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The roles of CKI in circadian rhythm

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“Interruption of circadian rhythm by genetic mutation or environmental factors such as working in shifts may lead to a number of diseases such as sleep disorders, cardiovascular disease, cancer and diabetes.”

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Circadian rhythms affect behavior, physiology and metabolism in mammals. The rhythms are tightly regulated by cellular clock genes and proteins. The phosphorylation of clock proteins is also important in the regulation of circadian rhythm. PER protein phosphorylation by CKI is influenced in proteasomal degradation and nuclear translocation of PER proteins. Therefore, treatment using CKI inhibitors can modulate cellular and mammalian circadian rhythms. In this editorial, we will focus on the circadian clock gene network and the role of CKI in circadian rhythms.

Circadian rhythm & the circadian clock gene network

Many organisms exhibit daily changes under the control of genetically determined biological rhythms. The rhythm affects mammalian behavior and physiology including the sleep/wake cycle, body temperature, hormone secretion, metabolism and blood pressure [1–3]. Circadian rhythm is autonomously controlled by a cellular circadian clock [4]. Many circadian rhythms are persistently maintained, even without any external time cues. Perturbation of clock function can cause a change in the circadian period. In the mammalian circadian clock, circadian oscillators comprise a hierarchical organization inside cells. In the brain, suprachiasmatic nuclei (SCN) generate the central circadian rhythm that can control behavioral rhythms including the sleep/wake cycle and feeding pattern [5]. Peripheral circadian rhythms are located in most of the major organs and control the retina visual process, blood glucose levels, blood pressure and heart rate [6,7]. Clock core genes and their products are involved in a transcription-translation feedback loop. The period (*PER*) and cryptochrome (*CRY*) genes are activated by translational activators such as CLOCK and ARNTL1 (more commonly known as BMAL1). The CLOCK and Bmal1 proteins actually form CLOCK–BMAL1 heterodimers. These heterodimers attach to E-box motifs and activate *PER* and *CRY* gene transcription. PER and CRY proteins move into the nucleus and repress their own transcription [8]. In addition, the stability of clock proteins is regulated by post-translational modifications such as phosphorylation and ubiquitination [9,10]. The clock proteins are phosphorylated by protein kinase, which can control the circadian mechanism. The CKI ϵ -mediated phosphorylation of the PER protein leads to the recruitment of the F-box proteins, β -TrCP1 and β -TrCP2, to specific site and PER protein degradation through the ubiquitin and proteasome pathways [11]. Ubiquitination has been known to regulate the stability of clock proteins. In particular, FBXL3 causes the ubiquitination of CRY proteins, which is necessary for timely degradation of CRY protein and reactivation of *BMAL1-CLOCK* gene [12].

CKI in the mammalian circadian rhythm

The function of CKI in circadian mechanism has been revealed by genetic and biochemical approaches. CKI δ and CKI ϵ are involved in the regulation of PER protein phosphorylation and degradation in the mammalian circadian mechanism. Therefore, mutation of the CKI ϵ gene leads to destabilization of PER proteins and causes

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a short period behavior rhythm, an effect which has been observed in hamsters [6]. However, knock out of *CKIδ* gene shows period lengthening effects, whereas knock out of *CKIε* gene shows no effects *in vivo* [13]. CK2 kinase also induces degradation of mouse PER protein by phosphorylation of Ser53 and collaboration with *CKIε* [14]. In human circadian phenotype, mutation of *PER2* and *CKIδ* genes cause human familial advance sleep phase syndrome (FASPS). FASPS can interfere with daily social and work schedules and result in shortened sleep duration and excessive daytime sleepiness [15]. As *CKIδ/ε* are functionally important to the circadian rhythm, the known *CKIδ/ε* inhibitors, including IC261, CKI7 and D4476, have been demonstrated to cause period lengthening effects in cultured cells. In a recent study, new casein kinase inhibitors (PF-670462 and PF-4800567) developed by a target-based approach could manipulate the cellular circadian rhythm and mouse circadian rhythm. In this research, the *CKIδ/ε* inhibitor PF-670462 and *CKIε* selective inhibitor PF-4800567 showed the minimal role of *CKIε* and the important role of *CKIδ* in period regulation [16]. Unbiased cell-based phenotypic screening revealed that *CKIα* is also involved in degradation of PER [17]. Oshima *et al.* identified a potent CK2 inhibitor that enables the modulation of circadian rhythms and cancer cell growth [18]. These selective kinase inhibitors may identify novel functions for kinases in the mammalian circadian clock.

Circadian rhythm role in disease

The circadian rhythm can modulate mammalian behavior and the physiological processes. Interruption of circadian rhythm by genetic mutation or environmental factors such as working in shifts may lead to a number of diseases such as sleep disorders, cardiovascular disease, cancer and diabetes. In animal models, mutation of core clock gene or coding proteins leads to altered sleep-wake timing. For instance, the mutation of *CKIδ* or PER protein can induce FASPS, which display abnormal sleep pattern including excessive fatigue in the afternoon, early wake and insomnia [19,20]. In circadian gene expression study's, metabolism and metabolic gene expression are regulated by circadian system. Therefore, misalignment of circadian oscillations causes glucose intolerance and hyperlipidemia, which may result in diabetes and cardiovascular disease [21–24].

Circadian rhythms and sleep dysfunction are also related to neurodegenerative conditions such as Alzheimer's (AD), Parkinson's and Huntington's diseases. In various type of mouse models of AD, Parkinson's disease and Huntington's disease, sleep abnormality has been frequently found. Sleep deprivation induces unfold protein response, which is related to endoplasmic reticulum stress and potential neuronal injury. Therefore, insufficient sleep could promote inflammation, reactive oxygen species increase and synaptic damage, which results in neurodegeneration [25]. Alternation in behavior of circadian rhythms is a common symptom among patients with neurodegenerative condition. In Alzheimer's disease patents, it is presumed that loss of critical neuron in SCN of AD patents is correlated with disruption of behavior of circadian rhythm [26]. Amyloid-β also promotes Bmal1 and CBP degradation in Alzheimer's disease. These findings suggest an underlying mechanism of circadian disruption [27]. Therefore, chronic circadian disruption can be a risk factor of neurodegenerative diseases.

Summary

Since circadian disruption is a pivotal biological process, several studies have been conducted with the aim to correct the dysregulation of circadian rhythm. Cell-based phenotypic screening approaches identified potent compounds

Table 1. Protein kinases inhibitors capable of changing circadian period.

Compounds	Molecular targets	Period effects	Ref.
IC261	<i>CKIδ/ε</i>	Period lengthening	[11]
CKI-7	<i>CKIδ/ε</i>	Period lengthening	[28]
D4476	<i>CKIδ/ε</i>	Period lengthening	[29]
Roscovitine	CDK, <i>CKIδ/ε</i>	Period lengthening	[30]
PF-4800567	<i>CKIε</i>	Period lengthening	[16]
PF-670462	<i>CKIδ/ε</i>	Period lengthening	[16]
LH846	<i>CKIδ</i>	Period lengthening	[31]
Longdaysin	<i>CKIα/δ</i>	Period lengthening	[17]
NCC007	<i>CKIα/δ</i>	Period lengthening	[32]
Indirubin-3-oxime	CDK, GSK3β	Period shortening	[33]
Kenpaulone	CDK, GSK3β	Period shortening	[33]

that modulate circadian rhythm by inhibiting CKIs, CK2 and GSK3 β . These studies help to understand the molecular circadian clock. Furthermore, these potent compounds can serve as novel candidates for the development of selective kinase inhibitor drugs. Structural optimization of these compounds to improve druglikeness will indicate the possibility of a pharmacological approach to chronotherapy.

Conclusion

For about 20 years, the circadian rhythms and molecular circadian clock network has been studied. Although research of circadian rhythms and diseases are still beginning, academic and economic possibility of a circadian field is likely to be high in comparing with other medical science field. Recently, circadian-related diseases including sleep disorders, metabolic disorders and diabetes have been gradually increased among population. Growing knowledge of the link between diseases and disrupted circadian rhythm may lead to the development of treatments for these diseases. Several circadian drug candidates have been attempted to clinical trials for treatment of insomnia, sleep disorder – among others. Recent advance of electronic wearable devices can be utilized for the measurement of sleep-wake cycle or personalized circadian rhythms. These advances in circadian research field will provide a new therapeutic strategy for treatment of circadian-related diseases (Table 1).

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