【Grant-in-Aid for Specially Promoted Research】

Biological Sciences

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

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## 【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.

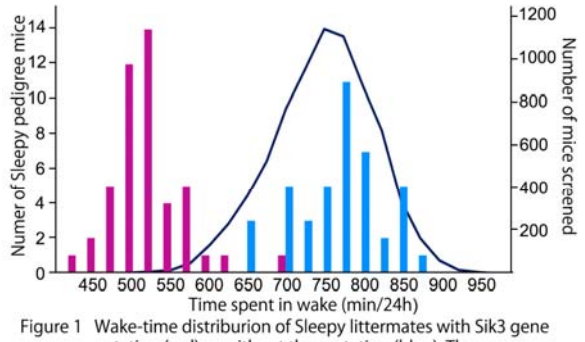


Figure 1　Wake-time distriburion of Sleepy littermates with Sik3 gene mutation (red) or without the mutation (blue). The curve indicates wake-time distribution of all mice screened.

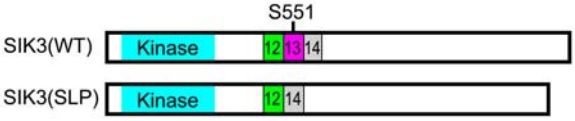


Figure 2　SIK3 is a protein kinase. The sleepy mutant skips exon 13 which encodes 52 amino acids containing S551, a well conseved PKA phosphorylation site.

## 【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