Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 187809, 8 pages
http://dx.doi.org/10.1155/2015/187809

Review Article

Circadian Control of Global Transcription

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Received 9 September 2015; Accepted 4 November 2015

Academic Editor: Giuseppe Piccione

Circadian rhythms exist in most if not all organisms on the Earth and manifest in various aspects of physiology and behavior. These rhythmic processes are believed to be driven by endogenous molecular clocks that regulate rhythmic expression of clock-controlled genes (CCGs). CCGs consist of a significant portion of the genome and are involved in diverse biological pathways. The transcription of CCGs is tuned by rhythmic actions of transcription factors and circadian alterations in chromatin. Here, we review the circadian control of CCG transcription in five model organisms that are widely used, including cyanobacterium, fungus, plant, fruit fly, and mouse. Comparing the similarity and differences in the five organisms could help us better understand the function of the circadian clock, as well as its output mechanisms adapted to meet the demands of diverse environmental conditions.

1. Introduction

Circadian rhythms, controlled by endogenous circadian clocks, are rhythmic oscillations in our behavior and physiological processes with a period close to 24 h. Circadian rhythm exists in diverse organisms on the Earth ranging from bacteria and fungi to plants and animals, allowing adaptation to light and temperature changes caused by the self-rotation of the Earth [1, 2]. In all kingdoms of life, the circadian clock regulates a wide variety of physiological activities such as cyanobacteria cell division [3], fungal sporulation [2], plant growth and flowering time [4, 5], and sleep/wake cycles in animals [6].

The circadian clocks are organized around three major physiological components: an input pathway that receives environmental cues and entrain the oscillator, a central oscillator that keeps circadian time and generates rhythms, and an output pathway that generates manifested rhythmic processes throughout the body [7]. The central oscillator in eukaryotic organisms is similar in different kinds of organisms, consisting of transcriptional and posttranscriptional negative feedback loops. In fungi, fruit flies, and mammals, the positive elements of the circadian negative feedback loops are heterodimeric complexes of two PER-ARNT-SIM domain-containing transcription factors that activate the transcription of negative elements. Moreover, the negative elements repress their own expression by inhibiting the activity of the positive elements. The cyclic activation, repression, and reactivation of circadian negative elements generate circadian rhythmicity, which regulates the circadian output pathway by driving downstream clock-controlled gene (CCG) expression [8–10].

2. The Core Molecular Clock

Circadian clocks in diverse organisms are composed of molecular feedback loops, but these are coordinated in somewhat different ways with different factors that are presented in detail here, based on our knowledge from five widely used model systems: the freshwater cyanobacterium *Synechococcus elongatus*, the filamentous fungus *Neurospora crassa*, the thale cress *Arabidopsis thaliana*, the fruit fly *Drosophila melanogaster*, and the house mouse *Mus musculus*.

The cyanobacterial clock is regulated by the activity of three genes, *kaiA*, *kaiB*, and *kaiC*. Inactivation of any of the *kai* genes abolishes clock function [11]. The phosphorylation status of KaiC exhibits robust circadian oscillation [12]. KaiC also exhibits ATPase activity, which correlates with clock speed [13]. Kai proteins interact with each other and regulate the rhythmic supercoiling/condensation status of the chromosome [14, 15]. This supercoiling/condensation

status of the chromosome rhythmically changes such that it becomes an oscillating nucleoid, or oscilloid, which globally regulates rhythmic gene expression [16].

In the core *Neurospora* circadian clock, the positive element is the heterodimeric White Collar Complex (WCC) consisting of WC-1 and WC-2, and the key negative element is the FREQUENCY- (FRQ-) FRQ RNA helicase (FRH) complex [17–19]. WCC binds to *frq* promoter and activates *frq* transcription. Meanwhile, the FRQ-FRH complex (FFC) recruits the casein kinases to phosphorylate WC proteins which lead to dissociation of WCC from the *frq* promoter, thereby inhibiting transcription of *frq* and closing the negative feedback loop [20–23]. FRQ undergoes progressive phosphorylation by several kinases and is degraded through the ubiquitin proteasome pathway [20, 24]. After the degradation of FRQ protein, WCC reactivates *frq* transcription, thereby initiating a new circadian transcriptional cycle. The cyclic activation, repression, and reactivation of *frq* expression bring about circadian oscillation, which is the major basis of the rhythmic expression of CCGs. In addition to the role in repressing WCC function in the negative feedback loop, FRQ functions to promote the steady-state levels of WC-1 and WC-2, forming a positive feedback loop [25]. These interconnected feedback loops are essential to maintain the robust and stable oscillation in *Neurospora*.

In *Arabidopsis*, the central oscillator consists of three interlocked transcriptional feedback loops, the core loop, the morning loop, and the evening loop. The core loop consists of two single MYB transcription factors, Circadian Clock Associated 1 (CCA1) and Late Elongated Hypocotyl (LHY), which repress the expression of evening-phased pseudoresponse regulator (*PRR*) and *Timing of CAB Expression 1* (*TOC1*) [26–30]. TOC1 originally was reported to activate *CCA1* and *LHY* expression [26], but more recent reports have confirmed that TOC1 also inhibits the expression of *CCA1* and *LHY* [31, 32]. Members of the PRR family (PRR5, PRR7, and PRR9) bind to promoters of their activators, *CCA1* and *LHY*, and repress their expression, forming a second interlocked morning loop [33, 34]. The evening loop is composed of TOC1, GIGANTEA (GI), and the evening complex (EC) including Early Flowering 3 (ELF3), ELF4, and LUX ARRYTHMO (LUX)/PHYTOCLOCK 1 acting at dusk as a transcriptional repressor of *PRR9* expression [35–37].

The *Drosophila* circadian oscillator is composed of two interlocked feedback loops. In the core feedback loop, *period* (*per*) and *timeless* (*tim*) transcription are activated when CLOCK (CLK) and its heterodimeric partner CYCLE (CYC) bind E-box elements in *per* and *tim* promoters [38–41]. As *per* mRNA accumulates to peak levels around dusk, PER accumulates in the cytoplasm, where it binds TIM and then translocates into the nucleus, thereby inhibiting CLK/CYC activity and subsequently repressing the transcription of *per* and *tim* [42–44]. Once TIM is induced to degrade early in the light phase, thus "deprotecting" PER which is also targeted for degradation, CLK/CYC binds to E-boxes again to initiate the next cycle of *per* and *tim* transcription [45–47]. In the second feedback loop, CLK/CYC drives the transcription of *vrille* (*vri*) and *PAR-domain protein 1ε* (*Pdp1ε*), whose protein products repress and activate the transcription of *clk*, respectively [48, 49].

The circadian oscillator in mouse is built on a series of feedback loops highly similar to that in *Drosophila*. The core feedback loop contains a heterodimer of transcriptional activators formed by brain and muscle ARNTL-like protein 1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK), which directs transcription of three Period genes (*Per1*, *Per2,* and *Per3*) and two Cryptochrome genes (*Cry1* and *Cry2*) by binding to E-box sites within their promoters [50–53]. PER and CRY translocate into the nucleus and inhibit the transcriptional activity of CLOCK/BMAL1 [54–56]. Targeted degradation of PER and CRY proteins enables the reactivation of CLOCK/BMAL1, and a new cycle begins [54, 57, 58]. In an additional coupled feedback loop, CLOCK/BMAL1 activates the transcription of retinoic acid-related orphan receptors, Rorα and Rev-erbα, which activates and represses transcription of *Bmal1*, respectively [53, 59]. In certain tissues, neuronal PAS domain protein 2 (NPAS2) functions as a CLOCK analog [60].

3. A Significant Portion of the Transcriptome Is CCGs

As previously mentioned, many genes are rhythmically expressed. In cyanobacteria *Synechococcus elongatus* PCC7942, about 30% to 64% of the transcriptome is expressed in a circadian manner based on results from microarray studies [61, 62]. Circadian genes peak mostly at dawn and dusk, with ~30% more genes peaking at dawn than dusk. Genes that belong to the central intermediary metabolism, including glycoprotein and polysaccharide synthesis, transcription, and energy metabolism, are enriched among the rhythmically expressed transcripts [61].

In *Neurospora*, high-density microarrays demonstrated that roughly 20% to 25% of the transcriptome can be expressed under circadian control [63, 64]. Very recently, RNA sequencing (RNA-Seq) revealed that from 10% to as much as 40% of the transcriptome is under the control of the clock [65, 66]. Oscillating genes are enriched in pathways involving metabolism, protein synthesis, stress responses, cell signaling, and development [63–66]. Similar to cyanobacteria, the peak time of *Neurospora* CCG expression is also clustered in either dawn or dusk [65, 66]. In general, dawn-phased genes are mainly participating in catabolic processes of energy production and precursor assembly, whereas dusk-phased genes are mostly involved in anabolic processes of cellular components and growth.

In *Arabidopsis*, between 6% and 15% of the transcriptome is regulated by the circadian clock [67–69], and by combining the three data sets and thus improving the strength of the analysis, between 31% and 41% of the expressed genes are believed to oscillate [70]. This is consistent with an enhancer trap study showing that roughly one-third of the genome is rhythmically regulated [71]. Another study investigated the transcriptome under different thermocycles, photocycles, and circadian conditions and found that 89% of the transcripts oscillate in at least one of the conditions [72]. CCGs

are overrepresented among all of the classical plant hormone and multiple stress response pathways, as well as cell cycle and protein synthesis [70, 72].

Based on microarray studies, approximately 1% of genes from *Drosophila* head exhibit circadian expression pattern [73–77]. Recently, RNA-Seq assays revealed that close to 2% of the genes in the fly head and 4% in the fly brain demonstrate rhythmic expression, including several noncoding RNAs that were not identified in microarray studies [78, 79]. To distinguish transcriptional versus posttranscriptional regulations in the transcriptome, nascent RNAs from fly heads were isolated and subjected to high-throughput sequencing (Nascent-Seq) [79]. 130 robust cycling transcriptional units were detected, which is about 1% of the genome, and more than 1/3 of these transcripts exhibit oscillation in mRNA analysis. The reverse comparison indicates that 19% of the cycling mRNAs are identified to be cycling in the Nascent-Seq data, implicating significant contribution of posttranscriptional modifications that contribute to rhythmic expression of CCGs [79]. CCGs in fly heads are associated with diverse biological processes, including areas of metabolism, detoxification, signal transduction, and immunity [73–77, 79].

In mouse, approximately 5% to 25% of the expressed genes in central and peripheral tissues were identified as oscillating according to microarray and RNA-Seq studies [80–84]. Despite being greatly informative, most of these studies have analyzed only one or two organs/tissues. A recent study, which profiled the transcriptomes of 12 different mouse organs, reported that 43% of all protein coding genes exhibit circadian rhythms of mRNA abundance somewhere in the body, largely in an organ-specific manner, and 32% of conserved noncoding RNAs oscillate as well [85]. Consistent with the findings in *Drosophila*, Nascent-Seq demonstrated that roughly 15% of all detected genes are rhythmically transcribed in the mouse liver, but of which only 42% exhibit mRNA oscillations [86]. On the other hand, about 70% of the genes that show rhythmic mRNA expression do not show transcriptional rhythms, indicating the existence of substantial posttranscriptional regulation that leads to mRNA cycling. Mouse CCGs are involved in diverse biological pathways, particularly various metabolic pathways, along with many others [81–83, 86].

4. Transcriptional Regulation of CCGs

How are these CCGs rhythmically transcribed? Based on our current understanding, this is accomplished by coordinated efforts of rhythmic activities of transcription factors at promoter elements in the genome and rhythmic epigenetic modifications, such as chromatin remodeling through post-translational modifications (PTMs). This will be described in detail below.

In cyanobacteria, the KaiC-containing protein complex regulates circadian gene expression via multiple protein-dependent pathways [87]. In one pathway, KaiC interacts with a histidine kinase SasA, which contains a KaiB-like sensory domain [88]. KaiC increases the rate at which SasA autophosphorylates, and the autokinase activity of SasA is crucial to its function [88–90]. SasA phosphorylates and activates a transcription factor RpaA, which regulates the expression of a small set of circadian effectors that orchestrate genome-wide transcriptional rhythms [87, 89, 91]. More specifically, RpaA functions to promote dusk-like expression state [89]. In parallel to SasA, low amplitude and bright (LabA) is also believed to signal to RpaA and represses circadian gene expression [92]. A third pathway involving CikA exerts repressive effects on circadian gene expression, possibly by promoting dephosphorylation and suppressing RpaA activity [89, 92]. The phosphatase activity of CikA is enhanced by KaiB/C at a time that is distinct from the activation of SasA by KaiC [89]. The RpaA paralog, RpaB, is recently shown to bind rhythmically to several promoters, including *kaiBC* promoter in the subjective night, and repress transcription [92]. This binding may be terminated by RpaA to activate transcription during the subjective day. Moreover, the core clock proteins KaiA and KaiC exert opposite effects on global circadian gene expression [93]. KaiA overexpression activates "dusk genes" and represses "dawn genes," whereas KaiC overexpression results in the opposite effect, that is, repression of "dusk genes" and activation of "dawn genes." In addition to protein-dependent pathways, kai proteins regulate chromosome compaction rhythm and oscillation in the superhelical status of the DNA [90, 94]. The topological state of the chromosome is highly correlated with gene expression, and depending on the AT content in the promoter regions, some genes are activated while some genes are repressed by chromosome relaxation [62, 95]. An oscilloid model proposes that topological changes of the chromosome mediated by KaiABC oscillator drive cyclic expression of genes at a global scale, and specific promoter elements are believed to be not essential [96].

In *Neurospora*, microarray and RNA sequencing studies demonstrate that 10–40% of the genome is circadianly expressed, and these genes contain WCC binding sites and light response elements (LRE), as well as a number of other motifs in their promoter regions [63–65, 97]. Chromatin immunoprecipitation (ChIP) results show that WC-2 is physically associated with 300 to over 400 regions in the genome, depending on the environmental conditions [65, 98]. Interestingly, the expression of 8–20% of transcription factor genes is regulated by WC-2 [65, 98, 99]. The authors propose that these transcription factors regulated by WCC can control downstream target genes, thus establishing a hierarchical system that shapes genome-wide rhythmic expression of CCGs. One such transcription factor driven by WCC is CSP1, a transcriptional repressor [100]. Genes controlled by CSP1 are rhythmically expressed with a peak in the evening and are predominantly involved in anabolic processes, whereas genes strongly dependent on WCC peak in the morning and are mainly involved in catabolic processes [97, 100]. The *Neurospora* clock has also been shown to regulate the phosphorylation and thus activation of mitogen-activated protein kinases MAK-1 and MAK-2 [101]. MAK-1 is required for normal expression of at least 145 CCGs. Moreover, RNA polymerase II (Pol II) is rhythmically recruited to over 1300 genes and about 25% of these genes display rhythms in transcript levels [97].

In *Arabidopsis*, promoter analyses of genes with cycling mRNA levels, in combination with luciferase reporter and enhancer trap assays, identified the evening element (EE), which is overrepresented in the promoters of evening-phased genes and both necessary and sufficient for transcription occurring in the evening [69, 71, 102, 103]. CCA1-binding site (CBS), which is only one base pair different from the EE, is important for morning-phased transcription [71, 103]. Two additional *cis*-regulatory elements, the morning element and protein box, mediate transcription in the morning and midnight, respectively [102, 104]. Recently, using ChIP followed by deep sequencing, over 500 genomic regions were found to be targeted by PRR5 and over 1000 regions by CCA1 [105, 106]. PRR5 direct targets are enriched in transcription factors, providing a means for PRR5 to control clock output, that is, CCGs [106]. PRR5 represses the expression of its direct targets from noon to midnight, possibly in conjunction with PRR7 and PRR9. The majority of the genes associated with strongest CCA1-binding peaks contain EE and show a rhythmic pattern with the peak of expression in the evening [105].

In *Drosophila*, a study using ChIP tiling array assays has demonstrated that CLK binds to ~1500 sites in the genome, and association with at least ~60% of these sites appears to be rhythmic [107]. These target regions are enriched for canonical or degenerate E-boxes. CYC is detected and PER binds to CLK/CYC about 4–6 h later at most of these target sites, which suggests that these target genes are regulated similarly to core clock genes. Pol II binds rhythmically to approximately ~30% of the target genes, leading to cyclic synthesis of RNA [107].

In the mouse, most of the core clock proteins, including BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, and REV-ERBs, have all been shown to bind to over 1000 sites in the genome with a circadian rhythm [81, 108, 109]. These target sites are enriched in E-boxes, as well as binding motifs for CEBPA and a number of nuclear receptors. Pol II is cyclically recruited to the genome with a peak that coincides with the peak of global rhythms in nascent transcription [81]. Genome-wide analysis revealed that the majority of the expressed genes undergo circadian histone modifications regardless of whether RNA oscillation can be detected and the recruitment (and initiation) of Pol II may contribute to variation in the amplitude of histone marks [81, 84, 110]. Valekunja and colleagues identified the histone-remodeling enzyme mixed-lineage leukemia 3 (MLL3) as a CCG that can modulate over 100 epigenetically targeted circadian output genes. Moreover, inactivating the methyltransferase activity of MLL3 severely impairs cyclic methylation at the promoters of core clock genes [111]. Genome-wide histone acetylation also exhibits a diurnal rhythm, contributing to rhythmic expression of CCGs [112]. This is believed to be orchestrated at least in part by histone deacetylase 3 (HDAC3), which is rhythmically recruited to the genome by REV-ERBα. Another study employed chromosome conformation capture on chip technology and demonstrated oscillation in spatial and temporal chromosomal organization, which is driven by the clock [113]. This may lead to rhythmic generation of genomic environments that promote rhythmic gene expression. Recently, enhancer RNAs have also been identified which modulate genome-wide rhythmic expression of CCGs [114].

5. Conclusion and Discussion

CCGs consist of a substantial portion of the genome. Although they participate in diverse biological processes that differ in different organisms and tissues/organs, genes involved in metabolism appear to be circadianly regulated throughout the phylogeny. This implicates an evolutionarily conserved function of the circadian clock in regulating rhythmic metabolic processes. This also means that it is particularly important for metabolic processes to be synchronized to the external day/night cycle.

The transcription of CCGs is regulated by rhythmic activities of transcription factors accompanied by rhythmic alterations of the chromatin in organisms ranging from cyanobacteria to plants and mammals, reflecting evolutionarily conserved mechanisms controlling circadian gene transcription. However, the complexity of the regulations increased by orders of magnitude. In the unicellular prokaryote cyanobacteria, CCGs are regulated by a few transcription factors as well as rhythmic changes in the topology of the chromosome, and no specific promoter sequences are believed to be involved. Apparently, this is not sufficient for more complex organisms to adapt to the daily cycle, and thus specific *cis*-elements evolved, along with many more transcription factors and various posttranslational modifications of the chromatin to modulate rhythmic expression of CCGs. The combined actions of these processes result in unique circadian transcriptomes adapted to the needs of each organism, as well as each tissue/organ within an organism. It would be of interest to identify common and distinct mechanisms employed by diverse organisms and tissues/organs. The former shall reveal fundamental pathways adopted by the clock output system, whereas the latter will reflect unique output mechanisms that are a result of unique clock-environment interactions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] J. C. Dunlap, "Molecular bases for circadian clocks," *Cell*, vol. 96, no. 2, pp. 271–290, 1999.

[2] J. C. Dunlap, "Closely watched clocks: molecular analysis of circadian rhythms in *Neurospora*and *Drosophila*," *Trends in Genetics*, vol. 6, no. 5, pp. 159–165, 1990.

[3] T. Mori and C. H. Johnson, "Independence of circadian timing from cell division in cyanobacteria," *Journal of Bacteriology*, vol. 183, no. 8, pp. 2439–2444, 2001.

[4] M. J. Dowson-Day and A. J. Millar, "Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*," *Plant Journal*, vol. 17, no. 1, pp. 63–71, 1999.

[5] T. Imaizumi and S. A. Kay, "Photoperiodic control of flowering: not only by coincidence," *Trends in Plant Science*, vol. 11, no. 11, pp. 550–558, 2006.

[6] R. Y. Moore, "Circadian rhythms: basic neurobiology and clinical applications," *Annual Review of Medicine*, vol. 48, pp. 253–266, 1997.

[7] M. Merrow, K. Spoelstra, and T. Roenneberg, "The circadian cycle: daily rhythms from behaviour to genes," *EMBO Reports*, vol. 6, no. 10, pp. 930–935, 2005.

[8] D. Bell-Pedersen, V. M. Cassone, D. J. Earnest et al., "Circadian rhythms from multiple oscillators: lessons from diverse organisms," *Nature Reviews Genetics*, vol. 6, no. 7, pp. 544–556, 2005.

[9] D. Bell-Pedersen, J. C. Dunlap, and J. J. Loros, "Distinct cis-acting elements mediate clock, light, and developmental regulation of the *Neurospora crassa* eas (ccg-2) gene," *Molecular and Cellular Biology*, vol. 16, no. 2, pp. 513–521, 1996.

[10] M. W. Young and S. A. Kay, "Time zones: a comparative genetics of circadian clocks," *Nature Reviews Genetics*, vol. 2, no. 9, pp. 702–715, 2001.

[11] M. Ishiura, S. Kutsuna, S. Aoki et al., "Expression of a gene cluster *kaiABC* as a circadian feedback process in cyanobacteria," *Science*, vol. 281, no. 5382, pp. 1519–1523, 1998.

[12] J. Tomita, M. Nakajima, T. Kondo, and H. Iwasaki, "No transcription-translation feedback in circadian rhythm of KaiC phosphorylation," *Science*, vol. 307, no. 5707, pp. 251–254, 2005.

[13] K. Terauchi, Y. Kitayama, T. Nishiwaki et al., "ATPase activity of KaiC determines the basic timing for circadian clock of cyanobacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 41, pp. 16377–16381, 2007.

[14] H. Kageyama, T. Kondo, and H. Iwasaki, "Circadian formation of clock protein complexes by KaiA, KaiB, KaiC, and SasA in cyanobacteria," *The Journal of Biological Chemistry*, vol. 278, no. 4, pp. 2388–2395, 2003.

[15] Y. Xu, T. Mori, and C. H. Johnson, "Cyanobacterial circadian clockwork: roles of KaiA, KaiB and the KaiBC promoter in regulating KaiC," *The EMBO Journal*, vol. 22, no. 9, pp. 2117–2126, 2003.

[16] T. Mori and C. H. Johnson, "Circadian programming in cyanobacteria," *Seminars in Cell and Developmental Biology*, vol. 12, no. 4, pp. 271–278, 2001.

[17] P. Cheng, Y. Yang, K. H. Gardner, and Y. Liu, "PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*," *Molecular and Cellular Biology*, vol. 22, no. 2, pp. 517–524, 2002.

[18] P. Cheng, Y. Yang, and Y. Liu, "Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 13, pp. 7408–7413, 2001.

[19] A. C. Froehlich, J. J. Loros, and J. C. Dunlap, "Rhythmic binding of a WHITE COLLAR-containing complex to the frequency promoter is inhibited by FREQUENCY," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 10, pp. 5914–5919, 2003.

[20] Q. He, H. Shu, P. Cheng, S. Chen, L. Wang, and Y. Liu, "Light-independent phosphorylation of WHITE COLLAR-1 regulates its function in the *Neurospora* circadian negative feedback loop," *The Journal of Biological Chemistry*, vol. 280, no. 17, pp. 17526–17532, 2005.

[21] C. I. Hong, P. Ruoff, J. J. Loros, and J. C. Dunlap, "Closing the circadian negative feedback loop: FRQ-dependent clearance of WC-1 from the nucleus," *Genes and Development*, vol. 22, no. 22, pp. 3196–3204, 2008.

[22] J. J. Loros and J. C. Dunlap, "Genetic and molecular analysis of circadian rhythms in *Neurospora*," *Annual Review of Physiology*, vol. 63, pp. 757–794, 2001.

[23] T. Schafmeier, A. Haase, K. Káldi, J. Scholz, M. Fuchs, and M. Brunner, "Transcriptional feedback of *Neurospora* circadian clock gene by phosphorylation-dependent inactivation of its transcription factor," *Cell*, vol. 122, no. 2, pp. 235–246, 2005.

[24] Y. Yang, P. Cheng, and Y. Liu, "Regulation of the *Neurospora* circadian clock by casein kinase II," *Genes and Development*, vol. 16, no. 8, pp. 994–1006, 2002.

[25] K. Lee, J. J. Loros, and J. C. Dunlap, "Interconnected feedback loops in the *Neurospora* circadian system," *Science*, vol. 289, no. 5476, pp. 107–110, 2000.

[26] D. Alabadí, T. Oyama, M. J. Yanovsky, F. G. Harmon, P. Más, and S. A. Kay, "Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock," *Science*, vol. 293, no. 5531, pp. 880–883, 2001.

[27] A. Matsushika, S. Makino, M. Kojima, and T. Mizuno, "Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: insight into the plant circadian clock," *Plant and Cell Physiology*, vol. 41, no. 9, pp. 1002–1012, 2000.

[28] A. Pokhilko, P. Mas, and A. J. Millar, "Modelling the widespread effects of TOC1 signalling on the plant circadian clock and its outputs," *BMC Systems Biology*, vol. 7, article 23, 2013.

[29] R. Schaffer, N. Ramsay, A. Samach et al., "The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering," *Cell*, vol. 93, no. 7, pp. 1219–1229, 1998.

[30] Z.-Y. Wang and E. M. Tobin, "Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression," *Cell*, vol. 93, no. 7, pp. 1207–1217, 1998.

[31] J. M. Gendron, J. L. Pruneda-Paz, C. J. Doherty, A. M. Gross, S. E. Kang, and S. A. Kay, "*Arabidopsis* circadian clock protein, TOC1, is a DNA-binding transcription factor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 8, pp. 3167–3172, 2012.

[32] W. Huang, P. Pérez-García, A. Pokhilko et al., "Mapping the core of the *Arabidopsis* circadian clock defines the network structure of the oscillator," *Science*, vol. 335, no. 6077, pp. 75–79, 2012.

[33] E. M. Farré, S. L. Harmer, F. G. Harmon, M. J. Yanovsky, and S. A. Kay, "Overlapping and distinct roles of PRR7 and PRR9 in the *Arabidopsis* circadian clock," *Current Biology*, vol. 15, no. 1, pp. 47–54, 2005.

[34] N. Nakamichi, T. Kiba, R. Henriques, T. Mizuno, N.-H. Chua, and H. Sakakibara, "PSEUDO-RESPONSE ReGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock," *Plant Cell*, vol. 22, no. 3, pp. 594–605, 2010.

[35] B. Y. Chow, A. Helfer, D. A. Nusinow, and S. A. Kay, "ELF3 recruitment to the *PRR9* promoter requires other Evening Complex members in the Arabidopsis circadian clock," *Plant Signaling & Behavior*, vol. 7, no. 2, pp. 170–173, 2012.

[36] A. Helfer, D. A. Nusinow, B. Y. Chow, A. R. Gehrke, M. L. Bulyk, and S. A. Kay, "LUX ARRHYTHMO encodes a nighttime repressor of circadian gene expression in the *Arabidopsis* core clock," *Current Biology*, vol. 21, no. 2, pp. 126–133, 2011.

[37] D. A. Nusinow, A. Helfer, E. E. Hamilton et al., "The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth," *Nature*, vol. 475, no. 7356, pp. 398–402, 2011.

[38] R. Allada, N. E. White, W. V. So, J. C. Hall, and M. Rosbash, "A mutant *Drosophila* homolog of mammalian *Clock* disrupts circadian rhythms and transcription of *period* and *timeless*," *Cell*, vol. 93, no. 5, pp. 791–804, 1998.

[39] T. K. Darlington, K. Wager-Smith, M. F. Ceriani et al., "Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim," *Science*, vol. 280, no. 5369, pp. 1599–1603, 1998.

[40] P. E. Hardin, "The circadian timekeeping system of *Drosophila*," *Current Biology*, vol. 15, no. 17, pp. R714–R722, 2005.

[41] J. E. Rutila, V. Suri, M. Le, W. V. So, M. Rosbash, and J. C. Hall, "Cycle is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of Drosophila period and timeless," *Cell*, vol. 93, no. 5, pp. 805–814, 1998.

[42] D. C. Chang and S. M. Reppert, "A novel C-terminal domain of *Drosophila* PERIOD inhibits dCLOCK:CYCLE-mediated transcription," *Current Biology*, vol. 13, no. 9, pp. 758–762, 2003.

[43] C. Lee, K. Bae, and I. Edery, "PER and TIM inhibit the DNA binding activity of a *Drosophila* CLOCK-CYC/dBMAL1 heterodimer without disrupting formation of the heterodimer: a basis for circadian transcription," *Molecular and Cellular Biology*, vol. 19, no. 8, pp. 5316–5325, 1999.

[44] O. T. Shafer, M. Rosbash, and J. W. Truman, "Sequential nuclear accumulation of the clock proteins period and timeless in the pacemaker neurons of *Drosophila melanogaster*," *The Journal of Neuroscience*, vol. 22, no. 14, pp. 5946–5954, 2002.

[45] I. Edery, L. J. Zwiebel, M. E. Dembinska, and M. Rosbash, "Temporal phosphorylation of the *Drosophila* period protein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 6, pp. 2260–2264, 1994.

[46] H. W. Ko, J. Jiang, and I. Edery, "Role for Slimb in the degradation of *Drosophila* Period protein phosphorylated by Doubletime," *Nature*, vol. 420, no. 6916, pp. 673–678, 2002.

[47] H. Zeng, P. E. Hardin, and M. Rosbash, "Constitutive overexpression of the *Drosophila* period protein inhibits period mRNA cycling," *The EMBO Journal*, vol. 13, no. 15, pp. 3590–3598, 1994.

[48] S. A. Cyran, A. M. Buchsbaum, K. L. Reddy et al., "vrille, Pdp1, and dClock form a second feedback loop in the *Drosophila* circadian clock," *Cell*, vol. 112, no. 3, pp. 329–341, 2003.

[49] N. R. J. Glossop, J. H. Houl, H. Zheng, F. S. Ng, S. M. Dudek, and P. E. Hardin, "VRILLE feeds back to control circadian transcription of *Clock* in the *Drosophila* circadian oscillator," *Neuron*, vol. 37, no. 2, pp. 249–261, 2003.

[50] S. A. Brown, J. Ripperger, S. Kadener et al., "PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator," *Science*, vol. 308, no. 5722, pp. 693–696, 2005.

[51] K. Padmanabhan, M. S. Robles, T. Westerling, and C. J. Weitz, "Feedback regulation of transcriptional termination by the mammalian circadian clock PERIOD complex," *Science*, vol. 337, no. 6094, pp. 599–602, 2012.

[52] R. J. Thresher, M. H. Vitaterna, Y. Miyamoto et al., "Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses," *Science*, vol. 282, no. 5393, pp. 1490–1494, 1998.

[53] D. K. Welsh, J. S. Takahashi, and S. A. Kay, "Suprachiasmatic nucleus: cell autonomy and network properties," *Annual Review of Physiology*, vol. 72, pp. 551–577, 2009.

[54] S. I. H. Godinho, E. S. Maywood, L. Shaw et al., "The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period," *Science*, vol. 316, no. 5826, pp. 897–900, 2007.

[55] K. Kume, M. J. Zylka, S. Sriram et al., "mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop," *Cell*, vol. 98, no. 2, pp. 193–205, 1999.

[56] A. M. Sangoram, L. Saez, M. P. Antoch et al., "Mammalian circadian autoregulatory loop: a timeless ortholog and mPer1 interact and negatively regulate CLOCK-BMAL1-induced transcription," *Neuron*, vol. 21, no. 5, pp. 1101–1113, 1998.

[57] G. Asher, D. Gatfield, M. Stratmann et al., "SIRT1 regulates circadian clock gene expression through PER2 deacetylation," *Cell*, vol. 134, no. 2, pp. 317–328, 2008.

[58] L. Busino, F. Bassermann, A. Maiolica et al., "SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins," *Science*, vol. 316, no. 5826, pp. 900–904, 2007.

[59] S. M. Reppert and D. R. Weaver, "Molecular analysis of mammalian circadian rhythms," *Annual Review of Physiology*, vol. 63, pp. 647–676, 2001.

[60] M. Reick, J. A. Garcia, C. Dudley, and S. L. McKnight, "NPAS2: an analog of clock operative in the mammalian forebrain," *Science*, vol. 293, no. 5529, pp. 506–509, 2001.

[61] H. Ito, M. Mutsuda, Y. Murayama et al., "Cyanobacterial daily life with Kai-based circadian and diurnal genome-wide transcriptional control in *Synechococcus elongatus*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 14168–14173, 2009.

[62] V. Vijayan, R. Zuzow, and E. K. O'Shea, "Oscillations in supercoiling drive circadian gene expression in cyanobacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 52, pp. 22564–22568, 2009.

[63] A. Correa, Z. A. Lewis, A. V. Greene, I. J. March, R. H. Gomer, and D. Bell-Pedersen, "Multiple oscillators regulate circadian gene expression in Neurospora," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 23, pp. 13597–13602, 2003.

[64] W. Dong, X. Tang, Y. Yu et al., "System biology of the clock in *Neurospora crassa*," *PLoS ONE*, vol. 3, no. 8, Article ID e3105, 2008.

[65] J. M. Hurley, A. Dasgupta, J. M. Emerson et al., "Analysis of clock-regulated genes in *Neurospora* reveals widespread posttranscriptional control of metabolic potential," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 48, pp. 16995–17002, 2014.

[66] C. Sancar, G. Sancar, N. Ha, F. Cesbron, and M. Brunner, "Dawn- and dusk-phased circadian transcription rhythms coordinate anabolic and catabolic functions in *Neurospora*," *BMC Biology*, vol. 13, article 17, 2015.

[67] M. F. Covington and S. L. Harmer, "The circadian clock regulates auxin signaling and responses in *Arabidopsis*," *PLoS Biology*, vol. 5, no. 8, article e222, 2007.

[68] K. D. Edwards, P. E. Anderson, A. Hall et al., "*FLOWERING LOCUS C* mediates natural variation in the high-temperature response of the *Arabidopsis* circadian clock," *The Plant Cell*, vol. 18, no. 3, pp. 639–650, 2006.

[69] S. L. Harmer, J. B. Hogenesch, M. Straume et al., "Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock," *Science*, vol. 290, no. 5499, pp. 2110–2113, 2000.

[70] M. F. Covington, J. N. Maloof, M. Straume, S. A. Kay, and S. L. Harmer, "Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development," *Genome Biology*, vol. 9, no. 8, article R130, 2008.

[71] T. P. Michael and C. R. McClung, "Enhancer trapping reveals widespread circadian clock transcriptional control in *Arabidopsis*," *Plant Physiology*, vol. 132, no. 2, pp. 629–639, 2003.

[72] T. P. Michael, T. C. Mockler, G. Breton et al., "Network discovery pipeline elucidates conserved time-of-day-specific cis-regulatory modules," *PLoS Genetics*, vol. 4, no. 2, article e14, 2008.

[73] M. F. Ceriani, J. B. Hogenesch, M. Yanovsky, S. Panda, M. Straume, and S. A. Kay, "Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior," *Journal of Neuroscience*, vol. 22, no. 21, pp. 9305–9319, 2002.

[74] A. Claridge-Chang, H. Wijnen, F. Naef, C. Boothroyd, N. Rajewsky, and M. W. Young, "Circadian regulation of gene expression systems in the *Drosophila* head," *Neuron*, vol. 32, no. 4, pp. 657–671, 2001.

[75] Y. Lin, M. Han, B. Shimada et al., "Influence of the period-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 14, pp. 9562–9567, 2002.

[76] M. J. McDonald and M. Rosbash, "Microarray analysis and organization of circadian gene expression in *Drosophila*," *Cell*, vol. 107, no. 5, pp. 567–578, 2001.

[77] H. R. Ueda, A. Matsumoto, M. Kawamura, M. Iino, T. Tanimura, and S. Hashimoto, "Genome-wide transcriptional orchestration of circadian rhythms in *Drosophila*," *Journal of Biological Chemistry*, vol. 277, no. 16, pp. 14048–14052, 2002.

[78] M. E. Hughes, G. R. Grant, C. Paquin, J. Qian, and M. N. Nitabach, "Deep sequencing the circadian and diurnal transcriptome of *Drosophila* brain," *Genome Research*, vol. 22, no. 7, pp. 1266–1281, 2012.

[79] J. Rodriguez, C.-H. A. Tang, Y. L. Khodor, S. Vodala, J. S. Menet, and M. Rosbash, "Nascent-Seq analysis of *Drosophila* cycling gene expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 4, pp. E275–E284, 2013.

[80] M. E. Hughes, L. DiTacchio, K. R. Hayes et al., "Harmonics of circadian gene transcription in mammals," *PLoS Genetics*, vol. 5, no. 4, Article ID e1000442, 2009.

[81] N. Koike, S. Yoo, H. Huang et al., "Transcriptional architecture and chromatin landscape of the core circadian clock in mammals," *Science*, vol. 338, no. 6105, pp. 349–354, 2012.

[82] S. Panda, M. P. Antoch, B. H. Miller et al., "Coordinated transcription of key pathways in the mouse by the circadian clock," *Cell*, vol. 109, no. 3, pp. 307–320, 2002.

[83] K.-F. Storch, O. Lipan, I. Leykin et al., "Extensive and divergent circadian gene expression in liver and heart," *Nature*, vol. 417, no. 6884, pp. 78–83, 2002.

[84] C. Vollmers, R. J. Schmitz, J. Nathanson, G. Yeo, J. R. Ecker, and S. Panda, "Circadian oscillations of protein-coding and regulatory RNAs in a highly dynamic mammalian liver epigenome," *Cell Metabolism*, vol. 16, no. 6, pp. 833–845, 2012.

[85] R. Zhang, N. F. Lahens, H. I. Ballance, M. E. Hughes, and J. B. Hogenesch, "A circadian gene expression atlas in mammals: implications for biology and medicine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 45, pp. 16219–16224, 2014.

[86] J. S. Menet, J. Rodriguez, K. C. Abruzzi, and M. Rosbash, "Nascent-Seq reveals novel features of mouse circadian transcriptional regulation," *eLife*, vol. 2012, no. 1, Article ID e00011, 2012.

[87] N. Takai, M. Nakajima, T. Oyama et al., "A KaiC-associating SasA-RpaA two-component regulatory system as a major circadian timing mediator in cyanobacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 32, pp. 12109–12114, 2006.

[88] H. Iwasaki, S. B. Williams, Y. Kitayama, M. Ishiura, S. S. Golden, and T. Kondo, "A KaiC-interacting sensory histidine kinase, SasA, necessary to sustain robust circadian oscillation in cyanobacteria," *Cell*, vol. 101, no. 2, pp. 223–233, 2000.

[89] J. S. Markson, J. R. Piechura, A. M. Puszynska, and E. K. O'Shea, "Circadian control of global gene expression by the cyanobacterial master regulator RpaA," *Cell*, vol. 155, no. 6, pp. 1396–1408, 2013.

[90] R. M. Smith and S. B. Williams, "Circadian rhythms in gene transcription imparted by chromosome compaction in the cyanobacterium *Synechococcus elongatus*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 22, pp. 8564–8569, 2006.

[91] A. Gutu and E. K. O'Shea, "Two antagonistic clock-regulated histidine kinases time the activation of circadian gene expression," *Molecular Cell*, vol. 50, no. 2, pp. 288–294, 2013.

[92] Y. Taniguchi, M. Katayama, R. Ito, N. Takai, T. Kondo, and T. Oyama, "labA: a novel gene required for negative feedback regulation of the cyanobacterial circadian clock protein KaiC," *Genes and Development*, vol. 21, no. 1, pp. 60–70, 2007.

[93] Y. Xu, P. D. Weyman, M. Umetani et al., "Circadian yin-yang regulation and its manipulation to globally reprogram gene expression," *Current Biology*, vol. 23, no. 23, pp. 2365–2374, 2013.

[94] M. A. Woelfle, Y. Xu, X. Qin, and C. H. Johnson, "Circadian rhythms of superhelical status of DNA in cyanobacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 47, pp. 18819–18824, 2007.

[95] V. Vijayan and E. K. O'Shea, "Sequence determinants of circadian gene expression phase in cyanobacteria," *Journal of Bacteriology*, vol. 195, no. 4, pp. 665–671, 2013.

[96] M. A. Woelfle and C. H. Johnson, "No promoter left behind: global circadian gene expression in cyanobacteria," *Journal of Biological Rhythms*, vol. 21, no. 6, pp. 419–431, 2006.

[97] C. Sancar, N. Ha, R. Yilmaz et al., "Combinatorial control of light induced chromatin remodeling and gene activation in *Neurospora*," *PLoS Genetics*, vol. 11, no. 3, Article ID e1005105, 2015.

[98] K. M. Smith, G. Sancar, R. Dekhang et al., "Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for neurospora white collar complex," *Eukaryotic Cell*, vol. 9, no. 10, pp. 1549–1556, 2010.

[99] P. L. Lakin-Thomas, D. Bell-Pedersen, and S. Brody, "The genetics of circadian rhythms in *Neurospora*," *Advances in Genetics*, vol. 74, pp. 55–103, 2011.

[100] G. Sancar, C. Sancar, B. Brügger et al., "A global circadian repressor controls antiphasic expression of metabolic genes in *Neurospora*," *Molecular Cell*, vol. 44, no. 5, pp. 687–697, 2011.

[101] L. D. Bennett, P. Beremand, T. L. Thomas, and D. Bell-Pedersen, "Circadian activation of the mitogen-activated protein kinase MAK-1 facilitates rhythms in clock-controlled genes in *Neurospora crassa*," *Eukaryotic Cell*, vol. 12, no. 1, pp. 59–69, 2013.

[102] S. L. Harmer and S. A. Kay, "Positive and negative factors confer phase-specific circadian regulation of transcription in *Arabidopsis*," *Plant Cell*, vol. 17, no. 7, pp. 1926–1940, 2005.

[103] T. P. Michael and C. R. McClung, "Phase-specific circadian clock regulatory elements in *Arabidopsis*," *Plant Physiology*, vol. 130, no. 2, pp. 627–638, 2002.

[104] T. P. Michael, G. Breton, S. P. Hazen et al., "A morning-specific phytohormone gene expression program underlying rhythmic plant growth," *PLoS Biology*, vol. 6, no. 9, article e225, 2008.

[105] D. H. Nagel, C. J. Doherty, J. L. Pruneda-Paz, R. J. Schmitz, J. R. Ecker, and S. A. Kay, "Genome-wide identification of CCA1 targets uncovers an expanded clock network in *Arabidopsis*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 34, pp. E4802–E4810, 2015.

[106] N. Nakamichi, T. Kiba, M. Kamioka et al., "Transcriptional repressor PRR5 directly regulates clock-output pathways," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 42, pp. 17123–17128, 2012.

[107] K. C. Abruzzi, J. Rodriguez, J. S. Menet et al., "*Drosophila* CLOCK target gene characterization: implications for circadian tissue-specific gene expression," *Genes & Development*, vol. 25, no. 22, pp. 2374–2386, 2011.

[108] A. Bugge, D. Feng, L. J. Everett et al., "Rev-erbα and Rev-erbβ coordinately protect the circadian clock and normal metabolic function," *Genes and Development*, vol. 26, no. 7, pp. 657–667, 2012.

[109] G. Rey, F. Cesbron, J. Rougemont, H. Reinke, M. Brunner, and F. Naef, "Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver," *PLoS Biology*, vol. 9, no. 2, Article ID e1000595, 2011.

[110] G. Le Martelot, D. Canella, L. Symul et al., "Genome-wide RNA polymerase II profiles and RNA accumulation reveal kinetics of transcription and associated epigenetic changes during diurnal cycles," *PLoS Biology*, vol. 10, no. 11, Article ID e1001442, 2012.

[111] U. K. Valekunja, R. S. Edgar, M. Oklejewicz et al., "Histone methyltransferase MLL3 contributes to genome-scale circadian transcription," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 4, pp. 1554–1559, 2013.

[112] D. Feng, T. Liu, Z. Sun et al., "A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism," *Science*, vol. 331, no. 6022, pp. 1315–1319, 2011.

[113] L. Aguilar-Arnal, O. Hakim, V. R. Patel, P. Baldi, G. L. Hager, and P. Sassone-Corsi, "Cycles in spatial and temporal chromosomal organization driven by the circadian clock," *Nature Structural and Molecular Biology*, vol. 20, no. 10, pp. 1206–1213, 2013.

[114] B. Fang, L. J. Everett, J. Jager et al., "Circadian enhancers coordinate multiple phases of rhythmic gene transcription in vivo," *Cell*, vol. 159, no. 5, pp. 1140–1152, 2014.