes mature into vascularized glomeruli upon experimental transplantation. **J Am Soc Nephrol** 27: 1778-1791, 2016
・Taguchi A, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, and Nishinakamura R. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. **Cell Stem Cell** 14: 53-67, 2014

【Term of Project】 FY2017-2021

【Budget Allocation】 157,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.imeg.kumamoto-u.ac.jp/en/bunya_top/kidney_development/

Title of Project : Generation of neural network repair medicine

Toshihide Yamashita
(Osaka University, Graduate School of Medicine, Professor)

Research Project Number : 17H06178 Researcher Number : 10301269
Research Area : Medicine, dentistry, and pharmacy
Keyword : Neuroscience, neuronal network

【Purpose and Background of the Research】

Neurological disorders such as cerebrovascular disease, focal and central nervous system disorders from traumatic brain and spinal cord injury, higher brain dysfunction, and neuropathic pain, form their pathology and bring about spatiotemporal changes in the biological system which is made up not only of the nervous system but the immune system, the vascular system, and various organs. In this study, we analyze central neural circuit disorders and the subsequent restoration process from the viewpoint of the functional network of the biological system, and attempt to reveal in an integrated manner the control mechanism for the series of processes using the spatiotemporal dynamics of the biological system. The research particularly aims to uncover the control mechanism based on the linkage between the nervous system and each organ (Figure 1). We approach the process of central neural circuit disorders and functional recovery as the dynamics of the entire biological system and analyze the linkage between the nervous system and each system in an integrated manner to clarify the working principles of the living body with regard to central neural circuit disorders.

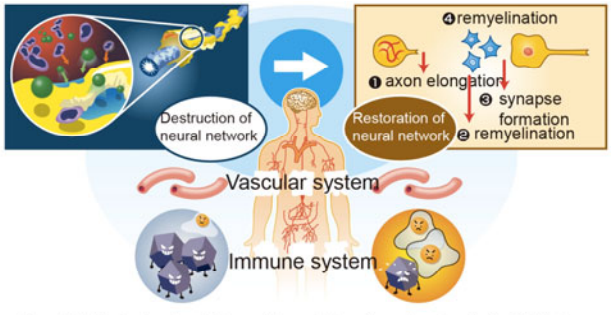


Figure 1 Biological systems that regulate rewiring of neural network after CNS injury

【Research Methods】

Unilateral cerebral cortex injury, local encephalomyelitis (EAE), and ADHD model mice are used. Using these pathological models, we analyze the dynamics of various organ cell groups and the spatiotemporal changes in gene expression. Furthermore, we continue analysis of the mechanisms of how immune cells, vascular cells, and organs control damage and repair of neural circuits. Along with these findings, we reveal the neural circuit repair mechanisms caused by activation of each cell group, and elucidate the working principles of the reactions of the living body.

【Expected Research Achievements and Scientific Significance】

Previous research has tended to identify the central nervous system as an independent organ and clarified the linkage between neural cells. Research that considers the central nervous system as an organ of the biological system and asks how the whole biological system is involved in the damage and repair of neural circuits is still in its early stages. We are generating research that understands neural circuit damage and the subsequent repair process reaction of living organisms as a 'scrap-and-build' strategy to reveal the mechanisms of this series of reactions and their significance, and we anticipate that this will create a new trend in life science research.

【Publications Relevant to the Project】

・Fujita, Y., Masuda, K., Nakato, R., Katou, Y., Tanaka, T., Nakayama, M., Takao, K., Miyakawa, T., Shirahige, K. and **Yamashita, T.** (2017) Cohesin regulates formation of neuronal networks in the brain. **J. Exp. Med.** 214, 1431-1452.
・Fujitani, M., Zhang, S., Fujiki, R., Fujihara, Y. and **Yamashita, T.** (2017) A chromosome 16p13.11 microduplication causes hyperactivity through dysregulation of miR-484/protocadherin-19 signaling. **Mol. Psychiatry** 22, 364-374.

【Term of Project】 FY2017-2021

【Budget Allocation】 158,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.osaka-u.ac.jp/pub/molneu/index.html

Title of Project : Identification of higher-order-epigenetic modification machineries and development of potential novel therapeutics in severe virus infection

Yumiko Imai
(National Institutes of Biomedical Innovation, Health and Nutrition, Laboratory for Regulation of Intractable Infectious Diseases, Project Leader)

Research Project Number : 17H06179 Researcher Number : 50231163
Research Area : Critical Care Medicine
Keyword : Virus, Epigenetics

【Purpose and Background of the Research】

Influenza viruses are a major cause of morbidity and mortality especially among humans with high risk factors, such as diabetes and cancers. Also, highly pathogenic strains (e.g. A/H5N1 virus) show high mortality rates up to 60% in humans. Patients with severe influenza show acute respiratory distress syndrome (ARDS), which requires critical cares in the ICU. To date, the therapies in patients with severe influenza are limited, with currently available anti-influenza drugs that primarily target viral proteins, showing disappointing results most likely due to the emergence of mutated viruses. Influenza virus is a RNA virus, and transcription and replication of the virus genome occur in the nucleus. It is important to find the strategies targeting rather host nuclear system less permissive to viral replication.

Recent high-resolution chromatin interaction maps using chromosome conformation capture (3C) techniques have defined units of chromatin, termed topologically associated domains (TADs). In addition, the insulator protein CCCTC-binding factor (CTCF) is known to act as borders of TADs and play a role in the formation and maintenance of long-range chromatin loops. However, it remains unknown the changes in host chromatin architectures and histone modifications to different pathogenisity of viruses. Nor is it known how such higher-order host epigenetic response can influence the pathogenesis of influenza virus infection.

【Research Methods】

Using different strains of influenza viruses (e.g. H1N1 and H5N1), 1) we plan to analyze the dynamic changes in higher-order epigenetic responses in the host cells to virus infection, which include histone modifications (ChIP-seq), higher-order chromatin structures (3C-based 4C-seq and Hi-C). The host nuclear proteins interacted with virus proteins (mass-spectrometry), as well as the chromatin domains associated with virus proteins (ChIP-seq) will be analyzed; 2) how such higher-order host epigenetic responses affect the pathogenesis of influenza virus infection using host genome-edited cell and mice, and genetically modified viruses; 3) based on the data from 1) and 2), we plan to establish the prediction system to identify the high risk factors for severe influenza and explore the potential for early diagnosis and preemptive cares; 4) the candidate compounds targeting higher-order epigenetic modifications will be screened and we plan to explore the potential for novel drug development. .

【Expected Research Achievements and Scientific Significance】

This project, for the first time can identify the changes in host higher-order epigenetic responses to different strains of influenza viruses. In addition, the possible mechanisms by which such epigenetic changes can influence the pathogenesis of severe influenza will be clarified. Those data could be important information to develop novel therapeutics for severe virus infections.

【Publications Relevant to the Project】

1. Morita M, Kuba K, Ichikawa A, Nakayama M, Katahira J, Iwamoto R, Watanebe T, Sakabe S, Daidoji T, Nakamura S, Kadowaki A, Ohto T, Nakanishi H, Taguchi R, Nakaya T, Murakami M, Yoneda Y, Arai H, Kawaoka Y, Penninger JM, Arita M, Imai Y. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell. 2013 28;153(1):112-25.
2. Haarhuis JHI, van der Weide RH, Blomen VA, Yáñez-Cuna JO, Amendola M, van Ruiten MS, Krijger PHL, Teunissen H, Medema RH, van Steensel B, Brummelkamp TR, de Wit E, Rowland BD. The cohesin release factor WAPL restricts chromatin loop extension. Cell. 2017 169(4):693-707.

【Term of Project】 FY2017-2021

【Budget Allocation】 150,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nibiohn.go.jp/