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Epigenetic Regulation of the Circadian Clock: Mechanisms and Clinical Implications

Jin Chang

Department Physics & Astronomy, McMaster University, ON, Canada

changjin889912@gmail.com

Abstract. The circadian clock, a fundamental timekeeping system, is regulated by complex epigenetic mechanisms. This review explores the role of DNA methylation, histone modifications, and the NAD+-dependent deacetylase SIRT1 in modulating circadian gene expression. Rhythmic epigenetic changes at clock gene promoters contribute to their periodic transcription, while interactions between circadian transcription factors and epigenetic modifiers ensure robust gene expression rhythms. Dysregulation of the circadian epigenome has been associated with various diseases, including cancer, where altered DNA methylation patterns of clock genes have been observed. Understanding the mechanisms and consequences of circadian epigenetic dysregulation may lead to novel diagnostic and therapeutic approaches for circadian-related diseases. To fully elucidate the intricate relationship between the circadian clock and the epigenome, a multidisciplinary approach combining experimental and computational methods is necessary. High-throughput analytical techniques, functional genomics, epigenetic editing tools, and systems biology modeling will provide a comprehensive view of the circadian epigenome. Ultimately, a deeper understanding of the epigenetic regulation of the circadian clock will advance our knowledge of biological timekeeping and inform the development of chronotherapeutic strategies for improving human health in the modern 24/7 society.

Keywords: circadian clock, epigenetics, DNA methylation, histone modifications, SIRT1.

1. Introduction

The circadian clock is an intrinsic timekeeping system that allows organisms to anticipate and adapt to daily environmental changes. In mammals, the central clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and is synchronized with oscillators in peripheral tissues [1]. The molecular clock mechanism consists of an interlocking transcription-translational feedback loop in which the CLOCK and BMAL1 heterodimeric transcription factors activate the expression of the Period (Per) and Cryptochrome (Cry) genes. In turn, the PER and CRY proteins form a complex that inhibits CLOCK-BMAL1 activity, thereby inhibiting their own transcription [2]. Additional feedback loops including ROR and REV-ERB nuclear receptor further regulates this core loop.

Notably, up to 10% of the transcriptome is under circadian control, and disturbances in clock function can have profound physiological consequences [3]. Accumulating evidence suggests that epigenetic mechanisms, such as DNA methylation and histone modifications, play a crucial role in regulating circadian gene expression. DNA methylation, which is often associated with transcriptional silencing, has been found to regulate clock genes. Rhythmic histone modifications have also been observed at the

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promoters of clock genes, with activation marks coinciding with the time of peak transcription [4]. The NAD+-dependent deacetylase SIRT1 has emerged as a link between metabolism and the circadian clock, acting on both histone and non-histone proteins [5]. These findings have important clinical implications, as circadian rhythm disturbances have been associated with a variety of diseases, including sleep disorders, metabolic syndrome, and cancer [6].

Notably, altered DNA methylation patterns of clock genes have been observed in cancer, suggesting that abnormal epigenetic regulation of the clock may contribute to disease development. Understanding the epigenetic mechanisms of the circadian clock will provide insights on how environmental and metabolic factors affect circadian rhythms and may open new avenues for the treatment of circadian rhythm-related diseases. This article reviews the latest understanding of epigenetic regulation of the circadian clock and discusses the clinical significance of these findings.

2. Theoretical studies

Theoretical and mechanistic studies have provided important insights into the epigenetic regulation of the circadian clock. The core circadian clock proteins CLOCK and BMAL1 have been shown to interact with a variety of epigenetic modifiers (as indicated in the Figure 1). CLOCK itself possesses histone acetyltransferase (HAT) activity and rhythmically acetylates histones H3 and H4 at clock gene promoters[7]. CLOCK also acetylates its binding partner BMAL1, which is important for circadian function[8]. Histone methyltransferases MLL1 and MLL3 have been found to interact with CLOCK-BMAL1and promote the rhythmicity of H3K4 trimethylation (an activating chromatin mark) at clock gene promoters[9]. In contrast, the histone deacetylase SIRT1 is recruited to clock gene promoters by CLOCK-BMAL1 and rhythmically deacetylates H3K9/K14, contributing to the repressive phase of the transcription cycle [10].

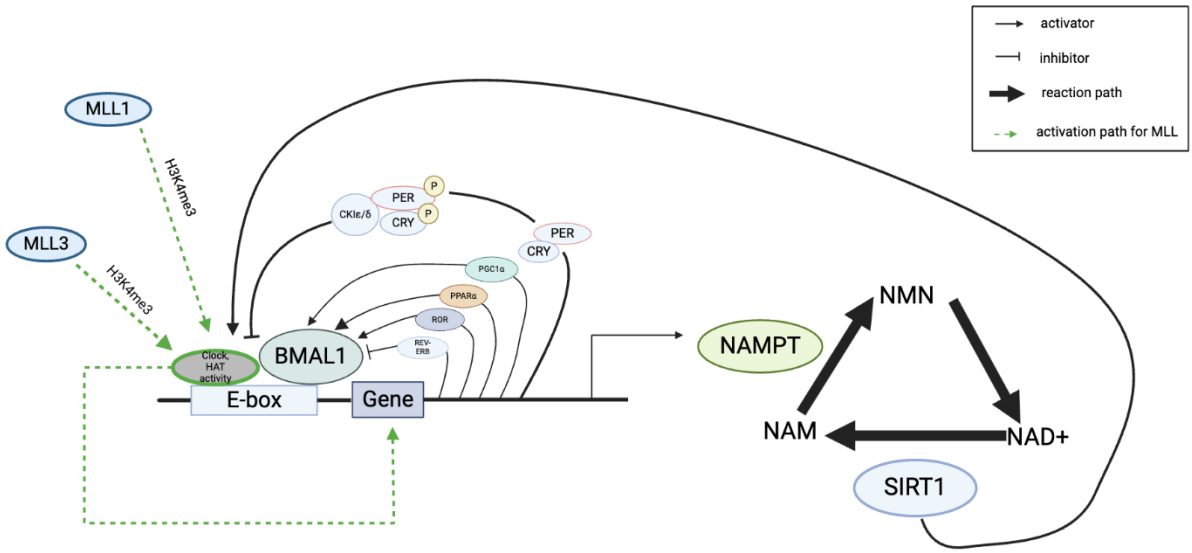

Figure 1. The core network of epigenetic regulation in circadian rhythm

Genome-wide studies have revealed extensive rhythmic epigenetic modifications in the liver, and a large portion of the cyclical transcriptome also exhibits circadian histone modifications and chromatin states[11]. DNA methylation has also been implicated in circadian regulation, with clock genes showing tissue-specific methylation patterns that correlate with the amplitude of their cycles[12]. Interestingly, DNA methylation was found to be rhythmic at a subset of CpG sites in mouse liver, suggesting dynamic regulation [13].

Theoretical models have been developed to describe how the interplay between circadian transcription factors and epigenetic modifications generates robust gene expression rhythms [14]. These models incorporate feedback loops between clock proteins, chromatin modifiers, and metabolic

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regulators. For example, the NAD+ biosynthetic enzyme NAMPT is controlled by circadian rhythms, and rhythmic NAD+ production in turn regulates SIRT1 activity, forming a metabolic feedback loop [15].

3. Existing Problems

Despite significant progress, several key issues regarding epigenetic regulation of the circadian clock remain unresolved. First, the exact mechanism by which clock proteins coordinate the recruitment of epigenetic modifiers to specific target genes is not fully understood. It is still unclear how the timing and specificity of these interactions are regulated and how they promote tissue-specific circadian transcriptional programs.

Second, although rhythmic epigenetic modifications have been observed in clock genes, their functional significance remains to be determined. Genetic deletion of individual histone modifiers often has only modest effects on circadian rhythms, suggesting the possibility of redundant or compensatory mechanisms [9]. More targeted approaches, such as specific manipulation of epigenetic marks at clock gene promoters, may be needed to directly assess their role in circadian regulation.

Third, the relationship between epigenetic modifications and clock-controlled physiological processes remains poorly understood. Although circadian disruption has been associated with altered DNA methylation patterns in cancer, causality remains unclear. It is unclear whether epigenetic dysregulation is a cause or consequence of circadian dysfunction in disease states.

Finally, the potential for targeting epigenetic mechanisms in the treatment of circadian-related diseases remains largely unexplored. Although some studies have shown that inhibition of histone deacetylases can alter circadian rhythms and alleviate metabolic disorders [16], the therapeutic potential of specific epigenetic modulation of the biological clock requires further investigation.

4. Potential solutions

To address these outstanding questions, a combination of experimental and computational approaches is needed. High-resolution epigenetic modification analysis of clock-controlled genes in different tissues and time points can provide a more comprehensive understanding of the circadian epigenome. Chromatin immunoprecipitation sequencing (ChIP-seq) and whole-genome bisulfite sequencing can map histone modifications and DNA methylation on a genome-wide scale [11,13]. These data can be integrated with RNA sequencing and metabolomics to understand the functional consequences of epigenetic changes on gene expression and metabolic output.

Genetic manipulation of epigenetic regulators in a tissue-specific and time-controlled manner can help to dissect their role in circadian regulation. CRISPR-based epigenetic editing tools, such as CRISPR-dCas9 fused to histone modifying enzymes or DNA methyltransferases, can target epigenetic states at specific loci [17]. Applying these tools to clock gene promoters can directly test the causal role of specific epigenetic marks on circadian transcription. Inducible knockout or overexpression of key epigenetic enzymes (such as SIRT1 or MLL1) in specific tissues can also provide insights into their circadian functions.

Computational modeling and systems biology approaches that integrate multi-omics data and generate testable hypotheses will be essential [18]. Machine learning algorithms can be used to predict the epigenetic state of DNA sequence features and identify cis-regulatory modules that drive circadian gene expression. Network analysis can uncover key regulatory hubs and feedback patterns in the circadian epigenome. These models can guide experimental design and help prioritize targets for therapeutic intervention.

Translational research in animal models and human samples is essential to understanding the clinical relevance of circadian epigenetics. Comparison of the epigenetic profiles of clock genes in normal and diseased tissues (e.g., tumor samples) can reveal the role of epigenetic dysregulation in pathogenesis [19]. Longitudinal studies tracking epigenetic changes and circadian disruption over time can help establish causal relationships. Testing the effects of drugs that modulate the circadian clock (e.g.,

melatonin or metabolic modulators) on the epigenome and disease outcomes can provide insights into potential therapeutic strategies.

5. Conclusion

Epigenetic mechanisms, including histone modifications and DNA methylation, play a key role in regulating the circadian clock. Rhythmic epigenetic changes at the promoters of clock genes contribute to their periodic transcription, and the interaction between circadian transcription factors and epigenetic modifiers ensures a robust gene expression rhythm. The NAD+-dependent deacetylase SIRT1 has emerged as a key link between cellular metabolism and the circadian clock, highlighting the close connection between epigenetics and metabolic status. Importantly, dysregulation of the circadian epigenome is associated with a variety of diseases, including cancer. Altered DNA methylation patterns of clock genes have been observed in several cancer types, suggesting that epigenetic disruption of circadian rhythms may contribute to disease occurrence.

Understanding the mechanisms and consequences of circadian epigenetic dysregulation may lead to novel diagnostic and therapeutic approaches for circadian-related diseases. To fully elucidate the complex interaction between the circadian clock and the epigenome, a multidisciplinary approach combining experimental and computational methods is needed. High-throughput analytical techniques, functional genomics, and epigenetic editing tools will provide a comprehensive view of the circadian epigenome and enable targeted manipulation of specific regulatory elements. Systems biology modeling and machine learning will be necessary to integrate multidimensional data and generate predictive models of circadian gene regulation.

Ultimately, a deeper understanding of the epigenetic regulation of the circadian clock will not only advance our fundamental understanding of biological timekeeping but will also inform the development of chronotherapeutic strategies for a wide range of diseases. By harnessing the power of the circadian epigenome, we may be able to optimize the timing and efficacy of medical interventions and improve human health in an increasingly 24/7 society.

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