Computational and Structural Biotechnology Journal 18 (2020) 1914–1924

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Review

Genome-wide circadian regulation: A unique system for computational biology

Lingying Sun [a], Junjie Ma [a], Christoph W. Turck [b], Pin Xu [c,*], Guang-Zhong Wang [a,*]

[a] CAS Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Shanghai Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 200031, China
[b] Max Planck Institute of Psychiatry, Department of Translational Research in Psychiatry, Munich 80804, Germany
[c] Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390-9111, USA

ARTICLE INFO

Article history:
Received 30 November 2019
Received in revised form 30 June 2020
Accepted 2 July 2020
Available online 10 July 2020

Keywords:
Circadian rhythms
Cycling genes
Circadian transcriptome
Energetic cost
Circadian regulatory network

ABSTRACT

Circadian rhythms are 24-hour oscillations affecting an organism at multiple levels from gene expression all the way to tissues and organs. They have been observed in organisms across the kingdom of life, spanning from cyanobacteria to humans. In mammals, the master circadian pacemaker is located in the hypothalamic suprachiasmatic nuclei (SCN) in the brain where it synchronizes the peripheral oscillators that exist in other tissues. This system regulates the circadian activity of a large part of the transcriptome and recent findings indicate that almost every cell in the body has this clock at the molecular level. In this review, we briefly summarize the different factors that can influence the circadian transcriptome, including light, temperature, and food intake. We then summarize recently identified general principles governing genome-scale circadian regulation, as well as future lines of research. Genome-scale circadian activity represents a fascinating study model for computational biology. For this purpose, systems biology methods are promising exploratory tools to decode the global regulatory principles of circadian regulation.

Contents

Abbreviations: ABSR, Autoregressive Bayesian spectral regression; AMPK, AMP-activated protein kinase; AR, Arrhythmic feeding; ARSER, Harmonic regression based on autoregressive spectral estimation; BMAL1, The aryl hydrocarbon receptor nuclear translocator-like (ARNTL); CCD, Cortical collecting duct; CR, Calorie-restricted diet; CRY, Cryptochrome; DCT/CNT, Distal convoluted tubule and connecting tubule; DD, Dark: dark; eJTK_CYCLE, Empirical JTK_CYCLE; HF, High fat diet; JTK_CYCLE, Jonckheere-Terpstra-Kendall (JTK) cycle; KD, Ketogenic diet; LB, Ad libitum; LD, Light:dark; Liver-RE, Liver clock reconstituted BMAL1-deficient mice; LS, Lomb-Scargle; NAD, Nicotinamide adenine dinucleotides; ND, Normal diet; NR, Night-restricted feeding; PAS, PER-ARNT-SIM; PER, Period; RAIN, Rhythmicity Analysis Incorporating Nonparametric methods; RF, Restricted feeding; SCN, Suprachiasmatic nucleus; SREBP, The sterol regulatory element binding protein; TTFL, Transcriptional-translational feedback loop; WT, Wild type.

* Corresponding authors.
E-mail addresses: pin.xu@UTSouthwestern.edu (P. Xu), guangzhong.wang@picb.ac.cn (G.-Z. Wang).

https://doi.org/10.1016/j.csbj.2020.07.002
2001-0370/

1. Introduction

Rhythmic behavior is one of the earliest physiological phenomenons that humans are aware of. The famous Chinese pre-Qin poem "the song of ji rang", "beginning work at sunrise and resting at sunset " describes the circadian activity of a farmer. The scientific observation of rhythmic behavior dates back to the 17th century, with the French scientist Jean-Jacques d'Ortous de Mairan's observation of leaf movements[1]. de Mairan found that in constant darkness, the leaves of the mimosa plant retained the pattern of opening and closing that was observed with a light–dark cycle[1]. The molecular basis of circadian behaviors was not discovered until the 20th century. In 1971, Ron Konopka and Seymour Benzer published a milestone paper that reported abnormal circadian rhythms in the behavior of fruit fly mutants, with one mutant losing its circadian rhythm and another two mutants markedly changing their circadian period[2]. They discovered that both the eclosion rhythm and the locomotor activity of the mutants were different from those of the wild type flies, and all mutations were found at a single genetic locus[2]. These pioneering studies led to more research on identifying the genetic foundation of circadian rhythms. In 1984, Hall, Rosbash, and Young successfully cloned the DNA sequence of the period gene (*per*) [3–5]. Young's group was able to restore the circadian rhythmicity of fruit fly mutants by introducing the *per* transcript sequence into the genome of an arrhythmic fly [4]. With this finding the first circadian rhythm gene had been discovered.

Substantial progress has been made in the study of circadian clocks in the last 20 years of the 20th century. The negative feedback loop of *per* was proposed as the basic mechanism underlying the circadian regulatory network [6–8]. In addition, another important gene, timeless (*tim*), was discovered in *Drosophila* [9–12]. In mammals, the first circadian gene *Clock* was located and sequenced by Takahashi's group [13,14]. In *Neurospora crassa,* the frequency (*frq*) gene that is homologous to the *per* gene in *Drosophila* was sequenced [15]. Several plant circadian clock-related genes were also found, including that mutant strains in period were found in green algae *Chlamydomonas reinhardtii* [16], and the cab gene, CCA1 and LHY in Arabidopsis was reported [17–19]. The discovery of circadian genes in cyanobacteria [20–22] further added to our understanding of the circadian mechanisms, as this oscillation does not require transcription. This finding provided further evidence that the core circadian proteins KaiA, KaiB, and KaiC produce oscillation at the molecular level *in vitro* [23]. One note is the fact that the sequences of many core clock genes lack obvious similarity, supporting the multiple origin hypothesis of the clock[24]. The number of published articles related to biological rhythms has been steadily increasing over the past years (Fig. 1), as research continues to expand the boundaries of knowledge in this field.

Although the circadian regulation, which generates persistent rhythmic behavior under constant environmental conditions, can be detected in almost all living organisms, different species need to adapt to specific environmental factors for survival. Thus, the input and output of this regulation varies tremendously across

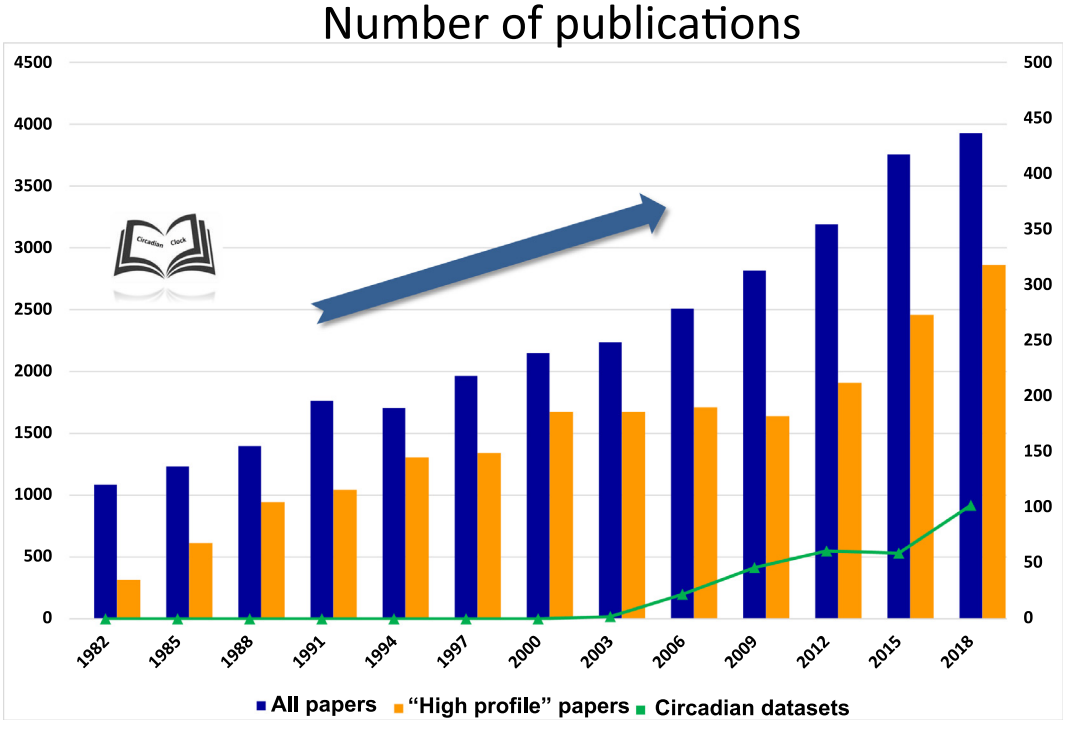

Fig. 1. The number of academic publications and datesets related to circadian rhythms increased dramatically from 1982 to 2018. Annual numbers of publications that contain the keywords "circadian" or "clock" were counted, which was obtained directly from PubMed's "Results by Year" option. The numbers of "high profile" (IF $\geq$ 10) papers among all publications related to circadian rhythms were calculated by PubMed Filters based on the impact factor in 2019. The number of circadian datesets (green lines) was obtained from GEO DateSets by using the "publication dates" filter. The x-axis represents the year, and the number of all publications (left y-axis), "high profile" papers (right y-axis) and circadian datesets (right y-axis) are displayed every three years. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

different organisms. This review emphasizes the impact of the different factors on transcriptome-wide activities in mammalian species (for more details of other model organisms, please refer to [25,26]). In mammals, circadian rhythms are composed of three major components: input factors, the central oscillator, and outputs [27–29]. The input factors refer to the environmental factors that affect the circadian clock, such as light, food intake, and temperature. The central oscillator, also known as the central clock, generates the oscillation behavior [28–30]. Both central and peripheral clocks consist of an interplay of positive and negative regulators, which affect transcriptional-translational feedback loops. The output of this system refers to the circadian output signals, which oscillate approximately 24 h in most organisms. In mammals, the clock system has a hierarchical structure, and suprachiasmatic nuclei (SCN) influences the peripheral clocks through the regulation of the nervous system and the endocrine system [30]. Therefore, output pathways exist between SCN and peripheral tissues at the system's level[30].

In the following, we will first elaborate upon the core molecular mechanisms that generate those rhythms in mammals (from several model organisms), followed by describing the external factors that affect biological rhythms. Finally, we will discuss the genome-wide circadian regulation and the general design principles of the output from the entire regulatory network. Biological rhythmic regulation is a good model for systems biology research because of the complexity of the input and output characteristics at the molecular level. Although the main factor affecting the rhythmicity of gene expression have been discovered recently[31], the systematic modeling of this regulatory system under different conditions is in its infancy. For instance, although many metabolic pathways are reported to be involved in rhythmically regulating gene expression in an experimental condition dependent manner, the common principles governing the regulation of these different pathways and individual condition remain unclear. For tissue specificity modeling, the ideal model should consider tissue specific regulatory mechanisms, and the key cycling pathways together with their pathological status. The present review illustrates the importance of the circadian system as a model for investigating molecular systems biology.

2. The central circadian clock in mammals

The core molecular mechanisms dictating biological rhythms have been the central topic in the circadian field. In mammals, the molecular mechanism of circadian oscillations is formed by an autoregulatory transcriptional-translational feedback loop (TTFL). In general, the positive elements of the feedback loop activate a set of negative regulators which in turn inhibit their own transcription.

In mammals, the positive elements of the circadian clock are CLOCK (and its paralog NPAS2) and BMAL1. These basic-helix-loop-helix -PER-ARNT-SIM (PAS) transcription factors form heterodimers and bind to regulatory element containing E-boxes in *Per1*, *Per2*, *Per3* and *Cry1*, *Cry2*, which are the negative elements. PER and CRY proteins dimerize and interact with CLOCK:BMAL1 to repress their own transcription. The feedback loop also involves the nuclear orphan receptors such as RORα/β/γ and REV-ERBα/β, which repress the transcription of *Bmal1* [32–34].

The absence or mutation of core clock genes can affect the molecular processes of the entire circadian regulatory network (Fig. 2). There are about 900 circadian-regulated genes in the wild-type mouse liver, and the rhythmicity of more than 90% of them was perturbed following the deletion of REV-ERBα/β[37]. The REV-ERBα/β knockout mice exhibit altered circadian wheel-running behavior and have lipid metabolism disorders[37]. In the islet beta cells 1,757 genes with altered expression levels were found in *Bmal1* ablation mice, with 1,074 genes decreased and 683 genes increased compared to wild-type mice[40]. The islet beta cells from *Bmal1* ablation mice no longer exhibit nutrient-responsive insulin secretion, and destruction of CLOCK and BMAL1 in the mouse pancreas causes hypoinsulinemic diabetes[40]. Rhythmic expression was observed in 5457 out of 37,681 transcripts (14.5%) in the liver of inducible *Bmal1* knockout mice. In WT mice, 716 out of 6,818 and 267 out of 7,824 genes were rhythmically expressed in the liver and skeletal muscle, respectively, and the majority (71% in liver and 78% in muscle) of these rhythmic genes had significantly different expression levels in *Clock* mutant tissue[35]. Total RNA sequencing and ribosome profiling data from the liver of *Bmal1* knockout mice revealed that loss of *Bmal1* expression affected mRNA accumulation at both the transcriptional and post-transcriptional levels[41].

3. Input factors

Environmental stimuli can affect the circadian rhythm as zeitgebers, such as light, temperature, hypoxia, and methamphetamine, etc. We will focus on light, temperature, and food intake, which have diverse effects in different species, and even for different developmental periods. They influence a great part of the transcriptome and are the most intensively studied stimuli so far.

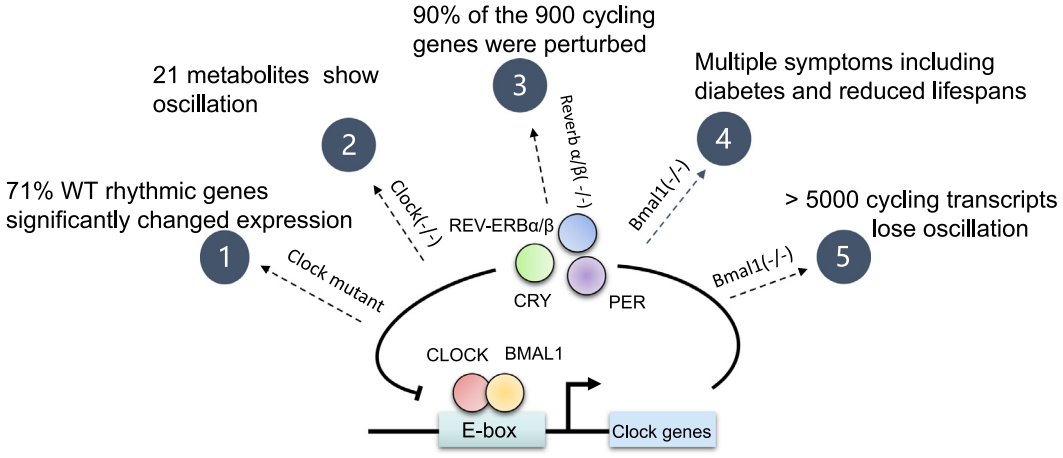

Fig. 2. The transcriptome-wide effects of core clock gene mutations (*Clock, Bmal1 and Rev-erb α/β*) on the circadian clock of mouse liver. Data source: *Clock* mutant [35]; Clock (-/-) [36]; Reverb α/β(-/-)[37]; Bmal1(-/-) [38]; Bmal1(-/-) [39].

3.1. Light

For most organisms, the light–dark cycle is the most apparent environmental factor that influences circadian behavior[42]. Completely blind individuals may have free-running circadian rhythms, although they can sense diurnal changes of environmental factors other than light, suggesting that light is one of the most important circadian stimuli[43]. In mammals, light signals are detected by retina, transmitted through retinohypothalamic tract to the SCN and change expression of clock genes (by cAMP response element-binding protein), causing daily phase changes. Light signals activate mitogen- and stress-activated protein kinase (MAPK) pathway and induce the expression of genes containing cAMP response elements in the promoter region through cAMP responsive element binding protein (CREB)[44]. In addition, the light signal regulates the phosphorylation of the translation initiation factor eIF4E by affecting MAPK/MNK pathway, and further regulates the translation of PER1 and PER2[45]. Early microarray analysis identified hundreds of cycling transcripts in the SCN under constant darkness, most of which are SCN-specific[46]. Specifically restoring *Clock* function in the brain of *Clock* mutants rescues the rhythmic expression of large numbers of cycling genes[47]. Interestingly, a much larger gene set (4,569 genes) was recently identified showing rhythmic expression in the SCN under light–dark condition, and an unexpected group of more than 700 genes was observed that peaked twice per day, indicating the complexity of its transcriptome[48]. In general, more genes show rhythmic expression under light–dark (LD) condition compared to dark-dark (DD) condition in peripheral tissues. For instance, 2960 and 2302 circadian transcripts were observed in epidermis in LD and DD conditions of wide type mouse, respectively[49], which indicates that different pathways are involved in regulating rhythmic gene expression under these two light conditions.

3.2. Temperature

Similar to light, temperature also changes rhythmically in the natural environment. Temperature has a fundamental effect on physiology, and variations in temperature are key to maintain the stability of circadian rhythms[50]. An important property of circadian clock is temperature compensation[51], i.e. the period of a free-running cycle remains relatively stable under different temperature conditions. The temperature compensation mechanism enables organisms to maintain the stability of the circadian clock under temperature fluctuations, thereby ensuring the consistency of physiological and behavioral rhythms. However, the mechanism of temperature compensation is not fully understood, and it may be related to the heat-shock pathway[52,53]. In mammals, temperature entrainment acts as a universal synchronizing factor, which resets circadian oscillations [54]. Subsequently, this signal acts as the input for the entrainment of other organs in the body.

3.3. Food intake

In addition to environmental cues, factors such as feeding, fasting, and social contact also affect the circadian clock. In the peripheral tissues, the circadian clock is an intrinsic time-keeping mechanism to cope with the changing external environment and maintain internal homeostasis. Food, including feeding and fasting, is an important synchronizer of the rhythmicity of gene expression, liver mass, and even cell size[36,55–58]. Restricted feeding (RF) can alter the circadian rhythms of fish, birds, and many mammals[59]. Time restricted feeding reduced body weight and cholesterol levels, and at the same time improved insulin sensitivity in rodents[60]. Under DD, about 15% of transcripts in the mouse liver are rhythmically expressed, which is driven by both food intake and the circadian regulatory network[56,61]. Time restricted feeding restored the oscillation of many transcripts in oscillator-deficient mouse livers[56]. Feeding- or fasting-induced transcripts accumulated as the amount of feeding or fasting time increased. After feeding, *Per1* mRNA levels were downregulated, while *Per2* expression levels were upregulated[56]. In *ad lib*-fed wild-type mice, 2997 transcripts had circadian expression patterns, while only 368 transcripts among them expressed circadian patterns under fasting conditions[56]. This indicates that temporal pattern of food intake in combination with circadian clock drive the expression of rhythmic genes in the liver[56].

The feeding activity of most animals has its own rhythmicity. This rhythm is important for maintaining the synchronization of metabolism and behavior. Under normal circumstances, multiple organs or tissues participate in the digestion, absorption, and metabolism of food after feeding. A suite of proteins are involved in this process, including NAD-dependent enzymes (e.g., sirtuins and poly[ADP-ribose] polymerases), and protein kinases (e.g., AMP-activated protein kinase) [48], which in turn affect the expression of rhythmic genes. Reducing the rhythmic expression of BMAL1 and REV-ERBα in the liver and muscle led to an inhibition of their target genes[62]. In both liver and muscle, around 70 (about 30%) differentially expressed BMAL1-target genes had higher expression levels under fasting conditions, and these genes were induced in a BMAL1-dependent manner[62]

A time-restricted feeding pattern produces a sharp feeding-fasting cycle that consolidates rhythmic expression of genes and activation of various metabolic pathways[63] (Fig. 3). Compared with cell-autonomous hepatic clock, signals provoked by rhythmic food intake were more potent at driving circadian gene expression in mouse liver[64]. In this study, mice were fed with an automatic feeding system according to three patterns: arrhythmic (AR) feeding, night-restricted (NR) feeding, and *ad libitum* (LB) feeding. More than 1000 genes (1454 in total) of the cycling transcriptome in the liver of *ad libitum* fed mice lost rhythmicity in AR fed mice, but core clock genes remained unchanged. Collectively, these observations suggest that arrhythmic feeding does not affect the rhythmicity of core clock genes (*Bmal1*, *Clock*, *Cry1*, and *Per1*), and the rhythmic feeding has a strong effect on the rhythmicity of gene expression in the liver[64].

4. The circadian transcriptome of peripheral clock

The circadian regulatory network exists in almost all organs and cells throughout the body and the autonomous circadian rhythmicity can be maintained independent of SCN[29,47,68–72]. Peripheral clocks were first reported in liver, kidney, heart, pancreas and then in other major organs [68–70]. It is important to note that usually 10–15% of transcribed genes are circadian regulated in one tissue, many of which are specific to the phenotype of that tissue. Although circadian regulatory network exists in every organ, in liver, rhythmic food intake (RFI) has a higher impact on the rhythmic gene expression, which indicates the independence of the cellular autonomic liver clock[64]. Furthermore, in mice with liver-specific deletion of BMAL1, the rhythmic expression of hepatic glucose-related genes is disturbed[73], which may be caused by BMAL1-related chromatin interactions[74]. There is a close connection between circadian rhythm and bone metabolism especially for the metabolic functions of osteoblasts, osteoclasts and chondrocytes. The regulation of circadian clock is important for bone and cartilage homeostasis [75]. Musculoskeletal shows decreased muscle force, high bone mass, arthritis and tendon calcification in mice deficient in various core clock genes [76]. For tendon, time-series microarray studies have shown that

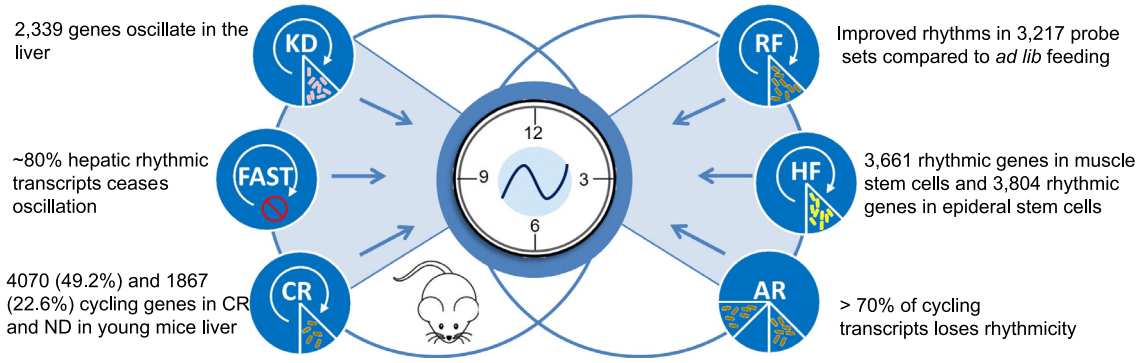

Fig. 3. The transcriptome-wide effects of differential feeding patterns of the circadian clock in mice. The small cubes represent the type of mouse food. Different colors of cubes are used to distinguish foods with different nutrients. Brown cubes: normal food; Yellow cubes: high fat diet; Pink cubes: ketogenic diet. Data source: AR, arrhythmic feeding[64]; CR, calorie-restricted diet[65]; RF, restricted feeding[56]; KD, ketogenic diet [66]; HF, high fat diet[67]; FAST[62]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

745 genes (4.6% of the expressed genes) show rhythmic oscillations[77]. For peripheral clocks, the microenvironment of cell or tissue stiffness impacts on circadian control[78]. Yang et al., identified 594 cycling genes in the breast of mice, and found that the older stroma becomes stiffer, which leads to a reduction in the amplitude of circadian clocks[79]. The disruption of tissue clocks damages tissue homeostasis, resulting in an increased risk of diseases such as metabolic disorder, age-related diseases and cancer [78].

The mechanism of communication between circadian clock genes in the mammalian peripheral tissue and the liver was studied by Koronowski et al.[80] using a tissue-specific clock mouse model in which the liver clock was reconstituted in BMAL1-deficient animals (liver-RE). They found that although the liver oscillated in the absence of other clocks *in vivo*, the circadian expression was reduced to 10% of normal rhythmic transcripts, i.e., 218 oscillating transcripts[80]. Many rhythmic behaviors were reduced or lost when liver-RE mice were placed under constant dark condition. The same phenomenon also occured in the epidermis of RE mice (epidermis-RE)[49], suggesting that the circadian clock in the liver operates independently to other clocks, yet remains dependent on the light–dark cycle, as light synchronizes circadian clocks in the liver without the presence of other Bmal1-dependent clocks. These results are consistent with previous reports that the circadian rhythmicity between the SCN and the liver can be decoupled by feeding[69], and interestingly, a phase shift of 10 h in 2 days can be achieved in the liver under restricted feeding conditions. The circadian rhythms of the liver, the SCN, and other peripheral tissues can be similarly aligned by feeding[69]. These findings are important for exploring the relationship between circadian rhythms in peripheral tissues. High-throughput transcriptomic and metabolomic analyses of mice with lung cancer revealed that a unique set of transcripts and metabolites were cycling exclusively in livers of tumor-bearing mice[81]. Further data shows that lung cancer had no effect on the core clock, but instead that the liver metabolism is reprogrammed by altered inflammatory responses through the STAT3-Socs3 pathway[81]. This process led to disruption of AKT, AMPK, and SREBP signaling, and resulted in changes in insulin, glucose, and lipid metabolism [81]. Collectively, these findings illustrate the complex regulatory connectivity between the liver and the lung, which have potential clinical importance during treatments of hepatic and lung diseases.

5. Large-scale analyses of rhythmically expressed transcripts

The regulatory targets of core circadian clock genes (*Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1* and *Cry2*) are many as each of these transcription factors can bind to tens of thousands of loci [32,61]. With the development of microarray and high-throughput sequencing technology, increasing numbers of rhythmically expressed genes have been detected, often across multiple biological processes.

The design of large-scale circadian experiments is not trivial, as experimental challenges exist in both the animal model generating process and circadian behavior design process. The precision and accuracy of the measurement and the degree of rhythmicity should be determined beforehand and are dependent on the specific experimental aims (for a more detailed discussion, refer to [82]). To ensure sufficient statistical power, the typical recommendations for the experimental design are: 1. Sampling at a 4-hour interval or even more frequently; 2. With at least 2 or 3 biological replicates per each time point or 2 cycles.

More sensitive algorithms are now applied to the identification of rhythmically expressed genes, many of which are high-quality methods to calculate related oscillation parameters, including period, phase, and amplitude[82]. These algorithms include COSOPT [83], JTK_CYCLE[84], ARSER[85], HAYSTACK[86], Lomb-Scargle [87], RAIN[88], CircWave[89], eJTK_CYCLE[90], ABSR[91], fisher's G test, cosine or sinusoidal wave-fitting algorithm, Fourier analysis, and BIO_CYCLE[92]. In Table 1 we show the genome-wide changes detected in different studies along with methods used. Of the most popular algorithms, there are >15 articles that make use of JTK_CYCLE, and many others where COSOPT, ARSER, or a wave-fitting algorithm were used to determine the number of rhythmic genes in transcriptomic datasets (Table 1 and Supplemental Table 1). Each algorithm has advantages and disadvantages. COSOPT is a popular method for fitting gene expression profiles with a series of modified cosine models. MMC-β is used to determine whether there is consistency between the experimental data and the reported model[83]. Lower MMC-β values denote data that is more consistent with the cosine model, in other words, that the gene is likely to be rhythmically expressed. Fisher's G test method is helpful if the dataset has unknown frequency[118]. Alternatively, the JTK_CYCLE algorithm is a powerful option and computationally efficient in calculating rhythmicity. JTK_CYCLE uses the Jonckheere-Terpstra (JT) test, a nonparametric test to reliably identify rhythmically expressed genes, with increased resistance to outliers[84]. Analysis of a standard time period dataset comprised of 48 time points across ~45 k transcripts (3 h (23–25 h) period range) using JTK_CYCLE takes 15~20 min (Intel Core 2 Duo P8800, 2.66 GHz, 4 GB RAM). Conversely, analysis of the same dataset using COSOPT takes several days to complete[84]. ARSER is a frequency-based algorithm that, while effective, requires a large number of test time points. ARSER cannot be applied if there are missing values in the dataset, limiting its applicability[82]. When

Table 1
Genome-wide impact of circadian regulatory networks and the corresponding computational methods used in typical mouse studies.

	Species	Number of rhythmic transcripts/genes	Organ	Condition	Genotype	Computational methods	Ref.
1	Mus musculus	2,960 1,107 1,018 2,302 476 836	Epidermis	12:12 LD 12:12 DD 	WT Reconstituted (RE) *Bmal1* KO WT RE *Bmal1* KO	JTK_CYCLE	[49]
2	Mus musculus	1,061 2,718 1,454	Liver	AR NR LB	WT	F24, Meta Cycle, RAIN, Harmonic Regression	[64]
3	Mus musculus	3,153 (15.3%) 2,146 (10.4%) 830 (4%) 3,384 (16.5%)	Liver Muscle 	LB Fast LB Fast	WT	JTK_CYCLE	[62]
4	Mus musculus	3,144 2,309 2,010 1,363 2,376 3,804 1,979 2,221 2,221 2,507 3,210 3,661	Epidermal stem cells Muscle stem cells 	Adult Aged Aged & ND Aged & CR Adult & ND Adult & HF Adult Aged Aged & ND Aged & CR Adult & ND Adult & HF	WT	JTK_CYCLE	[67]
5	Mus musculus	4,201 3,239 6,404 5,232 2,773 1,246	Liver Skeletal muscle (stem cells) Epidermal (stem cells)	Young & ND Old & ND Young & CR Old &CR Young &ND Young &ND	WT	JTK_CYCLE	[65]
6	Mus musculus	1,520 3,140 1,511 1,300	Liver Gut 	Control chow KD Control chow KD	WT	JTK_CYCLE	[66]
7	Mus musculus	5,457 (14.5%) 1	Liver	12:12 DD	WT *Bmal1* KO	JTK_CYCLE	[39]
8	Mus musculus	2,460 1,330	Liver	12:12 LD	WT Npy KO	MetaCycle	[93]
9	Mus musculus	1,502 1,220	Lung	12:12 LD	WT Lung-tumor-bearing mice	JTK_CYCLE	[81]
10	Mus musculus	328 4,063 810	NIH 3 T3 cells	12:12 LD	Normal	JTK_CYCLE RAIN ARSER	[94]
11	Mus musculus	2,869 (11%) 2,730 (10%)	Liver	12:12 LD	WT Nocturnin KO	JTK_CYCLE, ARSER	[95]
12	Mus musculus	3,905 (27%)	Islet	12:12 LD	WT	eJTK_CYCLE	[40]
13	Mus musculus	4,569 (24%)	SCN	12:12 DD	WT	JTK_CYCLE, DESeq2	[48]
14	Mus musculus	~3,100 (16%) ~25,00 (13%) ~2,300(12%) ~1,500(8%) ~1,100(6%) ~980(5%) ~790 (4%) ~790 (4%) ~790 (4%) ~790 (4%) ~790 (4%) ~620(3%)	Liver Kidney Lung Brown fat Heart Adrenal gland Aorta White fat Skeletal muscle Cerebellum Brainstem Hypothalamus	12:12 LD	WT	JTK_CYCLE	[96]
15	Mus musculus	1,197 1,285 1,794 2,217	Liver	12:12 LD	WT(SIRT6) SIRT6 KO WT(SIRT1) SIRT1 KO	JTK_CYCLE	[97]
16	Mus musculus	1,067	Lung	12:12 LD	WT	CYCLE, Autocorrelation, COSOPT	[98]
17	Mus musculus	684 13,59	Fast tibialis anterior skeletal muscles Slow soleus skeletal muscles	12:12 LD	WT	JTK_CYCLE	[99]

(*continued on next page*)

Table 1 (*continued*)

	Species	Number of rhythmic transcripts/genes	Organ	Condition	Genotype	Computational methods	Ref.
18	Mus musculus	1,261 (11%)	Liver	12:12 LD	WT	JTK_CYCLE	[100]
19	Mus musculus	284	Liver	12:12 LD	WT	A cosinor model	[101]
20	Mus musculus	1,016 (5%) 433(2%)	Telogen skin Anagen skin	12:12 LD	WT	ANOVASine-wave–based methods	[72]
21	Mus musculus	900	Liver	12:12 LD	WT	CircWave v3.3	[37]
22	Mus musculus	963(15.2%)	Liver	12:12 LD	WT	Amplitude, F24	[102]
23	Mus musculus	>3,000	Liver	12:12 DD	WT	COSOPT, Fisher's G-test	[57]
24	Mus musculus	2,997 4,960 368	Liver	LB Daytime-restricted feeding Fast	WT	COSOPT, Fisher G test	[56]
25	Mus musculus	356 504	Kidney (DCT/CNT) Kidney (CCD)	12:12 LD	WT	A cosinor model	[103]
26	Mus musculus	716 (10.5%) 267 (3.4%)	Liver Skeletal muscle	12:12 DD	WT	COSOPT	[35]
27	Mus musculus	274	Pituitary	12:12 DD	WT	COSOPT, Fisher's G-test	[104]
28	Mus musculus	1,606 2,421	Adrenal gland	12:12 DD	WT	COSOPT CircWave	[89]

dealing with low-resolution data, ARSER will have a lower false negative rate, while JTK_CYCLE will report phase less accurately, together with a lower number of cycling genes. However, when high-resolution time data are processed, JTK_CYCLE performs better than ARSER, with lower false positive rates[119].

Many transcriptomic datasets have been published with the development of high-throughput sequencing technology, and additional cycling genes have been identified in each tissue using these datasets[46,102,104]. An early study using microarray identified 650 cycling transcripts in the liver and SCN with only a small number overlapping with each other[46]. Mouse liver is one of the most used tissues for circadian research and 1371 intron and 2037 exon cycling transcripts were reported recently[61]. Oster H et al.[89] performed whole-genome microarray hybridization to identify circadian genes in the mouse adrenal gland, and found that about 5% of the genes are under the circadian control. To study the role of circadian rhythms on the physiology and behavior of mice, Zhang R et al.[96] combined RNA-seq and DNA array technology to obtain quantitative time-dependent transcription data of 12 mouse tissues, including both mRNAs and non-coding RNAs. They found that 43% of protein-coding genes display circadian oscillations in at least one mouse tissue, and that the expression of many rhythmic genes peaked before dawn and dusk. A well-controlled analysis using human skeletal muscle revealed that 5748 pre-mRNA/mRNA transcripts are rhythmically expressed[106]. Most of the rhythmic genes identified in multiple organs were found in mammals with nocturnal habits, and there are no time series expression datasets from different tissues or brain regions of humans or species that are closely related to humans. Based on this, Mure et al. [114] performed genome sequencing on 64 tissue samples from baboons, including 22 brain regions. They found that most protein-coding genes in baboons have rhythmic expression[114].

6. The design principles of the circadian network output

The increase in the number of known rhythmic genes in different species has contributed to our overall understanding of the characteristics of rhythmic genes, which supports deeper understanding of the design principles of a circadian clock system. So far, four important design principles have been revealed: (**a**) Several studies have indicated that the distribution of cycling genes is tissue-specific[35,46,114], with only a small overlap between tissues. The recently released baboon circadian transcriptome shows that less than 1% (<10) of rhythmic genes were shared among tissues [114]. Comparison of cycling genes in the mouse SCN and liver revealed that a total of 28 genes were shared between these two tissues[46]. (**b**) There is a strong positive correlation between expression level and the amplitude of rhythmically expressed genes across different tissues in mice (E-A correlation). Rhythmic gene expression can explain most of the variation observed in the amplitude of circadian genes, suggesting that it is a major factor in the regulation of rhythmic gene behavior[31]. (**c**) The energetic cost of expressing a cycling gene is significantly higher than that of a non-cycling gene in mice, fruit flies, and yeast[120], as the former has significantly higher expression levels than the latter. (**d**) In simulation experiments, switching between the sequence of cycling and noncycling genes led to an increased overall cost of expression for the whole transcriptome[120]. Moreover, the overall energy cost of expressing the whole transcriptome is increased if all the genes are shuffled in the genome. In summary, the higher the expression level of the cycling genes, the more energy is consumed during the transcription process. As a consequence transcriptional systems tend to downregulate these highly expressed genes when their function is not needed, and rhythmically regulating highly expressed genes can effectively reduce the energy cost of transcription[31,120]. This strategy is part of the overall design requirement for reducing the energetic cost of expressing the whole transcriptome. In addition, the circadian regulatory system tends to regulate paralogous copies that consume more energy through evolution[120].

7. Circadian disruption and disease

Disrupted circadian rhythms can result in many diseases and affect downstream gene expression. The factors that cause circadian rhythm disorders can be roughly divided into three types: gene-level factors, such as mutations in clock genes, physiological factors, such as obesity and aging, and environmental factors, such as irregular work schedules, jet lag, and shift works.

Mutations or deletions of core clock genes directly impact the circadian system, leading to circadian disorders. *Bmal1* gene knockout or conditional knockout mice lose behavioral rhythms, such as

wheel-running activity, and have diabetes and tendon calcification [38,121–123].RNA-seq analysis showed that the circadian expression of 5457 rhythmic genes in the liver of adult *Bmal1* knockout mice was lost [39] and *Bmal1* mRNA expression in the *Bmal1* knockout mice decreased by 80% compared with wild-type mice at Zeitgeber time 0 (ZT0) [39].

A strong link between obesity and circadian rhythm disorders has been widely reported, and both of feeding and fasting behavior can change the number of cycling genes in peripheral tissues. Studies have found that a high fat diet can alter the circadian rhythm in mice [124]. In mouse muscle stem cells 3210 cycling genes were detected in those fed with normal diet, of which 2283 genes lost rhythmicity when fed with high fat diet[125]. In contrast to the high fat diet, periodic fasting has many benefits. For example, fasting has the potential to reduce obesity, lower the risk of cancer, and delay aging[126,127]. In addition, fasting can promote great changes in ketogenesis, lipolysis and gluconeogenesis which results in a variety of biological responses, including improved cellular stress resistance and insulin sensitivity-[126,128]. Moreover, lower levels of oxidative stress and inflammation in the body and brain were observed in response to fasting[126]. In wild type mice, after fasting, 2353 rhythmically expressed hepatic genes lost rhythmicity, while 1528 genes gained rhythmicity[129].

The decline of circadian clock function and aging may interact with each other, resulting in the circadian expression of many genes changed. In the brain, 2475 cycling genes (~12% of the genome) in Brodmann's area 11 (BA11) and 1615 cycling genes (~8% of the genome) in Brodmann's area 47 (BA47) were identified in the human prefrontal cortex [107]. Aging may lead to a shift in the phase of molecular rhythm to a "morning" chronotype. In aging mice 1186 genes in BA11 and 1591 genes in BA47 were found to show changes in rhythmicity[107]. Moreover, the amplitudes of *PER1* rhythms were reduced and the acrophases had a shift from ZT5 (BA11) and ZT7 (BA47) to ZT1–ZT2 in the two brain regions [107]. Strong rhythmic expression was observed in 3144 transcripts of adult epidermal stem cells and 1979 transcripts of adult muscle stem cells, of which 76% and 72%, respectively, no longer oscillated in aged mice[125].

Sleep disorders is a type of circadian disorder. Shift work can lead to the lack of sleep which results in disorders in circadian rhythm. Genome-wide gene expression analysis showed that the number of significant rhythmic transcripts for night shifts was significantly reduced compared with the baseline [105]. Night shift caused a reduction of rhythmic probe sets from 444 at baseline to 62 probe sets [105]. The model selection approach showed that 3.0% of the human transcriptome is differentially regulated during night shifts[105]. Transcriptomic analysis revealed that lack of sleep also reduced the number of genes with circadian expression from 1,855 to 1,481, and the circadian amplitude of these genes also reduced [130]. Therefore, the regulation of the circadian rhythm of patients with these disorders may play a positive role in their overall treatment.

8. Summary and outlook

The circadian clock either directly or indirectly affects many behaviors. In animals, the circadian clock regulates the sleep-wake cycle and feeding behavior, and has a far-reaching influence on metabolic processes, which in turn affect growth and development.

The biological circadian system is an adaptive behavior to respond to the environment, and this regulation exists in all layers (gene, cell, tissue, organ, behavior, etc.). Many genetic factors can be affected through the regulation of the circadian network, including mRNA, noncoding RNAs, chromatin structures, and epigenetic regulatory factors[61,94,101,113,131,132]. The relationship between these elements and rhythmically expressed genes is not yet entirely clear. Further research is required to expand our understanding of these connections.

Biological rhythmic regulatory systems are involved in regulating metabolic processes, development, aging, and sleep [65,107–109,133–135]. Mutations in circadian genes are associated with diseases including major depressive disorder and cancer [110,136,137]. Accordingly, studies of circadian genes can help to understand the association between disorders of biological rhythms and complex diseases, especially sleep related disorders (Fig. 4). Molecular and cytological studies of biological rhythms can identify individual-specific disease characteristics that open up new opportunities for precision medicine[139–141], and provide customized methods to predict disease risk and prognosis to facilitate early and effective intervention[142]. This has facilitated chronotherapy that combines time biology with clinical treatment through drug administration according to circadian rhythms,

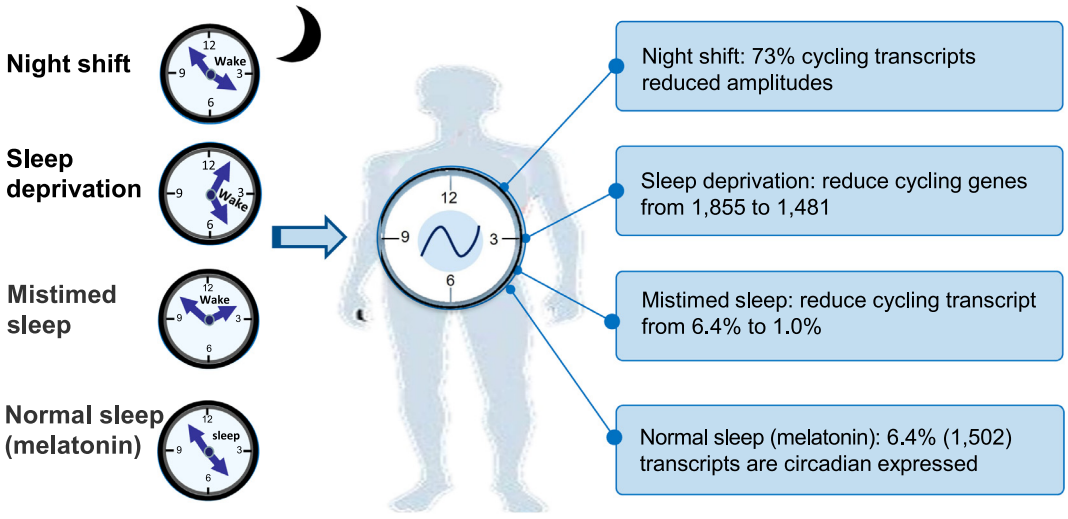

Fig. 4. The effects of four different sleep-wake cycles on the circadian transcriptome of human blood. Shift work or mistimed sleep or sleep loss can lead to transcriptome-wide circadian changes. The clock (left) represents the sleep or wake condition and right boxes represents changes in each condition. Data source: Night shift [105]; Sleep deprivation[109]; Mistimed sleep [138]; Normal sleep (melatonin)[138]

improving drug efficacy or reducing side effects [139–141]. A considerable number of rhythmic genes are targets for drug action [96]. Therefore, studies of circadian regulation are highly significant for drug development and disease treatment.

In summary, the circadian clock affects the expression of many different genes that are involved in various processes. Rhythmic genes are usually expressed at high levels, and are often involved in the metabolism of glucose, carbon, lipids, etc. The transcription system rhythmically regulates the highly expressed genes according to the needs of each biological process [120]. If these high-expression genes are downregulated when they are not in need, the overall energy cost of transcription and translation can be reduced. This strategy allows the energy distribution within the genetic system to be optimized, and homeostasis to be maintained. This review of biological rhythms promotes the understanding of the molecular mechanisms of circadian regulation at the system's level and highlights the importance of studying the circadian clock in systems biology as a model system.

Author statement

GZW conceptualized the manuscript. LS drafted the manuscript. JM curated the data. GZW and PX supervised the manuscript; LS, GZW, PX and CWT reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 81827901, 31600960 and No. 31871333) and the National Key R&D Program of China (2016YFC0901700 and 2016YFC1303100). We appreciate Luoying Zhang and Hung-Chun Chang for helpful feedback on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2020.07.002.

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