Title of Project : Elucidation of sleep/wakefulness regulation using forward genetic approach

Masashi Yanagisawa
(University of Tsukuba, International Institute for Integrative Sleep Medicine, Director and Professor)

Research Project Number : 17H06095 Researcher Number : 20202369
Research Area : Neuroscience
Keyword : Sleep, Forward genetics, mouse

【Purpose and Background of the Research】

Sleep is a ubiquitous behavior in animals, but the molecular mechanisms controlling sleep/wakefulness are unknown. To elucidate the sleep/wake regulatory mechanism, the principal investigator conducted a forward genetic screen on sleep using randomly mutagenized mice, which was an unprecedented project in the world. We found that the *Sleepy* mutants spent long time in NREM sleep and had a mutation in the *Sik3* gene that encodes a protein kinase (Figures 1 and 2). We also found that the *Dreamless* mutation in the nonselective cation channel, NALCN, shortens REM sleep (Funato, Yanagisawa et al., Nature 2016). In this research project, we 1) accelarate a large-scale forward genetic research on to identify additional novel genes that control sleep/wake, 2) elucidate the SIK3-signal cascade that controls sleep/wakefulness behavior, and 3) reveal the intracellular signaling system that regulates REM sleep. These studies will lead to a paradigm shift in sleep research.

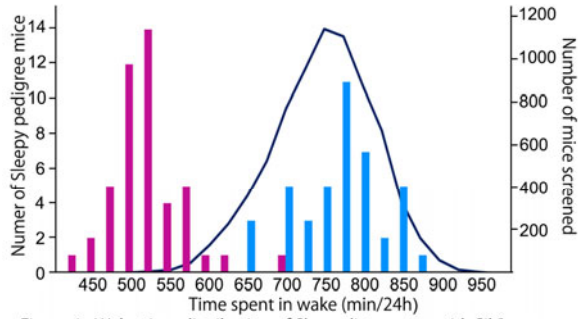


Figure 1 Wake-time distriburion of Sleepy littermates with Sik3 gene mutation (red) or without the mutation (blue). The curve indicates wake-time distribution of all mice screened.

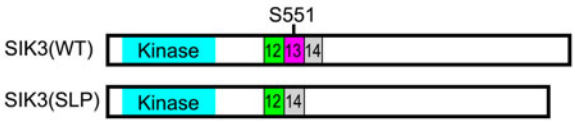


Figure 2 SIK3 is a protein kinase. The sleepy mutant skips exon 13 which encodes 52 amino acids containing S551, a well conseved PKA phosphorylation site.

【Research Methods】

1) We will conduct an EEG/EMG-based high-throughput screening of randomly mutagenized mice. After the establishment of pedigrees showing heritable sleep abnormalities, we will identify the mutation responsible for the sleep/wake phenotype through whole exome sequencing and genome editing. 2) To elucidate the SIK3 signal pathway regulating sleepiness, we identify neuronal groups responsible for determining sleep need by crossing Sik3 gene-modified mice with Cre driver mouse lines. We also try to identify the intracellular signal cascade that controls sleep/wakefulness by quantitative phosphoproteomics analysis of the FLAG-SIK3 mouse and the mutant FLAG-SIK3 (SLP) mouse. 3) To elucidate the mechanism to switch and terminate REM sleep episodes, we will identify the neuronal circuits controlling REM sleep using NALCN gene-modified mice. Furthermore, we will combine patch clamp recording and molecular biology methods to identify REM sleep control signal via NALCN.

【Expected Research Achievements and Scientific Significance】

By continuing the forward genetic analysis of mammalian sleep, we will be able to identify additional novel genes/pathways that control sleep/wakefulness. The identification of molecules that constitute the SIK3 signaling enables us to reveal intracellular signaling pathway of “sleep need.” We are also able to clarify the intracellular signaling system regulating REM sleep episodes through NALCN. Through these efforts, we will create a new research area, making a groundbreaking achievement in sleep research.

【Publications Relevant to the Project】

Chemelli, Yanagisawa et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98, 437-451, 1999.
Funato, Yanagisawa et al. Forward-genetics analysis of sleep in randomly mutagenized mice. Nature 539, 378-383, 2016.

【Term of Project】 FY2017-2021

【Budget Allocation】 423,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://sleepymouse.tsukuba.ac.jp

Title of Project : Molecular dissection of robust and flexible circadian clock and its control of animal physiology

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

Research Project Number : 17H06096 Researcher Number : 80165258
Research Area : Biology: Basic biology, Biological science
Keyword : Animal physiology and biochemistry, Transcriptional regulation, Post-translational modification, Circadian rhythm, Circadian oscillation mechanism

【Purpose and Background of the Research】

The circadian clock that governs daily rhythms in behaviors and physiology is characterized by its robustness of regulating stable daily rhythms. On the other hand, the circadian clock flexibly responds to a variety of external and internal signals in order to adjust its phase appropriately. In this project, we would explore a new mechanism that enables the stable oscillation of the molecular clock. We would also pursue a molecular mechanism that supports the flexibility of the entrainment.

In the later phase of this 5-years project, we would pay our attention toward interaction between the circadian clock and senescence. In addition to the well-recognized notion that the circadian clock is weakened during aging, a new idea will be examined whether aging could be one of the output of the circadian clock deformation. We would investigate bi-directional interaction between the circadian clock oscillation and aging.

【Research Methods】

In this project, we would perform extensive researches at molecular and cellular levels, and the outcomes will be fed back into behavioral analyses of mice that are genetically engineered or subjected to surgical or pharmacological manipulations. Based on the results obtained in the earlier phase of this project, we would make efforts to develop our research to the understanding of relationship of senescence and change in circadian clockwork during aging. Particularly we will explore molecular mechanism underlying bi-directional control between aging and circadian clock.

【Expected Research Achievements and Scientific Significance】

In individual cells, the clock genes and their encoding clock proteins form transcriptional and translational feedback loops, which generate a wide variety of oscillating transcripts. However, only a part of these oscillating transcripts has been recently shown to oscillate at the transcription level, while many other transcripts were constant at the de novo transcription (Koike *et al.,* 2012; Menet *et al.,* 2012). These observations strongly suggested important roles of post-transcriptional regulation in generating rhythmic transcripts, and indeed we have found an important role of A-to-I RNA editing rhythm (Terajima *et al.*, 2017). Furthermore, a number of studies such as Hirano *et al.* (2013) demonstrated that the robust and stable oscillation of the circadian clock requires post-translational modifications of the clock proteins, such as phosphorylation and ubiquitination. The protein modifications regulate the clock proteins in various aspects, such as their stabilities, cellular localization profiles, transcriptional activities, and protein-protein interactions. In this project, we would investigate potential roles of these unexplored steps of clock regulation toward understanding of the stable and flexible properties of the circadian clock at the molecular and cellular levels.

【Publications Relevant to the Project】

・Hirano *et al.* (2013) FBXL21 regulates oscillation of the circadian clock through ubiquitination and stabilization of cryptochromes. ***Cell***, *152,* 1106-1118
・Terajima, Yoshitane *et al.* (2017) ADARB1 catalyzes circadian A-to-I editing and regulates RNA rhythm. ***Nature Genet.*** *49,* 146-151

【Term of Project】 FY2017-2021

【Budget Allocation】 435,800 Thousand Yen

【Homepage Address and Other Contact Information】

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

Title of Project：In situ functional analyses of membrane proteins by NMR

Ichio Shimada
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number：17H06097　Researcher Number：70196476
Research Area：Structural biology
Keyword：NMR, G-protein coupled receptors, ion channels, transporters

【Purpose and Background of the Research】

Over the past decade, our structural understanding of membrane proteins has dramatically progressed, owing to the growing numbers of atomic resolution crystal structures of membrane proteins, including G-protein coupled receptors (GPCRs). However, the crystal structures represent static snapshots of proteins in the crystal lattice, and the observed conformations may not be the same as those in the environment where they actually function.

In this research project, we use the solution NMR, in order to obtain dynamic structural information of the membrane proteins under physiological conditions, which are directly related to their function.

【Research Methods】

In this research project, we will analyze the relationships between the dynamical structures and the functions for biologically important membrane proteins: 1) Biased-signaling of GPCRs, 2) Gating and activation mechanisms of potassium ion channels, and 3) Synergistic regulation of multi-drug resistant (MDR) system, with dynamical structures that are responsible for their functions. For these purposes, we will also develop NMR methodologies for studying the structures and dynamics of membrane protein.

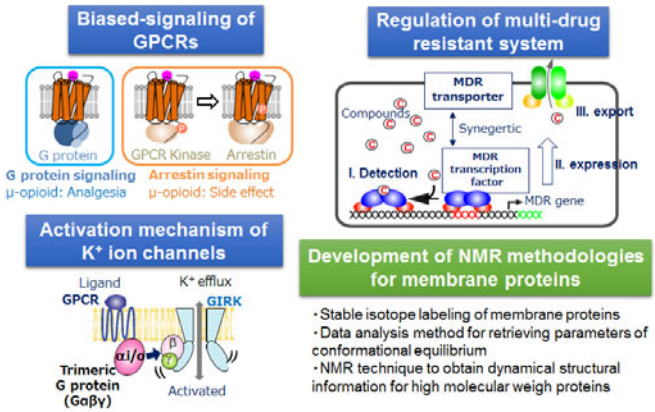


Figure 1　Overview of research project

【Expected Research Achievements and Scientific Significance】

Although the crystal structures of GPCRs bound to a G protein or arrestin are available, the mechanism underlying the biased signaling of GPCRs is still unclear. We will analyze the mechanism underlying the biased signaling of GPCRs using NMR. We expect that this NMR project will shed light on the conformational dynamics directly related to the biased-signaling, which will be valuable for the discovery of therapeutic agents targeted to GPCRs and other membrane proteins.

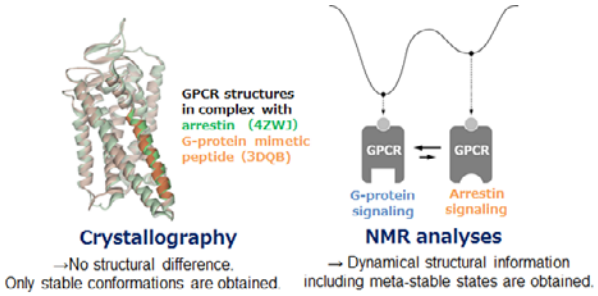


Figure 2　Scientific significance

【Publications Relevant to the Project】

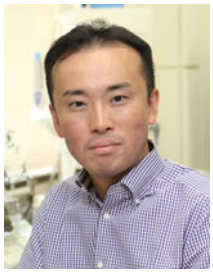
・Efficacy of the β_2-adrenergic receptor is determined by conformational equilibrium in the transmembrane region, Kofuku Y, Ueda T, Okude J, Shiraishi Y, Kondo K, Maeda M, Tsujishita H, Shimada I, Nat Commun. (2012) 3, 1045
・Dynamic regulation of GDP binding to G proteins revealed by magnetic field-dependent NMR relaxation analyses. Toyama Y, Kano H, Mase Y, Yokogawa M, Osawa M, Shimada I., Nat Commun. (2017) 8, 14523

【Term of Project】 FY2017-2021

【Budget Allocation】 354,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://ishimada.f.u-tokyo.ac.jp/public_html/index.html



Title of Project：Mechanism and Reconstitution In Vitro of Human Germ Cell Development

Mitinori Saitou
(Kyoto University, Graduate School of Medicine, Professor)

Research Project Number：17H06098　Researcher Number：80373306
Research Area：Medicine, dentistry, and pharmacy
Keyword：Germ Cells, Developmental medicine, Genome, Regulation of gene expression, Evolution

【Purpose and Background of the Research】

The germ cell lineage ensures the creation of new individuals, perpetuating/diversifying the genetic and epigenetic information across generations. Using mouse embryonic stem cells (mESCs)/induced pluripotent stem cells (miPSCs)], we have succeeded in reconstituting the development of germ cells in culture both in males and females: mESCs/miPSCs are induced into primordial germ cell-like cells (mPGCLCs), which contribute to spermatogenesis and oogenesis and to fertile offspring. We have also shown that human iPSCs (hiPSCs) with a primed pluripotency can differentiate into incipient mesoderm-like cells (iMeLCs) that robustly generate human PGCLCs (hPGCLCs). By combining the research using mice, cynomolgus monkeys, and humans, this project aims to further develop the in vitro reconstitution of human germ cell development.

【Research Methods】

We will perform four lines of highly related research sub-projects: 1) Establishment of a strategy for differentiating mPGCLCs into pro-spermatogonia or primary oocytes under defined conditions, 2) Induction of epigenetic reprogramming and sexually dimorphic differentiation of cynomolgus monkey PGCLCs, 3) Induction of epigenetic reprogramming and sexually dimorphic differentiation of hPGCLCs, 4) Induction of gonadal somatic cells from hPSCs.

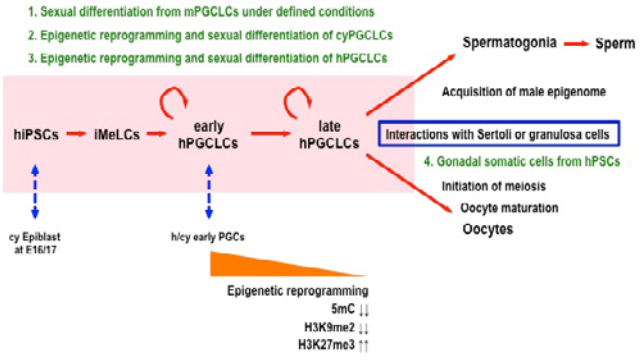


Figure 1. Outline of the Project

The combined achievements from these four lines of sub-projects will provide a strong foundation for human germ cell research (Figure 1).

【Expected Research Achievements and Scientific Significance】

The project is expected to generate robust foundations not only for extending our understanding of human germ cell development, but also for creating novel layers in human genetics and epigenetics; as such, its impact on the relevant fields is considered very high.

【Publications Relevant to the Project】

Nakamura, T., Okamoto, I., Sasaki, K., Yabuta, Y., Iwatani, C., Tsuchiya, H., Seita, Y., Nakamura, S., Yamamoto, T., and Saitou, M. (2016). A developmental coordinate of pluripotency among mice, monkeys, and humans, *Nature*, **537**, 57-62.

Sasaki, K., Yokobayashi, S., Nakamura, T., Okamoto, I., Yabuta, Y., Kurimoto, K., Ohta, H., Moritoki, Y., Iwatani, C., Tsuchiya, H., Nakamura, S., Sekiguchi, K., Sakuma, T., Yamamoto, T., Mori, T., Woltjen, K., Nakagawa, M., Yamamoto, T., Takahashi, K., Yamanaka, S., and Saitou, M. (2015). Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells, *Cell Stem Cell*, **17**, 178-194.

【Term of Project】

FY2017-2021

【Budget Allocation】

435,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://anat.cell.med.kyoto-u.ac.jp/index.html