Investigation of Sleep/Wake Regulatory Mechanisms Through the Sik3 Gene Identified by Forward Genetics

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学位論文題目	Investigation of Sleep/Wake Regulatory Mechanisms Through the *Sik3* Gene Identified by Forward Genetics （フォワード・ジェネティクスにより同定した新規睡眠覚醒制御遺伝子 *Sik3* の解析による睡眠覚醒制御機構の解明）

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論文の要旨
Abstract of thesis

The mechanism for homeostatic sleep/wakefulness regulation, as well as the neural substrate for “sleepiness,” remains as the biggest mystery in sleep biology. To make a breakthrough in this issue, the candidate’s laboratory has initiated a large-scale forward genetic screen of sleep/wake abnormalities in mice based on somnographic (EEG/EMG) measurements, which are the gold standard in mammalian sleep/wake assessment. The laboratory including the candidate has so far screened > 8,000 heterozygous ENU-mutagenized mice and established multiple pedigrees exhibiting heritable and specific sleep/wake abnormalities. By combining linkage analysis and whole-exome sequencing, the applicant identified two dominant mutations that strongly affect sleep/wakefulness (Funato H, Honda T, Yanagisawa M et al., Nature 2016). A splicing mutation in the Sik3 protein kinase gene (termed Sleepy mutation) causes marked hypersomnia (i.e., increased non-REM sleep), owing to an increase in inherent sleep need. A missense mutation in the sodium leak channel NALCN (termed Dreamless mutation) reduces the total amount and episode duration of REM sleep, apparently by increasing the excitability of REM sleep-inhibiting neurons. Since these dominant mutations cause severe and specific sleep abnormalities, the applicant expects that the mutated genes play central roles for regulating sleep/wake amounts.

To elucidate the molecular basis how Sik3 gene is involved in sleep/wake regulation, the applicant has performed the genetic and biochemical analysis. SIK3 has a serine-threonine kinase domain at the N terminus and a protein kinase A (PKA) recognition site (Ser551) in the middle portion. The skipping of exon 13 resulted in an in-frame deletion of 52 amino acids, encompassing the PKA site. From this point, it is hypothesized that, since Sleepy mutant mice lack the exon 13 including Ser551 (PKA recognition site), phosphorylation state could be constitutively defective form in vivo, which cause the prolonged NREM sleep.

To examine this hypothesis, the candidate generated the phosphorylation-defective SIK3 (Ser551Ala, termed S551A mutant) and constitutive phosphorylation-active SIK3 (Ser551Asp, termed S551D mutant) by CRISPR/Cas9 gene editing.

As a result, both S551A and S551D mutant mice exhibited the longer NREM sleep and decreased wakefulness similar to the phenotype of the exon skipped original Sleepy (ΔEx13) mutant. To elucidate the mechanism of their sleep/wake phenotypes, the candidate performed the biochemical analysis by using the FLAG/HA-tagged mice and the series of expression plasmids (WT, Sleepy (ΔEx13), S551A, S551D) to examine how the phosphorylation state on Ser551 effects on the signaling pathway and binding partners of SIK3. Through immunoprecipitation (IP) and western-blotting, the applicant confirmed that Sleepy (ΔEx13), S551A, S551D showed the similar binding patterns toward PKA and 14-3-3 which correspond to their similar sleep phenotypes. These results suggest that the phosphorylation state of Ser551 in Sik3 gene and its signaling pathway including PKA and 14-3-3 have a key role on sleep/wake regulation under normal physiological conditions. Notably, the exon 13-encoding region of Sik3, including Ser551, is highly conserved among vertebrate animals, which suggests the biological importance of the phosphorylation pathway PKA -> SIK3 (determined by Ser551) -> 14-3-3 in sleep/wake regulation. These findings provide the landmark information about the novel sleep/wake regulatory mechanisms connecting from the intracellular signaling pathway to the dynamic in vivo sleep/wake behaviors.

審査の要旨
Abstract of assessment result

【批評 Review】

The experimental performance of the applicant is outstanding. The candidate generated phosphorylation-defective SIK3 and constitutive phosphorylation-active SIK3 by CRISPR/Cas9 gene editing for behavioral and biochemical analysis. In the final examination, the applicant presented clear data on the sleep/wake behavior of the mutants and the intracellular signaling pathway of the mouse SIK3 phosphorylation site S551. His findings reveal a vital role of SIK3 in sleep/wake regulation.

The applicant will continue to study forward-genetic mutant mice with sleep abnormalities and learning and memory deficits as postdoctoral work and already secured a position at MIT and 2-year funding from the Japan Society for the Promotion of Science. Therefore, the PhD committee is confident to say that the applicant is an exceptional student and fulfills all necessities to graduate from the Human Biology PhD program.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 26 January, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.