REVIEW

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Circadian topology of metabolism

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Biological clocks are genetically encoded oscillators that allow organisms to anticipate changes in the light–dark environment that are tied to the rotation of Earth. Clocks enhance fitness and growth in prokaryotes, and they are expressed throughout the central nervous system and peripheral tissues of multicelled organisms in which they influence sleep, arousal, feeding and metabolism. Biological clocks capture the imagination because of their tie to geophysical time, and tools are now in hand to analyse their function in health and disease at the cellular and molecular level.

Benjamin Franklin's dictum "early to bed, early to rise" is built on the supposition that sleep is inevitable at night and waking should correspond to sunrise. However, some people are 'larks' and wake early, whereas others are 'night owls' and stay up late, hinting that there is a biological driver of sleep–wake rhythms. A triumph of modern genetics has been the identification of the molecular pathways that dictate the sleep–wake cycle and other 24-hour-circadian (derived from *circa diem*, about a day) rhythms (Fig. 1). Many of the principles of this system originally came from genetic studies of model-organism mutants with altered period phenotypes, and are described in this Review in the context of our current understanding of clock systems in mammalian tissues. These advances in understanding can also be considered in the context of human studies that suggest that artificial light, night work, a reduction in normal sleep time, shift work, travel and temporal disorganization, all of which are common in industrialized societies, have disrupted the pattern of alignment between the external light–dark cycle and the internal clock, which was set to a 24-hour day early in evolution (Fig. 2). Identification of the molecular clock may lead to insight into circadian and sleep disorders in humans. This Review highlights how advances in the field of molecular clocks could help in understanding the molecular pathogenesis of metabolic disorders across the lifespan.

Origins of circadian clocks

Consciousness of the temporal world has been a hallmark of civilization from prehistoric times — reflected in the iconic solar worship site at Stonehenge. Yet, the correspondence between biological and geophysical phenomena was not recognized until the early 1700s, when the French astronomer Jean-Jacques d'Ortous De Mairan demonstrated that the leaves of *Mimosa pudica* continue to open and close every 24 hours even when the plant was enclosed in a sealed box. Since then, other photosynthetic organisms, and nearly all forms of life on the surface of the planet, have been shown to exhibit similar circadian cycles. The principal criteria of circadian oscillators emerged from the work of Pittendrigh and Aschoff, who established the defining characteristics of biological clocks: a persistent and sustained period length under constant conditions, entrainment to environmental signals such as light, and stability across wide variations in temperature (referred to as temperature compensation).

The transcriptional motif

A leap forward in our understanding of molecular clocks came from the deliberate mutagenesis studies of Konopka and Benzer in the fruitfly *Drosophila melanogaster*. These studies screened for 24-hour rhythms in the fly's emergence from the pupal case, and provided a gateway to the modern era of circadian genetics[1]. A key advance in circadian genetics was the concept that clocks comprise a transcription autoregulatory feedback loop, with the forward limb encoding activators that promote transcription of a set of repressors, which feed back to inhibit expression and function — a cycle that repeats itself every 24 hours across divergent phyla[2,3] (Fig. 1). Indeed, transcriptional oscillators may have provided a selective advantage early in evolution by averting the DNA-damaging effects of sunlight. The presence of a photolyase domain in clock repressors indicates that the timing systems co-evolved with DNA repair[4].

A metabolic variation on transcription

Although the transcription feedback loop represents a conserved model of the circadian oscillator, recent advances point towards metabolic oscillators as an additional mechanism of circadian timekeeping that can be, in certain circumstances, independent of transcription. A remarkable series of test-tube experiments[5,6] has provided the most convincing evidence for a protein-based clock. A complex of just three proteins (KaiA, KaiB and KaiC) together with ATP undergo a self-sustaining 24-hour cycle of alternating phosphorylation and dephosphorylation. The phosphorylation cycle is both coupled to and directs gene transcription, although it can also be seen in the absence of transcription under certain circumstances[5]. The cycling of this kinase–phosphatase reaction remains constant at different temperatures — a defining feature of circadian oscillators[6] (Fig. 3). The turnover of ATP modulates the Kai phosphorylation cycle, suggesting that the clock may be coupled to metabolic activity[7]. An interdependence between circadian and metabolic oscillators has also been suggested by showing that the activity of clock transcription factors is sensitive to redox state[8] (Fig. 3).

More recently, periodic flux in metabolic cycles has been related to production of reactive oxygen species (ROS). Peroxiredoxin is a redox-sensitive protein, which has a reactive thiol within the active site that is involved in electron transfer from reactive oxygen. These proteins exhibit 24-hour oscillation in cells in the absence of transcription — as shown in erythrocytes[9] — and under conditions when transcription is arrested in the alga *Ostreococcus tauri*[10]. Peroxiredoxin proteins have been shown to exhibit self-sustained oscillation in many species of archaebacteria, plants and in other eukaryotic cells, suggesting that a redox cycle may be one of the most conserved 24-hour oscillators[11] (Fig. 3). Determining whether the peroxiredoxin proteins are themselves clocks or reporters of a more primary metabolic oscillator will require further genetic and biochemical analyses.

Given that eukaryotes first diverged from bacteria around 1.5 billion years ago, a provocative speculation is that oxygenation

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of the atmosphere conferred an adaptive advantage on organisms with a redox-based clock. If this speculation is true, then organisms existing in anaerobic conditions, such as deep-sea vaults, might have less pressure to eliminate ROS, and may be devoid of a metabolic clock. In mammals, oscillations in the peroxiredoxin redox state have been proposed to represent a means of rhythmically anticipating the generation of ROS. Conceivably, organisms that are capable of efficiently extinguishing ROS may have had a survival advantage during the oxygen expansion of the atmosphere. If this is true, then the peroxiredoxin clock may keep time at the organismal level by regulating the oscillation of ROS. Consideration of the origins of internal clocks remains central to our understanding of links between circadian and metabolic systems (Fig. 3): was it escape from sunlight, avoidance of toxic metabolites during respiration or elimination of inefficient metabolic cycles that drove these processes together? Identifying metabolic circadian clocks could determine whether there is a monophyletic origin of organisms that possess such oscillators, emphasizing the fundamental clues that clocks may yield for understanding evolutionary and functional relationships among species.

Clocks impact on fitness in multicellular organisms

A principle to emerge from genetic studies is that period length is genetically programmed. But how and why are the body's internal clocks set to 24 hours? Resonance studies are perhaps the best approach to address this question because they discriminate between oscillator-dependent and independent gene functions. Phenotypes such as growth and reproduction are monitored under conditions in which alignment between internal period length and the environmental light cycle are systematically varied (long- and short-period mutants are maintained under lengthened or shortened light cycles). In bacteria and plants, such studies suggest an advantage to period alignment[12]. But, what are the consequences of misalignment at the molecular level? One possibility is that misalignment reduces genome stability by shuffling the phase relationship between cycles of DNA damage and repair. Alternatively, misalignment may superimpose incompatible biochemical processes, such as the oxidative and reductive phases of the metabolic cycle. For instance, in yeast (*Saccharomyces cerevisiae*), the mutation rate increases when metabolic cycles are misaligned with DNA replication[13]. Furthermore, although circadian cycles are directly photosensitive in plants and even in the cells of *D. melanogaster*, in mammals the role of cryptochromes as both a timekeeper and a DNA-damage-repair agent is uncoupled. It is tempting to speculate that the coupling of DNA repair to circadian cycles may contribute to ageing even in higher eukaryotes.

Neural–clock sensory circuit and circadian–system ageing

Understanding how complex organisms detect light and synchronize the clocks in brain and other tissues to the environment remains a central challenge in circadian research. Sensory pathways within the brain have been identified that synchronize the clock independently of visual image formation. The light-response pathway also synchronizes neural and peripheral clocks, but the integrity of the clock network may decline with age.

Light–response pathway in the retina

A breakthrough in understanding the entrainment of oscillators in animals came with the discovery that even mice without the classic rod and cone visual photoreceptors are still able to synchronize their internal clocks to light. The light photoreceptors are expressed in a small number of retinal cells that express the photopigment melanopsin[14]. Mice that are genetically depleted of these few hundred cells still have normal vision, but they are unable to synchronize their clocks to light[15]. All connections to the hypothalamic pacemaker neurons in the suprachiasmatic nucleus go through these few melanopsin cells[16]. Suprachiasmatic-nucleus neurons comprise

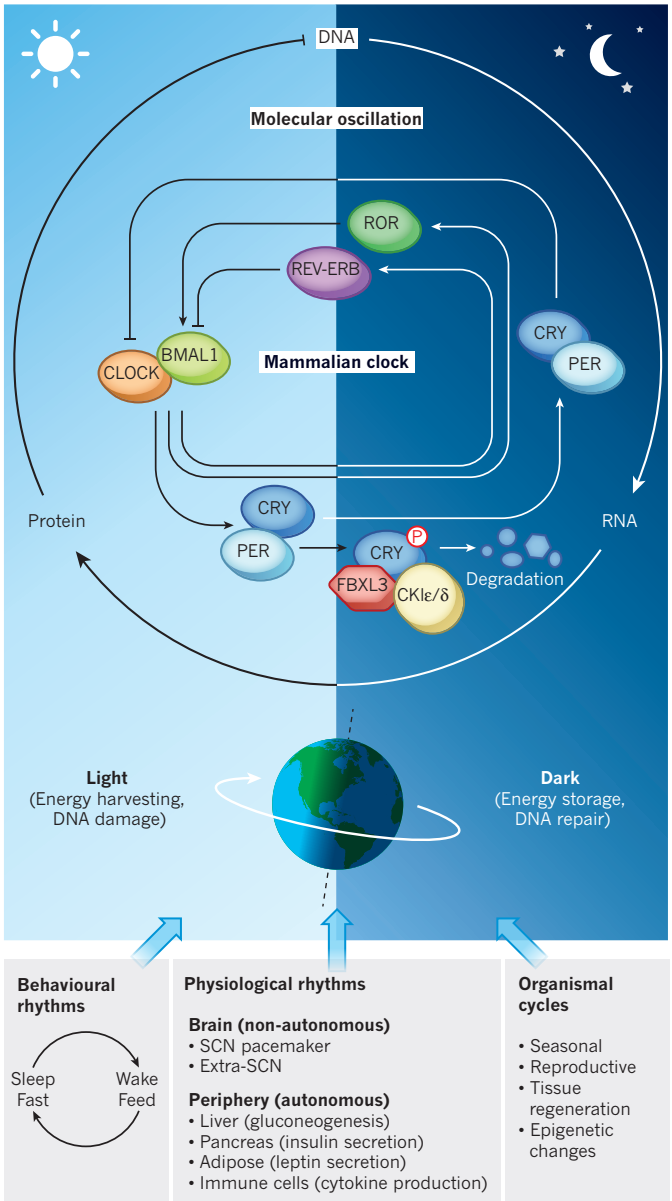

Figure 1 | Circadian adaptation as a unifying model that integrates behaviour and physiology. The circadian clock allows light-sensitive organisms to synchronize their daily molecular oscillations, behavioural rhythms, physiological rhythms and organismal cycles with the rotation of Earth on its axis. Core molecular pathways dictate behavioural and physiological cycles. This core molecular clock in mammals, expressed both in brain and peripheral metabolic tissues, comprises a series of transcription–translation feedback loops that include opposing transcriptional activators (CLOCK–BMAL1) and repressors (PER–CRY)[1]. The non-phosphorylated PER–CRY complex represses CLOCK–BMAL1; phosphorylation, in turn, results in the degradation of PER–CRY and the turnover of these repressors. In addition, CLOCK–BMAL1 induces transcription of REV-ERB and of ROR, which regulate BMAL1 expression. During the night, PER–CRY is degraded through the ubiquitylation of CRY by FBXL3. The circadian clock coordinates anabolic and catabolic processes in peripheral tissues with the daily behavioural cycles of sleep–wake and fasting–feeding. SCN, suprachiasmatic nucleus.

the central node in the clock network, which in turn synchronizes the hypothalamic control of energy balance, sympathetic outflow and the neuroendocrine systems[17]. A future goal will be to extend studies of the circadian neurosensory circuit to understand whether factors that affect sensory perception or nutrition in early development might alter synaptology within this circuit.

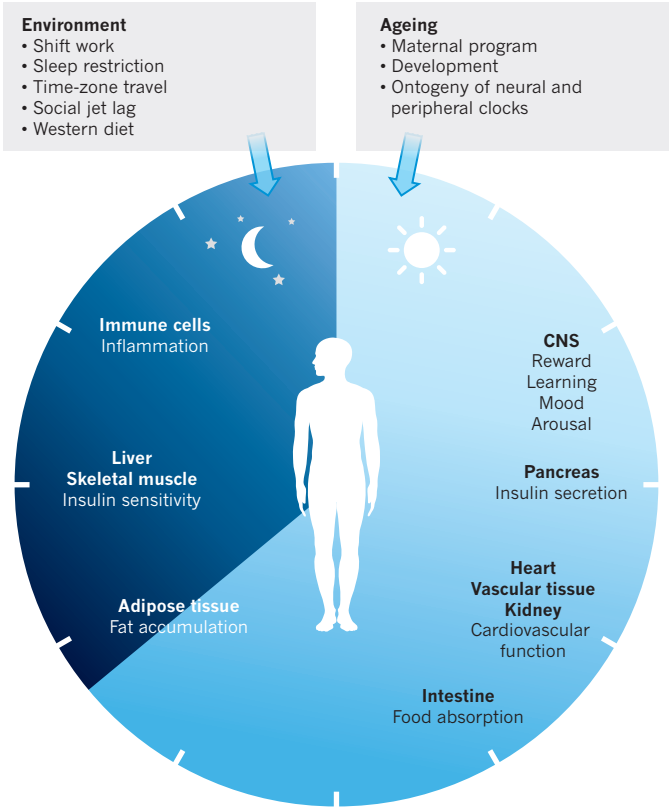

Figure 2 | Affect of ageing and environmental disruption on circadian control of metabolic processes. The circadian clock partitions metabolic processes within the peripheral tissues according to whether we are asleep or awake; for example, the pancreatic clock promotes insulin secretion during the wake–feeding period[52], but the adipose tissue clock promotes fat accumulation during the sleep as well as the wake period. Synchronization of peripheral tissue clocks and downstream metabolic processes with the environmental cycle is crucial for the maintenance of the health of the organism[35,39]. We are only just beginning to gain an appreciation of how both ageing[26,28] and environmental disruption (including changes in diet, time of feeding or jet lag) perturb the integration of the circadian and metabolic networks[100]. CNS, central nervous system.

Hypothalamic networks link circadian and energetic centres

The next question is how does the hypothalamic clock communicate with extra-pacemaker and peripheral tissues to produce a coherent phase in the circadian systems throughout the organism? An appreciation of the central role of the suprachiasmatic nucleus in coordinating sleep–wake behaviour, as well as physiological systems, pre-dated molecular advances in defining the molecular mechanisms of the clock. Master pacemaker cells were first localized to the anterior hypothalamus[18]. Transplantation of the suprachiasmatic nucleus with tissue from short-period mutants into animals with damage to the anterior hypothalamus imposed short periods on the host, proving that the suprachiasmatic nucleus is a master clock, not just a relay station[19]. These studies also suggested that secreted factors from the suprachiasmatic nucleus contribute to the synchronization of clocks, although the identity of these synchronizing factors has been elusive.

Among the areas receiving suprachiasmatic-nucleus projections, a large output is toward the dorsal medial hypothalamic (DMH) area[20]. This circuit has been implicated in a phenomenon known as food anticipatory activity (FAA), whereby animals increase their activity in response to food provided during the incorrect circadian phase, although it is possible that other hypothalamic regions may be necessary for FAA[21]. Moreover, the role of clock genes in FAA is controversial. Animals deficient in MC3R, a metabolic signalling receptor, have diminished FAA[22], suggesting that metabolic rather than clock factors may be the principal driver of FAA.

In addition to output to the DMH area, suprachiasmatic-nucleus projections synapse on orexin-expressing neurons within the lateral hypothalamic area. Orexin (also known as hypocretin) is a neuropeptide that has been found to stimulate arousal and increase energy expenditure with intracerebroventricular administration. Deficiency of either orexin or its receptor is a hallmark of both canine genetic[23] and human autoimmune forms of narcolepsy. Interestingly, narcolepsy is also correlated with elevated body-mass index[24]. Furthermore, ablation of orexin receptors increased susceptibility to diet-induced obesity, suggesting that the physiological role of the orexins is to promote arousal and antagonize weight gain[25].

Ageing and the circadian system

Given the extensive integration of the neuroendocrine and circadian systems, it is intriguing to note that suprachiasmatic-nucleus function declines with age[26] (Fig. 2). What molecular mechanisms might account for the observed effects of ageing on the integrity of circadian systems? Studies in circadian-clock-mutant animals have shown the susceptibility of certain tissues to damage, such as accelerated cataract formation and dermatitis[27]. In addition, *Bmal1*-knockout mice have a premature death, compared with control mice, that is correlated with increased accumulation of ROS[28]. Deficiency of cryptochrome, a repressor of the internal clock repressor, has also been associated with alterations in liver regeneration, emphasizing the coupling of circadian and cell-cycle pathways[29]. Although epidemiological evidence suggests there is a link between circadian disruption and cancer risk, a full understanding of the role of circadian systems in tumorigenesis remains an area for investigation. Given the emerging link between metabolic flux and cancer-cell survival, it will be especially interesting to determine whether circadian systems also intersect with oncogenic pathways through regulation of fuel selection (discussed later).

Circadian origins of metabolic disease

An emerging theme in both circadian and metabolic studies is that it is not only the central nervous system, but also the peripheral tissues that modulate sensory response to the environment. A key goal is to determine how the circadian cycle modulates homeostatic and nutrient responsive pathways to coordinate behaviour and energetics.

Feeding resets peripheral clocks

Shifting food availability to the incorrect circadian time (through restricted feeding) in animals entrained to a standard light–dark schedule causes a shift in the peripheral clock in the liver without altering pacemaker neuron clocks or overall behavioural rhythm[30]. One pathway that entrains the liver clock to feeding involves glucocorticoid signalling[31]. Temperature also entrains the clock through mechanisms involving transcriptional control of heat-shock factor protein 1 (refs 32 and 33) (Fig. 3). ADP-ribosylation also affects entrainment of the liver to feeding[34]. Together, these findings suggest that multiple levels of transcriptional regulation reinforce signalling from the neuronal pacemaker to the liver, with the net result of suprachiasmatic-nucleus input restraining the response of circulating hormonal or metabolite signals that are related to feeding.

Meal timing affects metabolic syndrome

Mounting evidence suggests that alignment between central behavioural rhythms and feeding time is important in metabolic health. Animals fed a high-fat diet shift their pattern of food intake and consume nearly all of the excess calories at the incorrect circadian time (during the rest period)[35]. A similar erosion of the partitioning of feeding between activity and rest is observed in *Clock*-mutant[36] and *Npas2*-knockout[37] mice, and a high-fat diet induces period lengthening in wild-type mice, which is a core property of the clock[35]. Similarly, mice fed a high-fat diet exclusively during the rest period have accelerated weight gain compared with animals fed during the correct circadian time[38], whereas restricting access to high-fat food ameliorates diet-induced metabolic syndrome in

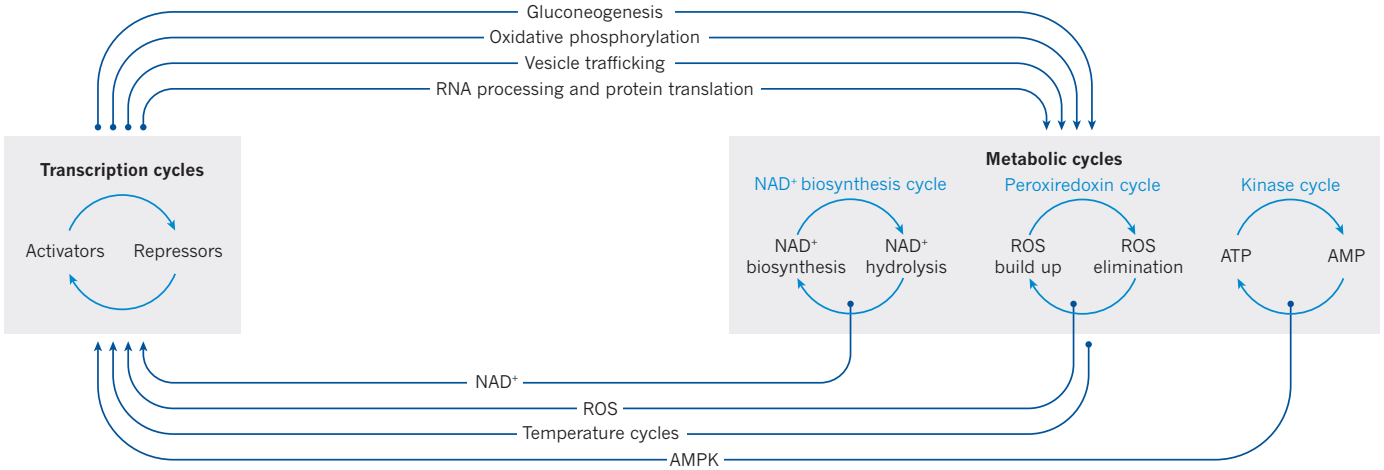

Figure 3 | Cross-talk between clock transcription and metabolic systems at the molecular and physiological levels. Cycling of circadian transcriptional activators and repressors[2,3] controls fundamental physiological cellular and metabolic processes, including gluconeogenesis, oxidative phosphorylation, RNA processing and translation, and vesicle trafficking[64]. This can occur in various tissue types, but there may be tissue-specific differences. In turn, metabolic cycles reciprocally affect the clock: the NAD^+ biosynthesis[81,82], peroxiredoxin[11] and kinase[6] cycles generate active intermediates that, along with cycles of temperature, provide feedback to regulate the core clock transcriptional network. AMPK, AMP-activated protein kinase; ROS, reactive oxygen species.

mice[39]. Even constant exposure to light causes increased insulin resistance[40]. An important goal will be to elucidate the neural and molecular basis of the links between altered timing and behavioural and metabolic disruption. Conceivably, alterations in the time of feeding may induce desynchrony between suprachiasmatic-nucleus firing rhythms and input from peripheral feeding responsive signals. Altered excitability and activity of certain cell groups may occur within restricted windows as a result of either cell-autonomous clock function or non-autonomous input from the suprachiasmatic nucleus. Although much of the experimental evidence that links timing to metabolism has emerged in rodent studies, both epidemiological and clinical investigations suggest parallel mechanisms may predispose humans to metabolic pathologies[41,42].

Function of clock genes in metabolic and vascular disease

Genetic tools to perturb the internal clock have created opportunities to analyse the molecular basis of the clustering of certain pathologies within limited time windows, including morning myocardial infarction and hypertensive crises[43] (Fig. 2). The basis of increased risk of myocardial infarction in the morning is probably multifactorial; circadian transcription factors have been shown to trans activate the promoter of the pro-thrombotic cytokine plasminogen activator inhibitor type 1 (PAI-1) through both REV-ERB and E-box motifs, corresponding to oscillation in thrombosis risk[44,45]. Arrhythmogenesis also occurs more frequently in the morning, and is associated with the control of potassium-channel expression by myocardial clock genes[46]. Cardiac clocks also influence myocardial contractility and oxidative metabolism[47]. Both autonomous and non-autonomous vascular effects of the clock cause variation in blood pressure across the light–dark cycle[48]. CLOCK expression in the vasculature affects the progression of atherosclerosis in xenograft models[49]. Similarly, dyslipidaemia arises in circadian mutants[36] and may be related to dysregulation of core clock genes[50] and the clock-controlled gene *Ccrn4l* (ref. 51).

Phase and tissue clocks determine physiological outcomes

Although the aforementioned studies have analysed mutations in multiple tissues, one complexity of the circadian system is that the set of clock transcriptional activators and repressors exerts opposing effects within different tissues at different times in the 24-hour cycle. This is most clearly evident in studies of glucose metabolism. For example, *Clock*-mutant mice are hyperglycaemic and have increased susceptibility to diet-induced obesity early in life, but also become hypoinsulinaemic with age[36]. An explanation for this finding was provided by studies in which animals with selective ablation of the circadian gene *Bmal1* within the endocrine pancreas were shown to have much more profound hyperglycaemia and β-cell failure than the multitissue mutants[52]. Indeed, whole body loss of *Bmal1* increases insulin sensitivity[53], and selective *Bmal1* ablation in the liver causes hypoglycaemia[54]. Taken together, these findings suggest that CLOCK–BMAL1 exerts opposing effects in the liver and the pancreas — within the liver, it promotes fuel mobilization during fasting, but in the pancreas this complex promotes post-prandial insulin exocytosis. The loss of the CLOCK–BMAL1 repressor CRY results in the opposite phenotype in the liver, with increased gluconeogenesis and enhanced responsiveness to both glucagon and glucocorticoids[55,56].

One challenge to studies of genetically modified circadian-mutant mice is whether the observed abnormalities are dependent on alterations in the core timing process or in downstream processes independent of the internal clock. Furthermore, because ablation of individual clock factors causes compensatory changes in the expression of other factors within the core loop — even when studying effects of individual gene manipulations — both loss and gain of function analyses become important to determine whether the effects are direct or indirect[57]. Studies in both activator and repressor mutants, and analyses across different circadian phases in synchronized tissues, will ultimately elucidate the link between gene function, timing and physiology.

Coupling of circadian and metabolic cycles

Extensive cross-talk between metabolic and circadian systems allows organisms not only to anticipate physiological needs in advance of the daily light–dark cycle, but also to adjust the phase of internal cycles in response to changes in the environment. The cross-talk between cellular oscillators and metabolic systems can be traced to overlapping transcriptional networks that encode circadian and nuclear-receptor signalling pathways (Fig. 4).

Transcription factors respond to metabolic flux

At the molecular level, multiple sequence alignment of core clock genes originally demonstrated the presence of the Per–Arnt–Sim (PAS) domain in the internal clock transcription factors. This conserved motif is also found within xenobiotic and hypoxaemic response transcription factors in addition to kinases and ion channels. PAS domains participate in both protein–protein interactions and direct detection of small molecules. Haem, a gas-responsive cofactor, binds to the PAS domain in a subset of these proteins and detects both carbon monoxide in the clock factor NPAS2 and oxygen in the bacterial PAS-containing histidine kinase[58,59] (Fig. 4). Haem has also been found to bind to the

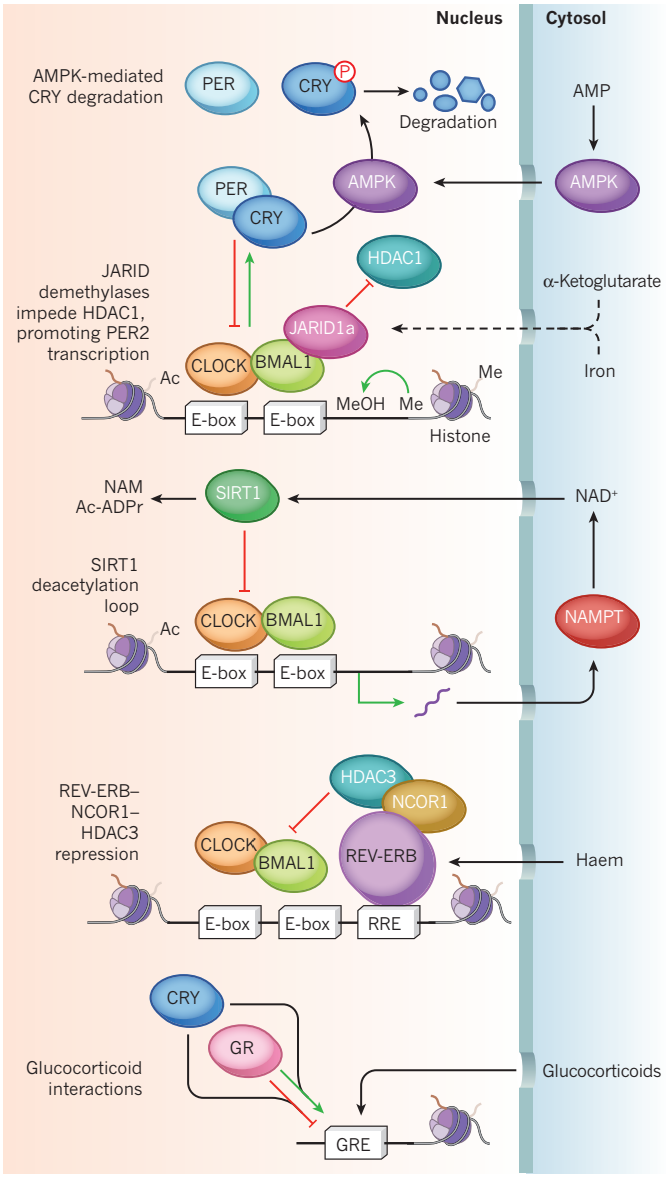

Figure 4 | Genomic and epigenetic links between circadian and metabolic systems. Research has highlighted the existence of a highly dynamic and multi-layered network of factors involved in epigenetic transitions across the circadian cycle. For example, AMP-activated protein kinase (AMPK)-mediated phosphorylation of CRY[63] controls proteolytic degradation of the negative arm of the central oscillator. Distinct from the 'core' oscillation shown in Fig. 1, which uses CKI to phosphorylate and therefore cause the degradation of CRY, AMPK entrains the circadian clock to the metabolic environment by using the same modification as CKI. The histone demethylase JARID1a (ref. 89) is involved in the activity of CLOCK–BMAL1 complexes (which regulate PER and CRY transcription) that are bound to E-box motifs. This activates the positive arm of the central oscillator by blocking HDAC1 activity, a function that may be tied to the use of α-ketoglutarate and iron, to hydroxylate and remove H3K4 methyl marks on histones. However, this demethylation activity seems to be dispensable in circadian regulation. NAD^+-dependent enzyme SIRT1 activity is dependent on NAD^+-regulating nicotinamide mononucleotide phosphoribosyltransferase (NAMPT) (a transcriptional product of the CLOCK–BMAL1 complex). SIRT1 in turn feeds back to inhibit the CLOCK–BMAL1 complex[79,80]. Deacetylation by SIRT1 generates nicotinamide (NAM) and O-acetyl-ADP-ribose (Ac-ADPr) as by-products. The REV-ERB–HDAC3–NCOR1 repressive complex is sensitive to haem levels, possibly activating the repressor activity[61,62], and binds directly to the promoters of clock-controlled genes (RRE), while binding directly to CLOCK–BMAL1 deacetylating nearby histones (not shown). Transcriptional activation and repression of glucocorticoid-responsive elements (GREs) by the glucocorticoid receptor (GR) are modulated by glucocorticoids and CRY[56].

PAS domain of the nuclear hormone receptor co-repressor proteins REV-ERB-α and REV-ERB-β (refs 60–62).

Although the hallmark of the circadian cycle is its self-sustained activity, changes in circadian gene transcription in response to energetic flux have also been demonstrated. One example of a chemical signal that couples internal clock function to nutrient state involves AMP kinase (AMPK), a protein that is activated following intracellular nutrient restriction. Stimulation of AMPK leads to the phosphorylation and subsequent proteasomal degradation of the repressor CRY[63]. An important question to ask is whether alterations in AMPK signalling modulate the output of pacemaker neurons to control behavioural and physiological rhythms, or whether AMPK signalling affects circadian transcriptional cycles in a restricted number of peripheral tissues.

Nuclear receptors connect endocrine and circadian physiology

Core clock genes can be defined as those that are necessary for circadian behavioural rhythmicity; however, it is intriguing to note that data mining suggests that a large fraction of the transcriptome exhibits circadian oscillation in tissues such as the liver, although the specific number of transcripts designated as oscillating depends on the threshold set for gene amplitude during bioinformatic extraction[64]. Indeed, many of the nuclear hormone receptors in mice exhibit circadian oscillation and, in turn, the timing of interaction between nuclear receptors and ligands may be considered a broad feature of the coupling between temporal and physiological systems[65]. The coupling of circadian and nuclear hormone receptor networks is reflected in the overlap between genome occupancy of the orphan nuclear receptor ERR-α and of BMAL1 (ref. 66), and in altered circadian function in mice that are deficient in the co-activator PGC-1α (ref. 67). Surprisingly, the circadian repressor PER2 binds to several nuclear hormone receptors[68], whereas CRY binds to the glucocorticoid receptor and modulates its function[56]. Glucocorticoid has also been shown to modulate the expression of REV-ERB-α (ref. 69), a regulator of *BMAL1* (also known as *ARNTL*) transcription[70]. REV-ERB-α agonists have been shown to influence circadian function and may protect against adverse metabolic consequences of diet-induced obesity[71]. Genetic studies in which REV-ERB is eliminated only in adults have reinforced the link between REV-ERB-α and its homologue REV-ERB-β in the co-regulation of circadian and metabolic homeostasis[72,73].

Genomic analyses have also begun to increase the evidence for co-repressors in coupling circadian and metabolic systems (Fig. 4). HDAC3, a class I histone deacetylase that is recruited to the REV-ERB-α–NCOR1 transcriptional complex[74], exhibits diurnal occupancy of the loci that encode the genes involved in lipogenesis and carbohydrate metabolism[75]. REV-ERB-α and REV-ERB-β occupy a spectrum of lipogenic genes in addition to core clock genes[73], although it remains unclear whether REV-ERB exerts the same effect on both classes of transcripts. For instance, BMAL1 occupies loci, encoding both internal clock and metabolic genes throughout the genome[76]. However, analysis of expressed transcripts indicates closer phase alignment between genome occupancy and RNA transcription for core clock genes. Moreover, although clock genes occupy many sites throughout the genome, the core clock genes generally exhibit greater variation in expression across the cycle compared with output genes. One exception is the very high amplitude rhythm of *DBP* and *TEF*, two output genes belonging to the proline and acidic amino-acid-rich basic leucine zipper (PAR-bZIP) family of transcription factors.

Although genomic analyses bring greater focus to studies of transcriptional cross-talk, future transcriptome analyses will be necessary to determine whether occupancy of loci throughout the genome is accompanied by changes in expression. Eventually, it will be important to extend chromatin immunoprecipitation and sequencing analyses to comparison of the transcriptome in wild-type and knockout mice for the factors in question to assess function. For instance, although steatosis is observed with hepatic ablation of both HDAC3 (ref. 74) and REV-ERB-α

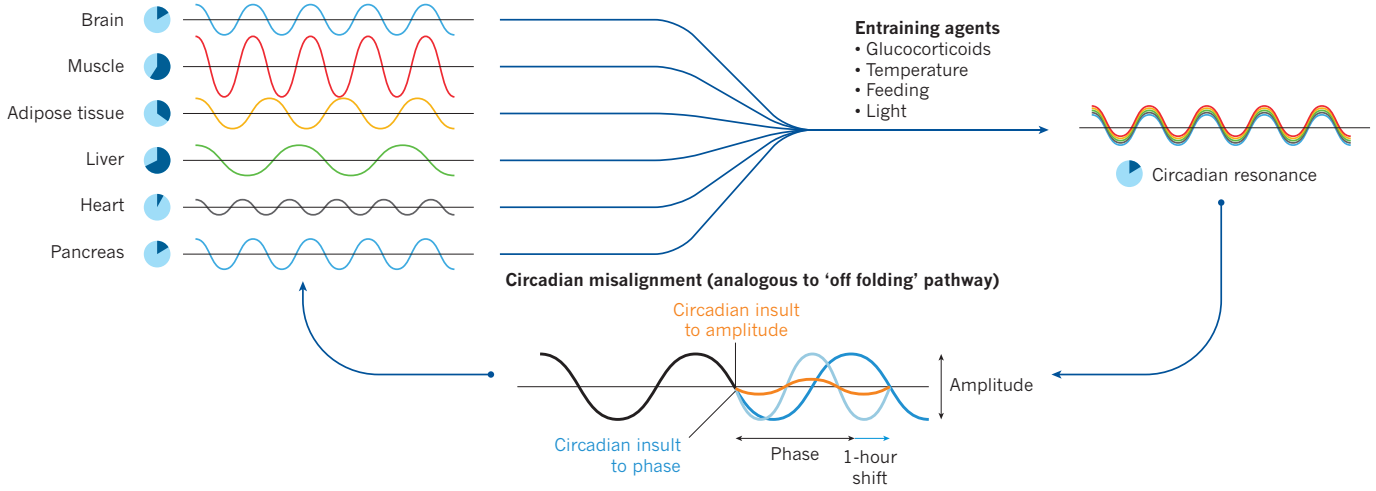

Figure 5 | Topological model of circadian physiology. I propose that the process of circadian synchronization is analogous to protein folding dynamics, with energy minima across the circadian landscape achieved during phase alignment of individual cells and tissues (asynchronous oscillators) and misalignment (analogous to misfolding traps) induced by either environmental or behavioural perturbation. Entraining agents promote synchronization and circadian resonance of individual tissue clocks, whereas circadian insults lead to off-synchrony pathways in which phase and amplitude are misaligned. Such misaligned states may be permanent (analogous to kinetic trapping of misfolded polypeptide) or re-aligned. I propose a concept of 'chronostasis' to describe the circadian synchrony landscape (just as proteostasis describes the folding landscape), with both *cis*- and *trans*-acting factors affecting achievement of energy minima and determining trajectory across the topological map.

(refs 72 and 73), delineating the relationship between circadian gene transcription changes and pathologies remains a challenge. The coincidence of circadian and metabolic perturbation in these mice may create a vicious cycle and augment the adverse effects of dual disruption in timing systems and metabolism.

Epigenetics and circadian cycles

Clocks also synchronize to the environment through post-translational modification of transcription factors and histones that tune gene expression rhythms to metabolic state (Fig. 5). NAD^+ oscillation, redox flux, ATP availability and mitochondrial function influence acetylation and methylation reactions, and may be important factors in circadian synchrony.

Chromatin transitions impact on core clock gene cycling

In liver tissue, the rhythmic acetylation of histone 3 corresponds with CLOCK–BMAL1-mediated transcriptional activation of *Per1* and *Per2* genes, and to recruitment of the histone acetyltransferase p300 — an event closely coupled to RNA polymerase II binding[77]. In an additional twist, CLOCK itself participates in histone acetylation[78]. Feedback repression by CRY proteins abrogates histone acetylation, which, together with rhythmic CLOCK–BMAL1 binding to DNA, probably contributes to circadian oscillation in gene expression[76]. Further investigation will be necessary to determine how the rhythmic assembly of activator and repressor complexes influences the kinetics of transcriptional oscillation, and to delineate how metabolic signals modulate dynamic transitions in the epigenetic state.

Histone deacetylases couple metabolic and circadian cycles

Studies of histone deacetylase activity have demonstrated mechanistic integration between circadian and metabolic processes at the level of post-translational protein modification and gene transcription (Fig. 4). As already noted, REV-ERB functions together with the co-repressor NCOR1 to rhythmically recruit the class I histone deacetylase HDAC3, and genetic abrogation of the NCOR1–HDAC3 interaction results in both metabolic pathologies and circadian disruption[75]. In addition, interactions occur between CLOCK–BMAL1 and the class III histone deacetylases belonging to the sirtuin (SIRT, silencer of information regulator) superfamily of chromatin-modifying enzymes[79,80]. SIRT1 association with the clock activator complex represents an additional mode of autoregulation because CLOCK–BMAL1 directly modulates the turnover of cellular NAD^+, a cofactor for deacetylase reactions[81,82]. NAD^+ functions as an electron shuttle in oxidoreductase reactions and as a cofactor in deacetylase and ADP-ribosylation modifications, raising intriguing questions concerning the role of NAD^+ in bidirectional interactions between circadian and metabolic signalling. Furthermore, the NAD^+-dependent sirtuins have been established as regulators of metabolic pathways in response to calorie restriction and as modulators of oxidative damage and DNA-repair processes that are central to lifespan regulation. As such, the nexus of circadian control of NAD^+ and sirtuin activity may have broad implications for ageing and oxidative metabolism, which are particularly relevant in view of the association between period length and longevity[83].

Histone methylation in metabolism and circadian rhythms

In addition to identification of histone acetylation as a key event in circadian cycles, biochemical pull down with PER1 has demonstrated that the clock repressor complex is also associated with factors involved in histone methylation, including WDR5 (ref. 84), whereas the mammalian methyltransferase EZH2 has been shown to participate in CRY-mediated repression[85] (Fig. 4). Conversely, rhythmic recruitment of the histone methyltransferase MLL1 participates in gene activation by CLOCK–BMAL1 (ref. 86). Rhythmic trimethylation of histone 3 lysine 4 (H3K4me3) is also involved in circadian activation of the clock-controlling gene *DBP*, whereas dimethylation of histone 3 lysine 9 (H3K9me2) corresponds to its repression[87]. The link between histone methylation and circadian cycles has also been identified in studies of the *Arabidopsis thaliana* clock[88]. CLOCK–BMAL1 activity has also been shown to involve cyclic association of the histone demethylase JARID1a (ref. 89), although a direct link to methylation was not observed. Activity of the jumonji C domain demethylases, such as JARID1a, is coupled to cellular redox and mitochondrial energetics because both Fe II and α-ketoglutarate are used as cofactors[90]. Whether changes in cellular metabolism may affect circadian systems through alterations in DNA methylation remains untested.

A range of additional mechanisms have been implicated in transduction of metabolic flux to co-activators and co-repressors, although the effect these may have on circadian gene transcription is not yet known (Fig. 4). For instance, CTBP1, a co-repressor associated with

NCOR1, is sensitive to the NADH:NAD^+ ratio, and CTBP1 itself is an NAD^+ reductase[91], making this a potential candidate in the communication between metabolic changes and circadian oscillations.

Chronobiology and health and management of disease

The integrative physiology of circadian and metabolic systems has emerged through a combination of biochemical and experimental genetic studies; however, emerging approaches in human analyses provide an important avenue for future work (Fig. 5). Monogenic disorders in sleep onset and waking have provided evidence that clock genes have an effect not only on subjective chronotype, but also on neurological pathways that regulate sleep in humans. Animal models of these coding mutations in humans may be a platform with which to investigate links between neuroendocrine homeostasis and circadian systems[92].

One of the more surprising results in human genetic analyses has been the association between variants of both *CRY2* and *MTNR1B* genes with glucose levels in humans[93–95]. Although the pharmacology of the melatonin receptor 1B in glucose homeostasis is complex, studies in rodents support a role for melatonin signalling in rhythms of insulin secretion[96]. Studies of Smith–Magenis syndrome, a haploinsufficiency disorder localized to *RAI1* and characterized by neural–behavioural abnormalities, intellectual deficit, obesity and circadian sleep disruption, has provided further evidence for a genetic link between *CLOCK* gene expression and energetic disorders[97]. Emerging human genetic work parallels evidence from animal-based experimental studies to strengthen the hypothesis that genetic signatures of circadian function may be used to predict risk for metabolic disorders in humans.

Although beyond the scope of this Review, both longitudinal population studies and clinical investigations have indicated there is an association between shift work and metabolic disease. For instance, a study of nurses, who are one of the best monitored cohorts with a large representation of individuals who work shifts, has associated sleep time and circadian disruption with a broad range of disorders — including type 2 diabetes, gastrointestinal disorders and cancer — that may also be modulated by circadian genotype[98]. Moreover, sleep loss and circadian disruption may be interacting risk factors for developing type 2 diabetes in individuals who are predisposed to the disease[99]. The public health implications may be quite broad given the frequency of circadian behavioural disruption; indeed, the habit of altering bedtime on weekends, or 'social jet lag', has been associated with increased body weight[100]. Laboratory models also suggest there is a direct causal role of circadian disruption on glucose tolerance[42], although separating the effects of circadian disruption from sleep reduction as a result of experimental regimens remains a challenge. Ultimately, a combination of clinical, genetic and animal paradigms will be needed to understand the links between circadian biology and metabolism and to tailor preventive interventions and therapies for humans.

Future horizons and implications of time

Summer 2012 marked a four-year cycle when we celebrated breaking physical boundaries at the Olympic Games; we also saw NASA's Voyager 1 spacecraft reach the farthest distance a man-made object has journeyed from our planet. Despite the marvel of progress that these images conjure, in our realistic moments we are reminded of the primeval constraints of our simple origins on the surface of Earth, none more fundamental than the daily alternation of light and darkness. Indeed, recognition of this environmental pressure has marked thinking about evolution ever since Darwinian times, despite the abstraction of space travel. What is the meaning of this pervasive timescale?

In green plants, circadian cycles represent an innovation to the dominant constraint of time. Namely, sessile plants use clocks to defend against DNA damage during exposure to sunlight, while optimizing oxygenic photosynthesis. In animals, the circadian system also provides flexibility in response to environmental challenge, but the solutions involve adaptations within both the nervous system and peripheral tissues. Nonetheless, some of the same toolkit has been deployed — including the PAS domain module, and the coupling of transcriptional oscillators to metabolic outputs. Epigenetic programs also fine-tune the clock across prokaryote and eukaryote lineages.

In the past century, we have also witnessed the invention of electric light, television, the jet engine and the Internet. But we are still unable to escape from the limits of inner time.

Although many gaps exist in our understanding, there is compelling evidence that points towards environmental disruptors of timing as agents of metabolic dysregulation (Fig. 5). Although abrogation through genetic mutation of the clock pathway is unlikely to explain common disease, the technology is now available to test how rare variants affect disorders such as type 2 diabetes, obesity and associated cardiometabolic complications. Clocks may inform stratification of risk of dyslipidaemia, microalbuminuria, retinopathy, neuropathy and cardiomyopathy. The discovery of circadian variants that affect glucose homeostasis, gluconeogenesis and β-cell function raises the possibility that pharmacological modification of molecular clock function may have therapeutic benefits. Given the window that circadian systems provide to understanding the partitioning and flux of fuel across different phases of the fasting–feeding cycle, it is likely that insight into the clock system may also provide an understanding of metabolic fate. This may include processes such as cellular regeneration and proliferation, and the switch from quiescent to active states in haematopoietic tissues. Although we are fixed in time by the clock in our genes, it is reasonable to predict that drugs, and even nutraceutical interventions, may soon be in hand to selectively alter time — even within specific tissues and cells — as a means of improving robustness, adaptability and health. ■

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