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Genetics of Circadian Rhythms

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SYNOPSIS

Nearly all organisms exhibit time dependent behavior and physiology across a 24 hour day known as circadian rhythms. These outputs are manifestations of endogenous cyclic gene expression patterns driven by the activity of a core transcription/translation feedback loop (TTFL). The TTFL is highly conserved across species and present in almost all mammalian cell types. This mechanism consists of a forward arm that drives gene expression and a negative arm that feeds back and inhibits the activity of the forward arm. Cyclic gene expression determines highly tissue specific functional activity regulating such processes as metabolic state, endocrine activity and neural excitability. Entrainment of these cellular clocks can be achieved through exogenous daily inputs such as light and food. Dysregulation of the TTFL has been shown to result in a wide range of disorders and diseases driving increased interest in circadian therapies.

Keywords

Circadian; Genetics; Entrainment; Clock

Introduction

Almost all organisms organize their physiology, behavior and metabolism according to the 24-hour solar cycle. Time dependent changes in these parameters have evolved in order to allow plants and animals alike to maximize their fitness according to external cues. Internal “clocks” evoke a set of anticipatory responses to changes in their environment, which we refer to as circadian rhythms.

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DISCLOSURE STATEMENT

The authors have nothing to disclose

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Circadian rhythms have four unique properties. First, these rhythms closely mirror the 24-hour solar day, hence the word “circadian”, which comes from the Latin words “circa” (close or about) and “dian” (day). The second principle dictates that even if there are no exogenous cues present, periodical patterns are still shown (1) indicating that these rhythms result from an internal time-keeping system. Thirdly, although circadian activity rhythms are derived from an endogenous clock, they can adjust to the exogenous signals such as light or heat. Lastly, the periodicity of rhythms is stable across a wide range of temperatures, a property referred to as “temperature compensation” (2). For humans, the most prominent circadian rhythm is the 24 h rhythm in the sleep-wake cycle.

Four widely divergent model systems that have been historically used to study the genetics underlying circadian rhythms: fruit flies (*Drosophila*), fungi (*Neurospora*), cyanobacteria and mouse (3). The discoveries found using these models have facilitated the understanding of the mechanism behind circadian rhythms and their significance to biology and disease. Remarkably, animal clocks are well conserved from insects to mammals, revealing an important role in basic animal models to understand the mechanistic basis of human circadian rhythms.

The Molecular Clock

Phasic expression of genes drives the physiological and behavioral manifestations of circadian rhythms at the organismal level. Nearly half of all protein coding genes show circadian dependent transcription in at least one tissue in mammals (4). While it is known that the specific genes that cycle are variable cross species as well as tissue dependent (5, 6), the mechanism that drives their phasic expression is consistent throughout the body and well conserved between species (7, 8).

Rhythmic expression of genes is accomplished through a cell intrinsic transcriptional/translational feedback loop (TTFL). The forward portion of the loop consists of a transcription factor(s) whose activity promotes the expression of genes that are the “negative” elements of the loop. Over the course of a day, levels of the negative arm increase until they are capable of translocating into the nucleus and repressing the activity of the positive arm. Once they are degraded, the positive arm is freed to restart the cycle. The activity of this loop creates a self-perpetuating oscillating pattern of gene expression with a near 24 hour period. Here we will present the mechanistic details of the fruit fly clock as it is where the clock mechanism was first discovered, is highly conserved with the human clock and which led to the discovery of the first genes involved in human circadian rhythms.

The *Drosophila* Molecular Clock

In *Drosophila* the forward transcription loop consists of master transcription factors CLOCK (dCLK) and CYCLE (dCYC) which heterodimerize and initiate transcription (9, 10). This is accomplished through binding to the E-boxes (CACGTG) at target promoters and activating them (7) (Fig. 1). The negative loop consists of *period* (*per*) (11) and *timeless* (*tim*) (12), which dimerize and accumulate slowly in the cytoplasm. In the morning, light causes TIM degradation and without TIM, PER is less stable and both are degraded by a proteasome dependent pathway (13). During the evening however PER/TIM accumulate and translocate

into nucleus (7, 14, 15). Once in the nucleus PER-TIM dimer binds to CLK-CYC, and inhibits their transcriptional activity (15) (Fig. 1). This simple negative feedback loop serves as the core principle and primary feedback loop of a 24 h biological clock.

In addition to this canonical loop, there are secondary loops that interact with and modulate clock activity. CLOCKWORK ORANGE (CWO) binds directly to E boxes, preventing CLK/CYC enhanced gene expression (7). PAR-DOMAIN PROTEIN 1 ϵ (PDP1 ϵ) and VRILLE (VRI) are two proteins that also act to regulate the forward loop (16). These circadian proteins feedback to act on the *Clk* promoter with PDP1 ϵ serving as an activator and VRI as an inhibitor (Fig. 1). Lastly the transcription factor Ecdysone-induced protein 75 (E75) has been found to repress *Clk* expression (17) and time-dependently enhance PER transcription (18). These additional feedback loops support the function of the primary feedback loop.

The Mammalian Molecular Clock

Mammalian and *Drosophila* TTFLs are very similar (6). In mouse and human the forward loop consists of CLOCK (homolog of dCLK) and BMAL1 (homolog of dCYC), which also form a heterodimeric transcriptional activator (Fig. 1). PERIOD and CRYPTOCHROME (CRY) homologs of which there are 3 and 2 respectively are functionally homologous to PER and TIM in flies respectively and serve as the negative loop (Fig. 1). *Drosophila* also uses a CRY that regulates transcription but also acts as a photosensor.

While primary transcriptional and translational mechanisms for the molecular clocks are largely conserved, the paralogs add a layer of complexity. In *Drosophila* PER acts as the primary negative regulator, however in mouse mPER1, mPER2 and mPER3 each play independent roles in the maintenance of molecular clocks (19). An example of this is that mPER3, has a negligible role in circadian rhythms in the brain (19), but plays a role in the regulation of circadian rhythms in peripheral tissues (20). CRY paralogs show similar complexity with double knockout mutant mice for both mCRY1 and mCRY2 displaying an arrhythmic phenotype while a loss of function for one or the other resulting in either shortened or lengthened period by 1 hour, respectively (21).

Secondary loops are present in mammals as well however with added complexity. The mammalian equivalent to the *Drosophila* PDP1 ϵ /VRI regulatory mechanism, consists of receptors REV-ERB and RETINOIC ACID RELATED ORPHAN RECEPTOR (ROR) which regulate CLK's heteromeric binding partner, BMAL1 instead of CLK itself (22) (Fig. 1). D-ELEMENT BINDING PROTEIN (DBP) and E4 BINDING PROTEIN 4 (E4BP4) each bind to the promoter sequence (D-Box) of *mPer* and alter its expression pattern (23). Lastly CLK-BMAL1 also facilitates expression of Deleted in esophageal cancer1,2 (*Dec1* *Dec2*), the structural and functional homologs of CWO in flies. Similar to CWO, mammalian DEC1 and DEC2 also repress E-box targets including mPER and their own site thus forming an interlocked feedback loop (24) (Fig. 1).

Posttranslational Modifications

Components of the TTFL are posttranslationally modified in a rhythmic manner to time their activity, subcellular localization, and/or stability. The major conserved mechanism of posttranslational modification is phosphorylation of the clock proteins (13). (Table 1)

In both flies and mammals a key protein kinase is DOUBLETIME (DBT)/CASEIN KINASE I delta and epsilon (CK1 δ/ϵ). In *Drosophila*, DBT binds to and phosphorylates PER, regulating its subcellular localization and signaling its rapid degradation by the proteasome (25, 26). The mammalian orthologs of DBT are CK1 δ/ϵ , which mediate phosphorylation and degradation of mPER2 as well as facilitating its entry into the nucleus (27). Thus, the CKI/PER relationship is conserved from flies to mammals. Even more remarkable is that mutations in CK1 δ in humans cause Familial advanced sleep phase disorder (FASPD) (28). Another critical kinase for PER activity is CASEIN KINASE 2 (CK2), whose kinase activity promotes PER nuclear localization in both mammalian and *Drosophila* cells (29, 30).

Phosphatases have been shown to modulate the clock as well. PROTEIN PHOSPHATASE 1 (PP1) dephosphorylates TIM, stabilizing it in the cytoplasm (31). PP1 also acts on the phosphorylation state of PER itself (32). In mammals however, PP1 dephosphorylates PER2 stabilizing it for reentry into the nucleus (33). The other major phosphatase involved in circadian rhythm modulation is PROTEIN PHOSPHATASE 2A (PP2A) (34). While PP1's primary function is stabilizing TIM and the PER/TIM complex, PP2A activity in *Drosophila* is involved in facilitating nuclear transport of PER/TIM through direct dephosphorylation of PER(34).

Molecular Clocks Drive Physiological Output in Multicellular Organisms

Neural Circadian Networks

In multicellular organisms the circadian activity of master pacemakers in the brain govern the daily rhythms of sleep and wake. The discovery of the molecular clock and relevant circuitry has allowed for the exploration of this phenomenon in mammals and *Drosophila* (6–8, 35). In mammals a hypothalamic structure known as the suprachiasmatic nucleus (SCN) acts as the master timekeeper. The SCN is thought to entrain various parts of the body through its output to certain regions of the hypothalamus which in-turn signal the rest of the body and brain (35, 36). *Drosophila* behavioral rhythms are generated by the activity of ~150 interconnected pacemaker neurons in each brain hemisphere (37). Independent groups within this network communicate with one another to integrate external cues and determine phase and period. Even in the highly divergent brains of insects and mammals, these clock neural networks consist of coupled oscillators (37, 38).

These master pacemakers drive behavioral rhythmicity via the rhythms of neuronal activity. Neuronal activity within the nuclei, in turn, is driven in part by the clock control of neuronal resting membrane potential. The resting membrane potential of clock neurons in both *Drosophila* and mouse, increases and decreases in a diurnal manner (39, 40). More positive resting membrane potential increases excitability and firing rates while more negative potentials have the opposite effect.

Peripheral Tissues

In addition to the master circadian pacemakers in the brain, clocks are found in virtually every tissue and organ in both flies and mammals. Clock genes are shown to be cycling in most nucleated mammalian cells except for the thymus and testis providing cyclic gene expression throughout the body (35, 41).

Some of the best-studied clocks in peripheral tissues are those in the pancreas and the liver due to their association with glucose homeostasis and metabolism. Mice with a pancreas-specific genetic inactivation of the essential clock component BMAL1 knockout show elevated glucose levels, impaired glucose tolerance and insulin secretion, suggesting a link between peripheral clock dysfunction and diabetes (42). Similar tissue specific knockout experiments in the liver showed the abolishment of the majority of clock controlled gene expression despite continued input from the master circadian clock in the SCN (43). These experiments and others demonstrate the significance of independent timing systems within the periphery whose regulation is critical for tissue function.

Entrainment of the Clock

In order to synchronize the clock to the 24 h environment, organisms use sensory cues that we refer to as zeitgebers (German for “time giver” or “synchronizer”). Light and food are two well studied zeitgebers that feedback to clock components to regulate their activity.

Light Entrainment

It is well known that most mammals receive visual input via retinal rod and cone photoreceptors, which send output to the retinal ganglion cells (RGC) that project into the brain. What is less well known and surprising is that rods and cones are not necessary for circadian entrainment. Mutant mice lacking rods and cones are still capable of entrainment to light-dark cycles (44). While most RGCs are incapable of directly perceiving light, a subset of them express the photopigment melanopsin, which responds to light independently of rod/cone system (45). These intrinsically photosensitive RGCs send primarily glutamatergic and pituitary adenylate cyclase activating peptide (PACAP) outputs to the SCN (46). Of note, flies also use non-visual photoreceptors (47). However, in an apparent point of divergence from mammals, flies also use CRY as a photoreceptor to synchronize both brain circadian pacemakers and peripheral tissue clocks.

Glutamate mediated effects on the clock genes within the SCN are well understood. Blockage of the glutamate receptor NMDA, using an NMDA antagonist block has been shown to block light induced phase shifts (48). Glutamate induced calcium influx through NMDA and voltage gated calcium channels leads to activation of the transcription factor cAMP-RESPONSE ELEMENT BINDING PROTEIN (CREB) (49) (Fig. 2). *mPer1* and *mPer2* contain CRE in their promoters and alter their expression in response to photic stimulation (50).

Food

Food quality/dietary composition and timing can directly entrain or influence molecular clocks specifically in peripheral tissues. (Table 2) Time-restricted feeding (TRF), where feeding is restricted to a particular time of day, causes peripheral clocks, such as those in liver, to be entrained without altering SCN clocks (51). High fat diet (HFD) can also reprogram peripheral circadian clocks in mice (52). Interestingly this mechanism appears to affect behavioral rhythms without affecting core clock genes in brain (53). HFD mediated circadian reprogramming is facilitated by nutrient-dependent chromatin remodeling. HFD impairs chromatin recruitment of CLK-BMAL1 to their target genes while at the same time it recruits supplementary transcription factors such as Peroxisome proliferator-activated receptor γ (PPAR γ) and Sterol regulatory element-binding protein 1 (SREBP1) to their target gene promoters (52). PPAR γ can directly repress transcription of CLK-BMAL1 (54), suppressing the endogenous clock while simultaneously transcribing a series of other non-clock controlled genes.

The molecular clock is also directly influenced by nutrient-sensing/sensitive pathways. An example of this is the regulation of clock by nutrient responsive AMP-activated protein kinase (AMPK). Administration of AMPK activators such as AICAR and metformin can reset the clock in mouse liver and activated AMPK mediates the degradation of CRY1 (55, 56). Another example of this is the NAD⁺-SIRT1-CLOCK feedback loop. CLK-BMAL1 facilitates circadian expression of Nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme for NAD⁺ salvage pathways. NAD⁺ also activates the deacetylase Sirtuin 1 (SIRT1). This enzyme influences clock rhythms through interacting directly with PER2 and BMAL1, destabilizing PER2 and decreasing the activity of the negative arm of the TTFL (36, 57).

Dysregulation of Clock and Disease States

Endogenous molecular clocks can become out of phase with the environment or the rhythms of other tissues within the body. Clocks acting out of phase or desynchronized may exacerbate a wide range of disorders and diseases (Fig. 3).

Sleep

Circadian rhythms and sleep are inextricably linked. Sleep is a vital process linked to a myriad of processes including metabolism and synaptic plasticity (58, 59). PER mutations in both mouse and human show that the circadian clock directly regulates the timing of sleep onset (60). Mutation of various clock genes also inherently leads to abnormal sleep patterning and properties (61, 62). FASPD, a disorder in which individuals display ~4 hours earlier sleep and awaking times, is directly associated with *Per* mutations. Specifically PER phospho-site mutations (63, 64) as well as mutations of CKI produce FASPD in humans (28). Clock genes have also been implicated in regulating sleep duration. Mutations to DEC2 in both humans and mice dice show increased wake and reduced rapid and non rapid eye movement sleep states (65).

Sleep timing and duration also have profound effects on the circadian clock. Insufficient sleep (sleep restriction) decreases the number of rhythmic genes in the human blood

transcriptome compared to a control group with sufficient sleep (66). Furthermore sleeping out of phase with the clock resulted in a 6-fold reduction in the rhythmic transcripts in blood, not only because it results in lower amplitude of gene cycling, but also there is suppression or enhancement of certain genes that manifest during sleep (67). The application of genomic approaches could yield new diagnostic tests for circadian or sleep-based disorders.

Metabolism

As mentioned earlier, metabolic processes interact with both central and peripheral clocks. One area in which this has been explored extensively is in the context of metabolic diseases, specifically diabetes. *Clk* mutant mice tended to be hyperglycemic and have increased susceptibility to diet induced obesity (68). Pancreatic clock activity is directly involved in insulin release and beta cell health (42). Work in other peripheral tissues such as liver (69), and adipose tissue (70) have furthered the idea that disruptions of CLK function may serve as a major culprit in metabolic syndrome.

In humans, CLK polymorphisms have been directly associated with obesity and metabolic syndrome (71). Furthermore type 2 diabetic patients showed down-regulation of *hPer2*, *hPer3*, and *hCry2* in their pancreatic islets relative to healthy patients (72). Individuals who undergo shift work show increased rates of type 2 diabetes (73), implicating disruption of circadian rhythms in the dramatic global increase of metabolic syndrome.

Cancer

Clock is involved in regulating a wide range of processes that are associated with cancer development including expression of numerous genes involved in DNA repair, cellular proliferation and even tumorigenesis (74). Furthermore clock genes have been shown to be involved in the generation and progression of tumors. Ectopic expression of PER in human cancer cell lines results in growth inhibition, cell cycle arrest and even apoptosis (75), while targeted inhibition of PER on the other hand has been shown to cause the induction of malignant lymphomas in mouse (76).

Ovarian cancer cells show circadian dependent S phase induction that is out of sync with that of the other human tissues (77). *hPer1* shows lower expression levels in familial forms of breast cancer compared to sporadic forms (78) and dysregulation of the molecular clock has been directly associated with the progression of these cancers (79). Disruption of the clock at the organismal level through has been directly associated with increased risk of cancer in shift workers (80). It is thought that disruption and dissociation of the peripheral and central clock mechanisms results in the unregulated cellular growth and activity characteristic to cancer. (Figure 3)

Immune

Beyond their role in preventing disease states, clock may also be vital for combating them. Both quantity of immune cells and their gene expression are rhythmic (81, 82). Furthermore mice display time-of-day dependent responses to bacterial endotoxins and exotoxins, and

pro-inflammatory cytokines (83, 84). Clock disruption studies have shown that T-cell antigen response is partially mediated by clock (84).

Treatments

A wide range of treatments has emerged regarding circadian rhythms in humans. Pharmacological methods center around melatonin, a rhythmically circulating hormone known to be important in the regulation of sleep and circadian rhythms. Melatonin directly affect the clock phase through activation of Melatonin type 2 receptors (85) and is prescribed for numerous sleep disorders (86). One of the more interesting directions circadian treatment is trending is known as “chronotherapeutics”. Treatments such as those related to cancer are applied in with the timing dictated by the molecular clock, allowing therapeutic actions to be maximized according with the transcriptional state of the target (87, 88).

References

1. Pittendrigh CS. Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb Symp Quant Biol.* 1960; 25:159–84. [PubMed: 13736116]
2. Eckardt NA. Temperature compensation of the circadian clock: a role for the morning loop. *Plant Cell.* 2010 Nov.22(11):3506. [PubMed: 21098732]
3. Rosbash M. The implications of multiple circadian clock origins. *PLoS Biol.* 2009 Mar 17.7(3):e62. [PubMed: 19296723]
4. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci U S A.* 2014 Nov 11; 111(45):16219–24. [PubMed: 25349387]
5. Ko CH, Takahashi JS. Molecular components of the mammalian circadian clock. *Hum Mol Genet.* 2006 Oct 15; 15(Spec No 2):R271–7. [PubMed: 16987893]
6. Panda S, Hogenesch JB, Kay SA. Circadian rhythms from flies to human. *Nature.* 2002 May 16; 417(6886):329–35. [PubMed: 12015613]
7. Allada R, Chung BY. Circadian organization of behavior and physiology in *Drosophila*. *Annu Rev Physiol.* 2010; 72:605–24. [PubMed: 20148690]
8. Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. *Annu Rev Physiol.* 2001; 63:647–76. [PubMed: 11181971]
9. Allada R, White NE, So WV, Hall JC, Rosbash M. A mutant *Drosophila* homolog of mammalian Clock disrupts circadian rhythms and transcription of period and timeless. *Cell.* 1998 May 29; 93(5):791–804. [PubMed: 9630223]
10. Rutla JE, Suri V, Le M, So WV, Rosbash M, Hall JC. CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of *Drosophila* period and timeless. *Cell.* 1998 May 29; 93(5):805–14. [PubMed: 9630224]
11. Konopka RJ, Benzer S. Clock mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci U S A.* 1971 Sep; 68(9):2112–6. [PubMed: 5002428]
12. Vossell LB, Price JL, Sehgal A, Saez L, Young MW. Block in nuclear localization of period protein by a second clock mutation, timeless. *Science.* 1994 Mar 18; 263(5153):1606–9. [PubMed: 8128247]
13. Bae K, Edery I. Regulating a circadian clock's period, phase and amplitude by phosphorylation: insights from *Drosophila*. *J Biochem.* 2006 Nov; 140(5):609–17. [PubMed: 17012288]
14. Curtin KD, Huang ZJ, Rosbash M. Temporally regulated nuclear entry of the *Drosophila* period protein contributes to the circadian clock. *Neuron.* 1995 Feb; 14(2):365–72. [PubMed: 7857645]
15. Hardin PE. Molecular genetic analysis of circadian timekeeping in *Drosophila*. *Adv Genet.* 2011; 74:141–73. [PubMed: 21924977]

16. Cyran SA, Buchsbaum AM, Reddy KL, et al. vril, Pdp1, and dClock form a second feedback loop in the Drosophila circadian clock. *Cell*. 2003 Feb 7; 112(3):329–41. [PubMed: 12581523]
17. Kumar S, Chen D, Jang C, Nall A, Zheng X, Sehgal A. An ecdysone-responsive nuclear receptor regulates circadian rhythms in Drosophila. *Nat Commun*. 2014; 5:5697. [PubMed: 25511299]
18. Jaumouille E, Machado Almeida P, Stahli P, Koch R, Nagoshi E. Transcriptional Regulation via Nuclear Receptor Crosstalk Required for the Drosophila Circadian Clock. *Curr Biol*. 2015 Jun 1; 25(11):1502–8. [PubMed: 26004759]
19. Bae K, Jin X, Maywood ES, Hastings MH, Reppert SM, Weaver DR. Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. *Neuron*. 2001; 30(2):525–36. [PubMed: 11395012]
20. Pendergast JS, Oda GA, Niswender KD, Yamazaki S. Period determination in the food-entrainable and methamphetamine-sensitive circadian oscillator(s). *Proc Natl Acad Sci U S A*. 2012 Aug 28; 109(35):14218–23. [PubMed: 22891330]
21. van der Horst GT, Muijtjens M, Kobayashi K, et al. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature*. 1999; 398(6728):627–30. [PubMed: 10217146]
22. Lowrey PL, Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Annual review of genomics and human genetics*. 2004; 5:407–41.
23. Cho C-H. Molecular mechanism of circadian rhythmicity of seizures in temporal lobe epilepsy. *Frontiers in cellular neuroscience*. 2012; 6:55. [PubMed: 23189039]
24. Kato Y, Kawamoto T, Fujimoto K, Noshiro M. DEC1/STRA13/SHARP2 and DEC2/SHARP1 coordinate physiological processes, including circadian rhythms in response to environmental stimuli. *Curr Top Dev Biol*. 2014; 110:339–72. [PubMed: 25248482]
25. Cyran SA, Yiannoulos G, Buchsbaum AM, Saez L, Young MW, Blau J. The double-time protein kinase regulates the subcellular localization of the Drosophila clock protein period. *J Neurosci*. 2005 Jun 1; 25(22):5430–7. [PubMed: 15930393]
26. Price JL, Blau J, Rothenfluh A, Abodeely M, Kloss B, Young MW. double-time is a novel Drosophila clock gene that regulates PERIOD protein accumulation. *Cell*. 1998 Jul 10; 94(1):83–95. [PubMed: 9674430]
27. Akashi M, Tsuchiya Y, Yoshino T, Nishida E. Control of intracellular dynamics of mammalian period proteins by casein kinase I epsilon (CKIepsilon) and CKIdelta in cultured cells. *Mol Cell Biol*. 2002 Mar; 22(6):1693–703. [PubMed: 11865049]
28. Xu Y, Padiath QS, Shapiro RE, et al. Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. *Nature*. 2005 Mar 31; 434(7033):640–4. [PubMed: 15800623]
29. Lin JM, Schroeder A, Allada R. In vivo circadian function of casein kinase 2 phosphorylation sites in Drosophila PERIOD. *J Neurosci*. 2005 Nov 30; 25(48):11175–83. [PubMed: 16319317]
30. Tsuchiya Y, Akashi M, Matsuda M, et al. Involvement of the protein kinase CK2 in the regulation of mammalian circadian rhythms. *Sci Signal*. 2009; 2(73):ra26. [PubMed: 19491384]
31. Fang Y, Sathyanarayanan S, Sehgal A. Post-translational regulation of the Drosophila circadian clock requires protein phosphatase 1 (PP1). *Genes Dev*. 2007 Jun 15; 21(12):1506–18. [PubMed: 17575052]
32. Garbe DS, Fang Y, Zheng X, et al. Cooperative interaction between phosphorylation sites on PERIOD maintains circadian period in Drosophila. *PLoS Genet*. 2013; 9(9):e1003749. [PubMed: 24086144]
33. Schmutz I, Wendt S, Schnell A, Kramer A, Mansuy IM, Albrecht U. Protein phosphatase 1 (PP1) is a post-translational regulator of the mammalian circadian clock. *PLoS One*. 2011; 6(6):e21325. [PubMed: 21712997]
34. Sathyanarayanan S, Zheng X, Xiao R, Sehgal A. Posttranslational regulation of Drosophila PERIOD protein by protein phosphatase 2A. *Cell*. 2004 Feb 20; 116(4):603–15. [PubMed: 14980226]
35. Hastings MH, Reddy AB, Garabette M, et al. Expression of clock gene products in the suprachiasmatic nucleus in relation to circadian behaviour. *Novartis Found Symp*. 2003; 253:203–17. discussion 102–9, 218–22, 81–4. [PubMed: 14712923]

36. Froy O. Metabolism and circadian rhythms--implications for obesity. *Endocr Rev.* 2010 Feb; 31(1):1–24. [PubMed: 19854863]
37. Nitabach MN, Taghert PH. Organization of the *Drosophila* circadian control circuit. *Curr Biol.* 2008 Jan 22; 18(2):R84–93. [PubMed: 18211849]
38. Pittendrigh CS, Daan S. Functional-Analysis of Circadian Pacemakers in Nocturnal Rodents .5. Pacemaker Structure - Clock for All Seasons. *Journal of Comparative Physiology.* 1976; 106(3): 333–55.
39. Colwell CS. Linking neural activity and molecular oscillations in the SCN. *Nat Rev Neurosci.* 2011 Oct; 12(10):553–69. [PubMed: 21886186]
40. Cao G, Nitabach MN. Circadian control of membrane excitability in *Drosophila melanogaster* lateral ventral clock neurons. *J Neurosci.* 2008 Jun 18; 28(25):6493–501. [PubMed: 18562620]
41. Alvarez JD, Sehgal A. The thymus is similar to the testis in its pattern of circadian clock gene expression. *Journal of biological rhythms.* 2005 Apr; 20(2):111–21. [PubMed: 15834108]
42. Marcheva B, Ramsey KM, Buhr ED, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature.* 2010 Jul 29; 466(7306):627–31. [PubMed: 20562852]
43. Kornmann B, Schaad O, Reinke H, Saini C, Schibler U. Regulation of circadian gene expression in liver by systemic signals and hepatocyte oscillators. *Cold Spring Harb Symp Quant Biol.* 2007; 72:319–30. [PubMed: 18419289]
44. Freedman MS, Lucas RJ, Soni B, et al. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science.* 1999 Apr 16; 284(5413):502–4. [PubMed: 10205061]
45. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* 2002 Feb 8; 295(5557):1070–3. [PubMed: 11834835]
46. Hannibal J. Neurotransmitters of the retino-hypothalamic tract. *Cell Tissue Res.* 2002 Jul; 309(1): 73–88. [PubMed: 12111538]
47. Veleri S, Rieger D, Helfrich-Forster C, Stanewsky R. Hofbauer-Buchner eyelet affects circadian photosensitivity and coordinates TIM and PER expression in *Drosophila* clock neurons. *Journal of biological rhythms.* 2007 Feb; 22(1):29–42. [PubMed: 17229923]
48. Moriya T, Horikawa K, Akiyama M, Shibata S. Correlative association between N-methyl-D-aspartate receptor-mediated expression of period genes in the suprachiasmatic nucleus and phase shifts in behavior with photic entrainment of clock in hamsters. *Mol Pharmacol.* 2000 Dec; 58(6): 1554–62. [PubMed: 11093796]
49. Tischkau SA, Mitchell JW, Tyan SH, Buchanan GF, Gillette MU. Ca²⁺/cAMP response element-binding protein (CREB)-dependent activation of Per1 is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. *J Biol Chem.* 2003 Jan 10; 278(2):718–23. [PubMed: 12409294]
50. Travnickova-Bendova Z, Cermakian N, Reppert SM, Sassone-Corsi P. Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. *Proc Natl Acad Sci U S A.* 2002 May 28; 99(11):7728–33. [PubMed: 12032351]
51. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 2000 Dec 1; 14(23):2950–61. [PubMed: 11114885]
52. Eckel-Mahan K, Sassone-Corsi P. Metabolism and the circadian clock converge. *Physiol Rev.* 2013 Jan; 93(1):107–35. [PubMed: 23303907]
53. Kohsaka A, Laposky AD, Ramsey KM, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab.* 2007 Nov; 6(5):414–21. [PubMed: 17983587]
54. Fontaine C, Dubois G, Duguay Y, et al. The orphan nuclear receptor Rev-Erbalpha is a peroxisome proliferator-activated receptor (PPAR) gamma target gene and promotes PPARgamma-induced adipocyte differentiation. *J Biol Chem.* 2003 Sep 26; 278(39):37672–80. [PubMed: 12821652]
55. Lamia KA, Sachdeva UM, DiTacchio L, et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science.* 2009 Oct 16; 326(5951):437–40. [PubMed: 19833968]
56. Um JH, Pendergast JS, Springer DA, et al. AMPK regulates circadian rhythms in a tissue- and isoform-specific manner. *PLoS One.* 2011; 6(3):e18450. [PubMed: 21483791]

57. Asher G, Sassone-Corsi P. Time for food: the intimate interplay between nutrition, metabolism, and the circadian clock. *Cell*. 2015 Mar 26; 161(1):84–92. [PubMed: 25815987]
58. Laposky AD, Bass J, Kohsaka A, Turek FW. Sleep and circadian rhythms: key components in the regulation of energy metabolism. *FEBS Lett*. 2008 Jan 9; 582(1):142–51. [PubMed: 17707819]
59. Wright KP, Lowry CA, Lebourgeois MK. Circadian and wakefulness-sleep modulation of cognition in humans. *Front Mol Neurosci*. 2012; 5:50. [PubMed: 22529774]
60. Jones CR, Huang AL, Ptacek LJ, Fu YH. Genetic basis of human circadian rhythm disorders. *Exp Neurol*. 2013 May; 243:28–33. [PubMed: 22849821]
61. Laposky A, Easton A, Dugovic C, Walisser J, Bradfield C, Turek F. Deletion of the mammalian circadian clock gene *BMAL1/Mop3* alters baseline sleep architecture and the response to sleep deprivation. *Sleep*. 2005 Apr; 28(4):395–409. [PubMed: 16171284]
62. Naylor E, Bergmann BM, Krauski K, et al. The circadian clock mutation alters sleep homeostasis in the mouse. *J Neurosci*. 2000 Nov 1; 20(21):8138–43. [PubMed: 11050136]
63. Toh KL, Jones CR, He Y, et al. An *hPer2* phosphorylation site mutation in familial advanced sleep phase syndrome. *Science*. 2001 Feb 9; 291(5506):1040–3. [PubMed: 11232563]
64. Xu Y, Toh KL, Jones CR, Shin JY, Fu YH, Ptacek LJ. Modeling of a human circadian mutation yields insights into clock regulation by *PER2*. *Cell*. 2007 Jan 12; 128(1):59–70. [PubMed: 17218255]
65. He Y, Jones CR, Fujiki N, et al. The transcriptional repressor *DEC2* regulates sleep length in mammals. *Science*. 2009 Aug 14; 325(5942):866–70. [PubMed: 19679812]
66. Möller-Levet CS, Archer SN, Bucca G, et al. Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. *Proceedings of the National Academy of Sciences*. 2013 Mar 19; 110(12):E1132–E41.
67. Archer SN, Laing EE, Möller-Levet CS, et al. Mistimed sleep disrupts circadian regulation of the human transcriptome. *Proceedings of the National Academy of Sciences*. 2014 Feb 11; 111(6):E682–E91.
68. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*. 2005 May 13; 308(5724):1043–5. [PubMed: 15845877]
69. Feng D, Liu T, Sun Z, et al. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. *Science*. 2011 Mar 11; 331(6022):1315–9. [PubMed: 21393543]
70. Paschos GK, Ibrahim S, Song WL, et al. Obesity in mice with adipocyte-specific deletion of clock component *Arntl*. *Nat Med*. 2012 Dec; 18(12):1768–77. [PubMed: 23142819]
71. Scott EM, Carter AM, Grant PJ. Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man. *Int J Obes (Lond)*. 2008 Apr; 32(4):658–62. [PubMed: 18071340]
72. Stamenkovic JA, Olsson AH, Nagorny CL, et al. Regulation of core clock genes in human islets. *Metabolism*. 2012 Jul; 61(7):978–85. [PubMed: 22304835]
73. Gan Y, Yang C, Tong X, et al. Shift work and diabetes mellitus: a meta-analysis of observational studies. *Occup Environ Med*. 2015 Jan; 72(1):72–8. [PubMed: 25030030]
74. Savvidis C, Koutsilieris M. Circadian rhythm disruption in cancer biology. *Mol Med*. 2012; 18:1249–60. [PubMed: 22811066]
75. Gery S, Gombart AF, Yi WS, Koeffler C, Hofmann WK, Koeffler HP. Transcription profiling of C/EBP targets identifies *Per2* as a gene implicated in myeloid leukemia. *Blood*. 2005 Oct 15; 106(8):2827–36. [PubMed: 15985538]
76. Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response in vivo. *Cell*. 2002 Oct 4; 111(1):41–50. [PubMed: 12372299]
77. Klevecz RR, Shymko RM, Blumenfeld D, Braly PS. Circadian gating of S phase in human ovarian cancer. *Cancer Res*. 1987 Dec 1; 47(23):6267–71. [PubMed: 3677075]
78. Winter SL, Bosnyan-Collins L, Pinnaduwa D, Andrulis IL. Expression of the circadian clock genes *Per1* and *Per2* in sporadic and familial breast tumors. *Neoplasia*. 2007 Oct; 9(10):797–800. [PubMed: 17971899]

79. Cadenas C, van de Sandt L, Edlund K, et al. Loss of circadian clock gene expression is associated with tumor progression in breast cancer. *Cell Cycle*. 2014; 13(20):3282–91. [PubMed: 25485508]
80. Davis S, Mirick DK. Circadian disruption, shift work and the risk of cancer: a summary of the evidence and studies in Seattle. *Cancer Causes Control*. 2006 May; 17(4):539–45. [PubMed: 16596308]
81. Born J, Lange T, Hansen K, Molle M, Fehm HL. Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol*. 1997 May 1; 158(9):4454–64. [PubMed: 9127011]
82. Mazzocchi G, Sothorn RB, Greco A, et al. Time-related dynamics of variation in core clock gene expression levels in tissues relevant to the immune system. *Int J Immunopathol Pharmacol*. 2011 Oct-Dec;24(4):869–79. [PubMed: 22230394]
83. Scheiermann C, Kunisaki Y, Frenette PS. Circadian control of the immune system. *Nat Rev Immunol*. 2013 Mar; 13(3):190–8. [PubMed: 23391992]
84. Fortier EE, Rooney J, Dardente H, Hardy MP, Labrecque N, Cermakian N. Circadian variation of the response of T cells to antigen. *J Immunol*. 2011 Dec 15; 187(12):6291–300. [PubMed: 22075697]
85. Dubocovich ML, Rivera-Bermudez MA, Gerdin MJ, Masana MI. Molecular pharmacology, regulation and function of mammalian melatonin receptors. *Front Biosci*. 2003 Sep 18;18:d1093–108. [PubMed: 12957828]
86. Pandi-Perumal SR, Trakht I, Spence DW, Srinivasan V, Dagan Y, Cardinali DP. The roles of melatonin and light in the pathophysiology and treatment of circadian rhythm sleep disorders. *Nat Clin Pract Neurol*. 2008 Aug; 4(8):436–47. [PubMed: 18628753]
87. Levi F, Okyar A, Dulong S, Innominato PF, Clairambault J. Circadian timing in cancer treatments. *Annu Rev Pharmacol Toxicol*. 2010; 50:377–421. [PubMed: 20055686]
88. Scheer FA, Wright KP Jr, Kronauer RE, Czeisler CA. Plasticity of the intrinsic period of the human circadian timing system. *PLoS One*. 2007; 2(8):e721. [PubMed: 17684566]

KEY POINTS

Circadian rhythms at the organismal level are driven by rhythmic expression of genes at the molecular level.

The conserved architecture of these circadian clocks is based on a transcriptional feedback loop with posttranscriptional and posttranslational regulation.

Tissue specific clocks are synchronized to local time by environmental cues such as light and food.

Dysregulation of circadian rhythms through mutation or misalignment with environmental time is shown to contribute to a wide range of disease states.

Figure 1. Canonical molecular clocks in flies and mammals

Simplified *Drosophila* and mammalian circadian circuits. Arrows indicate activation and bars denote inhibition. Wavy lines represent rhythmic transcription. **P** marks phosphorylation. See text for more detail.

Figure 2. Light input modulates clock gene expression in the SCN

Glutamatergic input to SCN activates NMDAR resulting in cascade to induce mPER1,2 expression.

Figure 3. Disruption of the molecular clock results in tissue dependent disease states
 Normal clock function aligns gene expression to 24-hour cycle. Disruption of the genetic elements of tissue specific clock results in diseases and disorders.

Table 1

Posttranslational Modification.

Posttranslational modification:	Target	Known Effects
<i>Kinases:</i>		
CKI	PER1–2, CRY	Nuclear retention or degradation
DBT	dPER	Degradation and nuclear localization
CK2	dPER, PER2	Degradation and nuclear localization
<i>Phosphatases:</i>		
PP1 (Mammalian)	PER2	Nuclear localization and stabilization
PP1 (Drosophila)	dPER, TIM	Stabilization
PP2A	dPER	Nuclear localization

Table 2

Nutrient intake modifies circadian rhythms

1	Food times provide entrainment mechanisms
2	Diet can modulate clock activity
3	Nutrient sensitive pathways act directly on peripheral clocks to alter timing.

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