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Kinase signaling in distinct neuronal populations in the mouse brain controls sleep homeostasis and the circadian clock

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Sleep is a vital physiological phenomenon for animals, and many researchers have tackled the underlying mechanism and neuronal circuits responsible for sleep. In 1982, Borbély proposed the "two-process model," in which a combined action of two independent but closely associated processes plays a dominant role in sleep regulation (1). In this model, a sleep-independent process termed "process C" represents the circadian rhythmic change of sleep propensity, and this widely recognized variation had been assumed to be regulated by a circadian clock. In addition to this process, a sleep-dependent process termed "process S" was postulated to exhibit an exponential decline during sleep and an exponential increase during wake. Hence, the homeostatic process S is determined by prior waking time at sleep onset and by prior sleeping time at wake onset. In the two-process model, the duration/quality of sleep and timing of sleep/wake onset are defined by a combined action of process C and process S, where process C sets the upper and lower thresholds of the level of process S leading to sleep and wake, respectively (1). Approaches to address the molecular mechanisms and physiological analysis connecting these two processes have been historically limited. On the other hand, tremendous efforts have been made to understand the sleep homeostatic process and the neuronal circuitry controlling the duration and transitions of sleep/wake states (2). In parallel, the molecular machinery of the circadian clock has been well characterized using forward genetics and molecular biology (3). Recently, a research group led by Yanagisawa tried to identify a key molecule to regulate sleep homeostasis using forward genetics screening in mice and succeeded in isolating one mutant pedigree named *Sleepy* with an altered sleep phenotype (4). The *Sleepy* mice have an exon-skipped mutation in the *salt-inducible kinase 3* (*Sik3*) gene, resulting in abnormally increased duration of nonrapid eye movement (NREM) sleep and elevated delta power (an indicator of sleepiness) in the electroencephalogram (EEG). Now, the same research group, Asano et al. (5) precisely investigated the signaling pathway mediated by SIK3 in the circadian clock and demonstrated that SIK3 in different neuronal populations has dual functions regulating process S and process C.

The involvement of SIK3 in the circadian clock was first reported by Hayasaka et al. (6) who demonstrated that the whole-body deficiency of the *Sik3* gene in mice caused a phase delay of the metabolism and lengthening of the circadian period of behavioral rhythm. However, the conventional knockout mice showed abnormalities not only in the circadian clockwork but also in lipid and glucose metabolism and skeleton development and many newborn pups die after birth (7). To overcome this difficulty, Asano et al. (5) generated neuronal population-specific knockout mice of *Sik3*. In mammals, the master oscillator of the circadian clock resides in the suprachiasmatic nucleus (SCN) in the hypothalamus, and the SCN is mainly composed of gamma-aminobutyric acid (GABA)-ergic neurons. Asano et al. (5) generated GABAergic neuron-specific knockout mice of *Sik3* by crossing a *Sik3* flox mouse with a Cre driver mouse, which specifically expresses Cre recombinase under the control of the vesicular GABA transporter (*Vgat*) gene promoter. They found that the conditional *Sik3* knockout mice exhibited a longer circadian period and delayed phase of sleep-wake rhythms. On the other hand, specific introduction of *Sleepy* mutation into the GABAergic neurons caused an opposite effect on the behavioral rhythms, i.e., shorter period and advanced phase, which are consistent with the fact that the *Sleepy* mutation is a gain-of-function mutation (4).

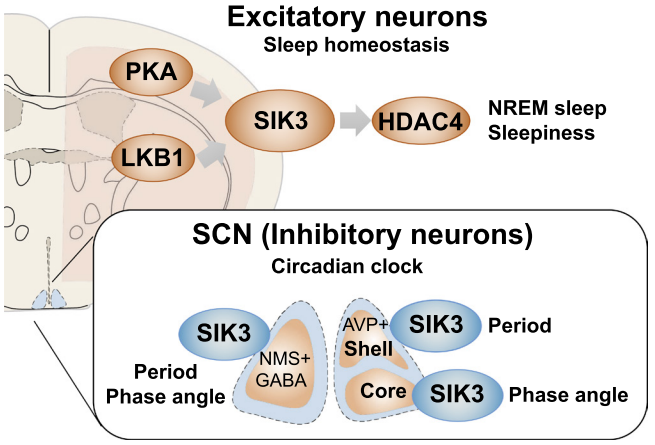

Fig. 1. Neuron population-specific physiological roles of SIK3 kinase in sleep of mice. SIK3 expressed in the glutamatergic (excitatory) neurons is mainly responsible for the sleep homeostatic process (process S), while SIK3 in the GABAergic (inhibitory) neurons overlapping with NMS-positive ones (10) in the SCN regulates the circadian period and phase angle of the circadian clock (process C). Particularly, SIK3 in the AVP neuron-enriched shell SCN (dorsal SCN) appears to play a role of keeping the normal circadian period, whereas in the core SCN (ventral SCN), SIK3 may be involved in normal phase response to light.

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SIK3 has multiple functions even in the central nervous system, and this property appears to be attributable to diverged expression of SIK3 in specific neuronal populations (Fig. 1). Of note, both loss-of-function and gain-of-function perturbation of *Sik3* in GABAergic neurons did not cause apparent abnormality in sleep homeostasis, suggesting a potential difference in how and where SIK3 plays key roles in between the sleep-wake rhythm and sleep homeostatic regulation. Previously, Dr. Yanagisawa and colleagues demonstrated that the *Sleepy* mutation in glutamatergic neurons increased sleepiness and NREM sleep time (8). Thus, SIK3 in the glutamatergic neurons is mainly responsible for the sleep homeostatic process S, while SIK3 in the GABAergic neurons in the SCN regulates the speed and phase angle of the circadian clock (Fig. 1). Interestingly, the SCN can be further classified into several neuronal populations defined by anatomical features and expression of neuropeptides such as neuromedin S (NMS) (9, 10), arginine vasopressin (AVP), and vasoactive intestinal polypeptide (11). SIK3 appears to play a role in keeping the circadian period in the dorsal part of the SCN (known as the shell SCN) where AVP-producing neurons are enriched. In the ventral SCN (known as the core SCN), SIK3 may be involved in phase response to light (5). Neuronal mechanisms are still to be elucidated as to how these *Sik3*-expressing neurons, i.e., glutamatergic (excitatory) neurons and GABAergic (inhibitory), control sleep homeostasis and circadian rhythm, respectively, particularly in terms of the neuronal properties (inhibitory or excitatory).

Asano et al., precisely investigated the signaling pathway mediated by SIK3 in the circadian clock.

SIK3-mediated regulatory mechanisms underlying the homeostatic process and the circadian process would be clarified by tracing upstream and downstream pathways of the SIK3 kinase signaling. In 2018, Yanagisawa's group found that Ser551 in SIK3 is a phosphorylation site catalyzed by protein kinase A (PKA). Intriguingly, the *Sleepy* mutation in SIK3 deletes the exon 13 encoding a region harboring Ser551. A point mutation at Ser551 attenuated the interaction of SIK3 with PKA and 14-3-3, leading to an increase in NREM sleep and EEG delta power during NREM sleep, i.e., an increase of sleepiness (12). In the sleep homeostatic regulation, PKA may sense the sleep history and modulate the phosphorylation of SIK3 based on the sleep need. Also, the time-of-the-day-dependent change in SIK3 activity may control the oscillation speed of the clock because adenosine 3′, 5′-cyclic monophosphate (cAMP) signaling fluctuates in a circadian manner in the SCN (13). Another regulatory kinase upstream of SIK3 is liver kinase B1 (LKB1). The knockout mice of *Lkb1* shortened the NREM sleep duration (8). For the downstream pathway of SIK3, a phosphoproteomic analysis demonstrated abundant phosphorylation in a series of synaptic proteins in the brain of *Sleepy* mutant mice and sleep-deprived mice (14). In addition, histone deacetylase 4 (HDAC4) is proposed as a candidate downstream of the SIK3-mediated sleep homeostatic regulation (8, 15), which was supported by the *Hdac4* mutant mice, *Sleepy2,* identified by another mutant screening of the same research group. All these findings are related to the sleep homeostatic process S. However, Asano et al. (5) demonstrate that the SIK3-HDAC4 pathway also functions in the circadian process C based on the phenotypic similarity in their mutant mice. Deletion of *Sik3* in GABAergic neurons and *Hdac4*^{S245A} mutation lacking the phosphorylation site at Ser245 by SIK3 caused an advanced sleep phase under the light–dark cycle. On the other hand, GABAergic neuron-specific *Sleepy* mutation and deficiency of *Hdac4* induced shorter circadian periods in constant darkness. However, either *Hdac4* mutant mice, phospho-resistant or deficient type, did not perfectly recapitulate the phenotypes of *Sik3* mutant mice, deficient or gain-of-function type, respectively, suggesting other substrates in the SCN. Interestingly, Hayasaka et al. (6) previously reported that SIK3 phosphorylates PERIOD2 (PER2) protein, which serves as a core molecular component of the circadian clock oscillation (6). They suggested that SIK3-dependent phosphorylation of PER2 caused destabilization of PER2 protein, leading to lengthening of the circadian period and impaired entrainment to the light–dark cycle in the *Sik3*-deficient mice. As well as the upstream signaling, the downstream of SIK3 may be multifaceted to simultaneously regulate the sleep homeostatic process S and the circadian clock, process C.

Since the discovery of *Sleepy* mutant mice, forward genetics, site-specific gene/neuronal manipulation and omics studies have significantly advanced sleep research. Therefore, it is time to consider the mechanism connecting sleep homeostasis and the circadian clock by integrating the series of findings together. For example, the phenotype in sleep duration and quality is more clearly manifest in the gain-of-function *Sleepy* mutation than in *Sik3* gene knockout mice, while the phenotype in the circadian clock is more obvious in the *Sik3* deficient mice than the *Sleepy* mutant (4, 6). These findings suggest that in the sleep homeostatic process, *Sik1* and/or *Sik2* may have a redundant role and compensate for the deficiency of *Sik3* (16). In the SCN, on the other hand, SIK3 is possibly dominant. However, because the SIK1-CREB-regulated transcription coactivator 1 pathway was previously shown to be involved in light entrainment (17), the double knockout of *Sik1* and *Sik3* may potentially cause a more drastic phenotype in the phase and period of the circadian clock than the single knockout of *Sik3*. In 2016, Funato and Yanagisawa's group claimed that the homozygous mutant mice having *Sleepy* alleles ubiquitously exhibited a normal circadian period (4). We also need to consider the contribution of glial cells and other populations of neurons to address the divergence between the GABAergic neuron-specific and whole-body *Sleepy* mutants. Overall, SIK family members have prominent roles in the regulation of sleep by modulating processes of sleep homeostasis and the circadian clock, and hence, they will continue to be an attractive target in this research field.

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