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Analysis of the role of clock genes in the sleep-wake cycle and other biological processes

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Abstract. Clock genes, forming the crux of the body's circadian system, underpin the molecular basis of circadian rhythms. These rhythms, following approximately 24-hour cycles, regulate an array of biological processes, enabling organisms to adjust to environmental shifts. The sleep-wake cycle, a fundamental manifestation of this, alongside crucial brain functions and basic physiological processes, demonstrates significant links to circadian rhythms. This paper explores the interplay between clock genes and the sleep-wake cycle, illustrating that these genes modulate the cycle by managing associated hormones and neurotransmitters. Conversely, disruptions to the sleep-wake cycle influence the expressions of clock genes. Furthermore, the bidirectional relationships between these genes and other processes are also examined. Clock genes exert direct or indirect influence on vital life processes, which in turn modulate clock gene expression in various ways. Ultimately, the paper concludes with an in-depth understanding of the underlying mechanisms and identifies potential avenues for future research. These insights significantly contribute to the knowledge of the genetic basis of circadian rhythms and their potential clinical implications.

Keywords: clock genes, sleep-wake cycle, biological process.

1. Introduction

The circadian rhythm, also known as “Circadian / Body clock”, is a natural and internal process that regulates the sleep-wake cycle and other essential physiological functions. It repeats roughly every 24 hours and is governed by the suprachiasmatic nucleus (SCN) in the hypothalamus, which is sensitive to the external factors, especially light and dark cues from the environment [1]. Correspondingly, for the internal regulation of circadian rhythm at the molecular level, it involves clock genes including CLOCK, BMAL1, PER, CRY and so on, which encode proteins and then interact in a complex network of positive and negative feedback loops [2]. Through these processes, clock genes play a crucial role in the sleep-wake cycle, controlling the timing and quality of sleep to ensure that individuals experience the necessary restorative processes for maintaining physical and mental health [3]. Moreover, much previous research also reported their functions in stress, memory, metabolism, and immune regulation [3, 4]. Therefore, a better understanding of clock genes, especially the interactions of gene products at the molecular level and relationship between these genes and important human activities, is of great significance for sleep genetic development and better quality of life.

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This paper first reviews the basic concepts and internal relationships of clock genes, then further discusses the interactions between these genes and the sleep-wake cycle in detail. Besides, their functions in fundamental brain processes, such as memory and stress response, as well as their role in normal physiological functions like metabolism and hormone release, are briefly described. Finally, this paper concludes the profound influence of clock genes, contributing to the advancement of sleep genetics and facilitating more effective prevention and treatment of various health issues instigated by rhythm disruptions.

2. Basic concepts of clock genes

Clock genes are a set of genes that play a crucial role in regulating the circadian rhythm, which drives the approximately 24-hour biological cycles governing behavior and physiology in most living organisms, such as sleep-wake patterns, metabolism, body temperature, and cell cycles [3]. Clock genes serve as the molecular basis for intracellular timekeeping systems throughout the body, involving interactions between CLOCK (circadian locomotor output cycles kaput, or Npas2), BMAL1 (brain and muscle ARNT-like protein 1), PER (period, including PER1, PER2, and PER3), and CRY (cryptochrome, including CRY1 and CRY2) [2].

There are three feedback loops in this timekeeping system, with the first being the core loop (Figure 1). In this core loop, CLOCK and BMAL1 genes encode their respective proteins, which are basic helix-loop-helix (bHLH) - Per-Arnt-Sim (PAS) transcription factors and form heterodimer complexes through their PAS domains [3]. These CLOCK-BMAL1 complexes bind to promoter region E-box sites via their bHLH domain, initiating the expression of PER (PER1, PER2 and PER3) and CRY (CRY1 and CRY2) genes [3]. Subsequently, PER and CRY proteins form respective heterodimers, which first accumulate in the cytoplasm, and then translocate into the cell nucleus upon reaching a certain threshold [3]. They directly interact with CLOCK-BMAL1 complexes and inhibit their transcriptional activities to suppress PER and CRY gene expression. Eventually, ubiquitin-mediated degradation of PER and CRY proteins relieves the inhibition on CLOCK-BMAL1 complexes, and a new feedback loop begins [3]. Besides the first core loop, two other feedback loops contribute to the circadian rhythm. The second loop involves nuclear receptors REV-ERBα/β and RORα/β/γ, which regulate BMAL1 gene transcription [2]. The third loop involves DBP and NFIL3, regulated by CLOCK/BMAL1 and CRY1, which bind to D-box elements on circadian promoters [2]. Governed by transcriptional, post-transcriptional, and post-translational regulatory mechanisms, these feedback loops take roughly 24 hours to complete and make up the "molecular clock" that maintains circadian rhythms [2]. Furthermore, external cues, particularly light and darkness, are essential for synchronizing these rhythms as well [1].

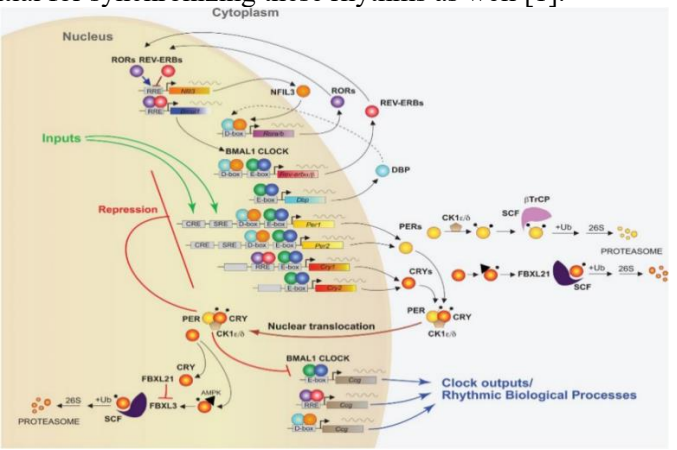

Figure 1. Components of the circadian clocks in mammals [2]. 1. The core feedback loop involves interactions between the CLOCK, BMAL1, PER and CRY genes and their proteins. 2. The second feedback loop provides both the ROR (positive) and REV-ERB (negative) regulation of the BMAL1 transcription. 3. The third feedback loop is mediated by the CLOCK/BMAL1-mediated transcription of DBP and the CRY1-mediated transcription of NFIL3.

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3. Interactions between clock genes and sleep-wake cycle

The sleep-wake cycle, a fundamental aspect of our daily lives, is orchestrated by a complex interplay of biological processes [4]. Among these, the circadian rhythm plays a leading role and is regulated by the intricate interactions of clock genes [1]. Thus, clock genes have a profound influence on the sleep-wake cycle. Conversely, the sleep-wake cycle also influences the expression levels of clock genes. Sleep changes or disorders can cause deviations in clock gene expression levels from their normal circadian oscillations [3]. As a result, there is a bidirectional relationship between clock genes and the sleep-wake cycle, and their interactions are discussed below in detail.

3.1. Role of clock genes in sleep-wake cycle regulation

The sleep-wake cycle regulation by clock genes in humans encompasses numerous complicated aspects, ranging from the external factors like light and darkness to the internal molecules of clock genes and their proteins, and finally to the substances like neurotransmitters and hormones that directly influence sleep and wake behaviors. Sunlight, as a cue to synchronize the body's internal circadian clock with the external environment, is detected by intrinsically photosensitive retinal ganglion cells (ipRGCs), which are specialized photoreceptive cells in the retina containing the photopigment melanopsin [5]. Light signals received by the ipRGCs are transmitted to the suprachiasmatic nucleus (SCN) in the hypothalamus, which serves as the central circadian clock [5]. Consequently, the expressions of the CLOCK, BMAL1, PER and CRY clock genes are impacted, creating a self-sustaining oscillation within a feedback loop that lasts approximately 24 hours as mentioned above [2]. Finally, the clock genes and their proteins in the SCN interact with other brain regions to regulate the sleep-wake cycle by influencing the production and release of various hormones and neurotransmitters [6]. Key brain regions include the sleep-promoting ventrolateral preoptic nucleus (VLPO), wakefulness-promoting orexin/hypocretin-producing neurons in the lateral hypothalamus and so on [6].

There are some essential hormones and neurotransmitters contributing to sleep-wake cycle regulation, as is shown in Table 1. First is melatonin, a sleep-inducing hormone produced by the pineal gland, whose secretion is inhibited because light exposure suppresses the activity of CLOCK-BMAL1 complexes [7]. Cortisol, released by the adrenal cortex and regulated by the HPA axis, is another important hormone influenced by the molecular clock, which helps maintain alertness with its peak levels in the early morning [7]. In terms of neurotransmitters, the VLPO contains GABAergic neurons that can secrete GABA (gamma-aminobutyric acid), an inhibitory neurotransmitter to promote sleep onset, which is indirectly regulated by clock genes through their regulation of the SCN and its connections with the VLPO [8]. Lastly, orexin (also known as hypocretin) is a neuropeptide also likely to be indirectly regulated by clock genes. It is produced by neurons in the lateral hypothalamus and can promote wakefulness and help maintain stable transitions between sleep and wake states [8]. In conclusion, the SCN receives environmental light and darkness signals, altering clock gene expression levels. Clock genes changes subsequently impact releasing of hormones and neurotransmitters in different brain regions, directly or indirectly, regulating sleep and wake behaviors. Through these complicated levels, our bodies realize the synchronization between the day-night cycle, circadian rhythms, and sleep-wake cycle.

3.2. Effects of sleep-wake cycle on clock genes expression

While clock genes play a pivotal role in sleep-wake cycle regulation, it still needs to be noted that the sleep-wake cycle, in turn, also has a huge effect on clock gene expression. A normal sleep-wake cycle, together with the appropriate light signals, is essential for regulating the proper clock gene expression and their typical feedback loops to maintain a healthy circadian rhythm [1]. However, alterations in the sleep-wake cycle, such as changes in sleep timing, duration and quality can lead to clock genes expression pattern deviating from the normal oscillation both in the central and peripheral clocks [3]. The effects of these sleep disturbances remain somewhat unclear, and the underlying mechanisms are different. As for sleep deprivation, the most prevalent form of sleep disturbance, it can be categorized into acute and chronic types. Acute sleep deprivation, characterized by short-term or single-night sleep

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loss, may attenuate PER and CRY expression in the SCN [9]. In contrast, chronic sleep deprivation, meaning sleep loss over a longer period, has been reported to affect CLOCK and BMAL expression in peripheral clocks throughout the body [10]. Both types can lead to disruptions of circadian rhythms and sleep-wake cycle, as well as other functional impairments like cognitive deficits and immune disorder [9]. Conversely, sleep recovery or an extended sleep period following deprivation can help restore normal clock gene oscillatory patterns [9]. Moreover, significant changes in sleep timing, such as shift work, can also profoundly impact clock gene expression, leading to feedback loop dysregulation [11]. This misalignment can further disrupt circadian rhythms, deteriorate sleep quality, and undermine overall health [11].

In conclusion, the intricate interplay between clock genes and the sleep-wake cycle is vital for maintaining a healthy circadian rhythm. Clock genes regulate the sleep-wake cycle by influencing the release of hormones and neurotransmitters. Conversely, alterations in the sleep-wake cycle can also disrupt clock gene expression, leading to circadian rhythms disturbances and impairments of other functions. Understanding the bidirectional relationship between the clock gene and the sleep-wake cycle is essential for promoting healthy sleep and mitigating the adverse effects of sleep disturbances.

Table 1. Four important hormones / neurotransmitters involved in the sleep-wake cycle regulation.

Types	Classification	Secretion / Synthesized	Function
Melatonin	Hormone	Primarily secreted by the pineal gland in the brain. Influenced by the circadian rhythm, higher during the night.	1. Regulate the sleep-wake cycle, helping to initiate and maintain sleep. 2. Have antioxidant properties and play a role in the immune system.
Cortisol	Hormone	Secreted by the adrenal glands on top of the kidneys. Regulated by the hypothalamic-pituitary-adrenal (HPA) axis.	1. As the "stress hormone", playing a role in the body's response to stress. 2. Regulate blood sugar levels, blood pressure and immune function. 3. Have a role in memory formation.
GABA	Neurotransmitter	Synthesized in the brain from the amino acid glutamate. Influenced by factors like genetics, age, stress and so on.	1. An inhibitory neurotransmitter to regulate neuronal excitability. 2. Help reduce anxiety, promote relaxation, and improve sleep quality.
Orexin	Neurotransmitter	Secreted by neurons in the hypothalamus. Influenced by factors like genetics, age, stress and so on.	1. Regulate the sleep-wake cycle, promoting wakefulness and arousal. (its deficiency is associated with narcolepsy) 2. Play a role in regulating apoetite and energy expenditure.

4. Functions of clock genes related to other aspects

In addition to regulating the sleep-wake cycle, circadian rhythms controlled by clock genes also play an important and extensive role in other human activities. Clock genes and their proteins have been found to engage in the fundamental brain processes of stress response and memory consolidation, as well as the basic physiological processes of metabolism regulation and the immune system [3, 12]. The relationships between clock genes and these four processes are discussed briefly below.

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4.1. Clock genes in stress response and memory consolidation

The stress response represents the body's reaction to various physical or psychological stressors, which also share an intricate and bidirectional relationship with clock genes. Clock genes and circadian rhythms play an important role in controlling the stress response through regulation of the HPA axis and cortisol secretion [12]. The HPA axis is central to the stress response, as the adrenal glands can produce cortisol, which aids the body in adapting to stress through energy resource mobilization, inflammation regulation and other essential processes [12]. Moreover, individual variations in clock genes can also impact stress resilience and susceptibility to mood disorders. For instance, polymorphisms in PER2 and PER3 have been linked to an increased risk of major depressive disorder [13]. Conversely, stress can disrupt circadian rhythms and lead to alterations in clock gene expression, which is likely to contribute to stress-related disorders like anxiety and depression [14]. As a result, a deeper understanding of the interactions between clock genes and stress response is essential for developing novel therapeutic strategies to address stress-related disorders and improve mental health related to stress responses.

The memory consolidation, which involves the conversion of newly acquired, unstable information into stable, long-term memories, predominantly occurs during sleep and is modulated by clock genes through both direct and indirect mechanisms. Directly, clock gene expression in memory-related brain regions like the hippocampus and amygdala is likely to modulate molecular processes underlying memory consolidation, such as synaptic plasticity and memory-related genes [15]. Indirectly, clock genes affect memory consolidation through regulation sleep structure and timing because neural activity patterns are replayed and connections between neurons are strengthened during sleep. Further research is essential for uncovering the exact mechanisms connecting clock genes and memory consolidation, as well as for exploring potential therapeutic applications for memory-related disorders.

4.2. Clock genes in metabolic homeostasis and immune reaction

Metabolic homeostasis refers to the preservation of a stable internal environment within the body, ensuring the necessary energy and nutrients are available for cellular processes, which is an extensive field encompassing various substances such as inorganic ions like $Mg2+$ and $Ca2+$, as well as organic nutrients like sugars and lipids. [1]. Clock genes play a crucial role in regulating the expression of metabolic genes, which in turn modulate metabolic pathways and processes. [16]. For instance, clock genes influence the body's insulin sensitivity to affect the regulation of carbohydrate and lipid metabolism, with implications for their synthesis, storage, and breakdown [16]. Disruptions in clock gene function or circadian rhythms can have detrimental effects on metabolic homeostasis, potentially leading to metabolic disorders like obesity, diabetes, and cardiovascular diseases [17]. Conversely, metabolic dysfunction can disrupt circadian rhythms as well, creating a feedback loop that exacerbates both conditions [17]. The interconnected relationship between clock genes and metabolic processes highlights the importance of maintaining proper circadian rhythms for overall health and well-being.

The immune system is a sophisticated network of molecules, cells and organs that collaboratively defend against foreign pathogens and maintain tolerance to our own components [18]. Its activities have been shown to be time-of-day dependent, as they are regulated by circadian rhythms, with clock genes influencing the functions of immune cells such as macrophages and lymphocytes [18]. Furthermore, disruptions of clock gene expressions also have a clear connection with an increased risk of inflammation and autoimmune disease [19]. In addition, normal sleep patterns are essential for maintaining a healthy immune response. By understanding the relationship between clock genes and the immune system, researchers may develop innovative therapeutic approaches for immune-related conditions and enhance vaccine strategies.

5. Conclusion

In conclusion, this paper has provided a comprehensive analysis of the pivotal function that clock genes, including CLOCK, BMAL1, PER and CRY, serve in the regulation of the sleep-wake cycle and various physiological and behavioral processes. The intricate bidirectional relationship between clock genes and the sleep-wake cycle has been meticulously explored, highlighting the significance of their protein

products and their interactions with hormones and neurotransmitters. Additionally, the associations between clock genes and other essential brain processes, such as stress response and memory consolidation, as well as basic physiological processes like metabolic homeostasis and immune reaction, have been briefly addressed.

Gaining a more profound understanding of the mechanisms underpinning these interactions is not only crucial for advancing our comprehension of the genetic foundation of circadian rhythms, but also holds promising implications for clinical applications. By investigating the role of clock genes in diverse physiological processes, researchers may potentially devise innovative therapeutic approaches and interventions for sleep disorders, mental health issues, metabolic diseases, and immune system dysfunctions. Future research should persist in exploring the complex interplay between clock genes and the sleep-wake cycle, while also considering the broader implications of these genes in other physiological and behavioral processes. This endeavor will ultimately contribute to a more holistic understanding of the intricate network of clock genes, their regulatory functions, and their potential impact on human health and well-being.

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