Crosstalk between metabolism and circadian clocks

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METABOLIC SIGNALLING

Crosstalk between metabolism and circadian clocks

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Abstract | Humans, like all mammals, partition their daily behaviour into activity (wakefulness) and rest (sleep) phases that differ largely in their metabolic requirements. The circadian clock evolved as an autonomous timekeeping system that aligns behavioural patterns with the solar day and supports the body functions by anticipating and coordinating the required metabolic programmes. The key component of this synchronization is a master clock in the brain, which responds to light–darkness cues from the environment. However, to achieve circadian control of the entire organism, each cell of the body is equipped with its own circadian oscillator that is controlled by the master clock and confers rhythmicity to individual cells and organs through the control of rate-limiting steps of metabolic programmes. Importantly, metabolic regulation is not a mere output function of the circadian system, but nutrient, energy and redox levels signal back to cellular clocks in order to reinforce circadian rhythmicity and to adapt physiology to temporal tissue-specific needs. Thus, multiple systemic and molecular mechanisms exist that connect the circadian clock with metabolism at all levels, from cellular organelles to the whole organism, and deregulation of this circadian–metabolic crosstalk can lead to various pathologies.

Phase
The relative position of the internal circadian clock time to the external time.

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Metabolism is the network of biochemical reactions that organisms employ to transform molecules with the purpose of generating energy and structural building blocks. Fundamental principles of metabolic regulation are highly conserved in all forms of life, and metabolic control transcends every aspect of cellular physiology, from the beginning of life to its end. Gross changes of cell fate as they happen during development and growth[1], tumorigenesis[2] or ageing[3] are accompanied by wholesale rewiring of cellular metabolism. Moreover, discrepancies between the metabolic requirements and the metabolic capacity of an organism are associated with a wide variety of pathologies, including metabolic syndrome and type 2 diabetes[4]. Overall, metabolic adaptability is essential to respond to external cues in a frequently changing environment.

All organisms rely on the ability to respond to environmental challenges, and the capacity to predict such challenges dictates how organisms deal with them. A singular intoxication with a food-borne pathogen, for example, invokes linear signal transduction cascades that trigger host defence pathways and ultimately lead to the elimination of the pathogen and its toxins by the immune system[5]. However, many foods contain low levels of naturally occurring toxins, which need to be neutralized by the animal to prevent pathology. Thus, as animals ingest food only during their activity phase, it is beneficial to activate certain detoxification mechanisms every day at roughly the same times that coincide with the feeding times[6]. Such events are highly predictable because of their periodic nature, and the timing of their recurrence largely depends on the solar cycle. However, an intrinsic, anticipatory mechanism is required to enable temporally programmed responses to environmental challenges. This mechanism is the circadian clock, which evolved in all light-sensitive organisms to provide rhythmic regulation to the vast majority of physiological and behavioural processes[7]. Circadian control is exerted at all levels, from the control of cellular and organellar metabolism[8] to the coordination of organ function in blood nutrient homeostasis[9] and the regulation of sleep–wake cycles in the brain[10]. However, not only does the circadian system impose rhythmicity on metabolic processes, metabolic signals and states strongly feed back to the circadian system. In other words, metabolism is not a mere output of circadian regulation but also provides important input to the circadian clock. This input–output feedback has turned out to be essential for providing circadian clocks with the necessary flexibility to adjust physiology to the metabolic requirements of cells, tissues and whole organisms.

In this Review, we focus on the reciprocal interplay between metabolism and the circadian clock and discuss various underlying mechanisms. The interactions between circadian rhythms and metabolism are extensive and can be covered from very different perspectives. We chose to provide the reader with regulatory principles of circadian metabolic rhythmicity and to place the involved processes

E-box elements
DNA elements (consensus sequence CANNTG) bound by transcription factors, most commonly basic helix–loop–helix domain-containing proteins.

into a broader systemic context. In doing so, we move from cellular to organ-specific and systemic regulation and point out circadian metabolic intersections at each level. We limit the discussion to mammalian clocks, and we highlight related human pathologies resulting from disturbances of circadian–metabolic crosstalk. Circadian metabolic regulation in other domains of life is the topic of many excellent review articles (see, for example, REFS[11–13]).

The mammalian circadian system

The molecular clockwork at the basis of circadian rhythmicity is present in most, if not all, fully differentiated cells of mammals. At the organismal level, circadian clocks are hierarchically structured and comprise distinct types of clock with different functions for the mammalian circadian system.

Core clock genes. The molecular circadian oscillator in all cells of the body relies on a transcriptional–translational feedback loop made of so-called clock genes (FIG. 1a). Period (*PER*) and cryptochrome (*CRY*) genes are activated by the transcription activators: circadian locomotor output cycles kaput (CLOCK), neuronal PAS domain protein 2 (NPAS2) and aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL1; more commonly known as BMAL1), which form CLOCK–BMAL1 and NPAS2–BMAL1 heterodimers. These complexes bind to E-box elements in promoter regions and activate the

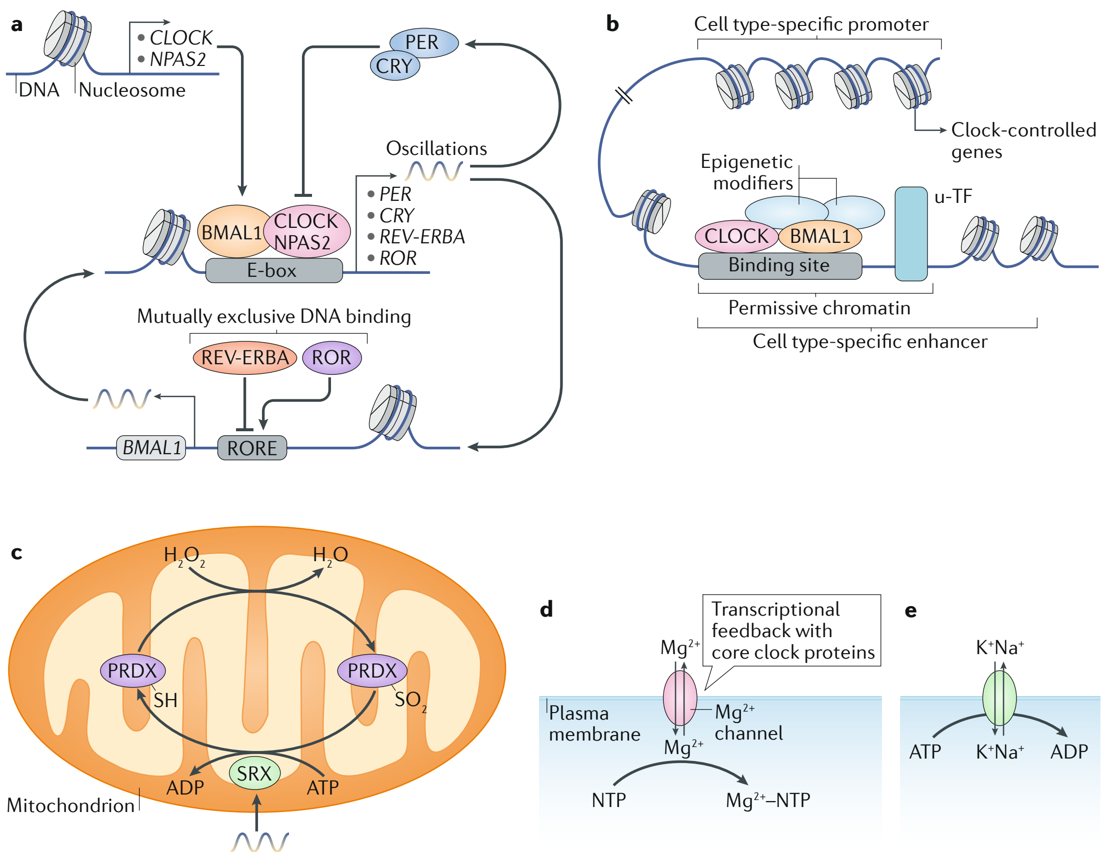


Fig. 1 | **Transcriptional and metabolic circadian oscillators. a** | Canonical transcriptional feedback loops in the core clock. Circadian locomotor output cycles kaput–aryl hydrocarbon receptor nuclear translocator-like protein 1 (CLOCK–BMAL1) and neuronal PAS domain protein 2 (NPAS2)–BMAL1 heterodimers activate the transcription of *PER*, *CRY*, *REV-ERBA* and *ROR* genes. Period (PER) and cryptochrome (CRY) proteins repress CLOCK–BMAL1-dependent and NPAS2–BMAL1-dependent transcription. Nuclear receptor subfamily 1 group D (REV-ERBA) and RAR-related orphan receptor (ROR) proteins drive rhythmic *BMAL1* transcription from ROR response elements (ROREs) in the promoter region. **b** | The clock proteins bind to cell-specific enhancers and establish complexes with various epigenetic regulators and universal transcription factors (u-TFs) to drive rhythmic expression of target genes. **c** | Peroxiredoxin (PRDX) oxidation cycles. PRDX is oxidized to the sulfinic acid form by hydrogen peroxide (H_2O_2) released from mitochondria. Hyperoxidized PRDX is reduced by sulfiredoxin (SRX), which is rhythmically imported into mitochondria. Hyperoxidized PRDX and SRX accumulate in antiphase in mitochondria with a period length of approximately 24 h[15,199–201]. **d** | Rhythmic magnesium (Mg^{2+}) ion accumulation. Oscillating Mg^{2+} levels appear to be primarily regulated via transcriptional regulation of magnesium channels by the canonical circadian oscillator and feed back to regulate its period, phase and amplitude. Mg^{2+} forms chemical complexes with nucleotide triphosphates (NTPs) and might thereby convey a circadian component to the availability of cellular energy equivalents[16]. **e** | Potassium (K^+) transport by the K^+/Na^+ pump is also cyclic and leads to circadian rhythms of membrane conductance and cytoplasmic conductivity[17]. The metabolic oscillations in parts **c**–**e** can take place in the absence of transcription and therefore function a priori independently of the canonical transcriptional feedback loop shown in part **a**. The mechanistic connections between metabolic and transcriptional oscillators as well as between the different metabolic oscillators have not been identified yet.

Box 1 | **Food consumption and the clock**

Food has an outstanding role in the mammalian circadian clockwork. First, rhythmic feeding–fasting cycles are dominant synchronizing signals for peripheral clocks. Most likely by simultaneously activating a plethora of molecular entrainment pathways, each of which is capable of resetting peripheral clocks by itself, the concerted action of all signals triggered by rhythmic food consumption is extremely potent. In fact, restricted feeding schedules, namely, limiting the food availability to a certain time of the day with phases different from the light–dark cycle, can fully uncouple peripheral oscillators from the master clock in the suprachiasmatic nucleus (SCN)[21,174] (see also FIG. 2b). The possibility to fully uncouple the phase of the core clock in the SCN from that of peripheral clocks by time-restricted feeding is often held as sufficient evidence that there is no metabolic feedback regulation to the master clock through peripheral metabolism. This view is challenged by the finding that core clock gene expression in the SCN can, in fact, be altered by hypocaloric time-restricted feeding[175] and that caloric restriction by itself induces mice to choose their own feeding times and enhances locomotor activity during the rest phase[176]. Therefore, metabolic cues, modulated by diet, seem to reach and signal to the SCN and modify selected output pathways of the clock. As an example, fibroblast growth factor 21 (FGF21) — a liver-derived starvation hormone with pleiotropic metabolic functions in carbohydrate and fatty acid metabolism[177] — binds to its receptors in the SCN to modify glucocorticoid secretion and alter circadian locomotor activity[178]. The SCN also expresses receptors for leptin[179] and ghrelin[180], and leptin-receptor mutant (*db/db*) mice are behaviourally arrhythmic and display altered photic responses in the SCN, in addition to being obese and diabetic[181]. Moreover, the SCN is bidirectionally connected to other hypothalamic nuclei, such as the arcuate nucleus, which relays metabolic states to the SCN and regulates feeding behaviour[20]. There is ample evidence that such hypothalamic areas in the immediate vicinity of the SCN are readily food entrainable under normocaloric conditions[182] and convey circadian signals to peripheral organs[183].

In peripheral tissues, food composition and timing of consumption have a profound influence on the circadian clock beyond the function of food as an entrainment cue. Mice fed a high-fat diet display changes at all levels of circadian organization[184]. The period length of their locomotor activity changes, their eating behaviour is altered and they consume food almost evenly throughout the day[75]. Additionally, metabolite rhythmicity is lost in the serum[185], and in cells, the expression levels of core clock and clock-dependent genes become deregulated[75]. Interestingly, these changes manifest themselves only when food is provided ad libitum. Time-restricted feeding prevents obesity, hyperinsulinaemia and hepatic steatosis associated with a high-fat diet, suggesting that the lack of regular feeding–fasting rhythms is likely a major reason for the adverse changes caused by a western diet in animal models[186,187]. Restricting food to the activity phase also alleviated pathological metabolic consequences in a rat model of shift work[188]. Even already manifested effects of a high-fat diet on the phase of the peripheral clocks can be reversed by switching back to a low-fat diet[189]. These results are in accordance with a study showing that direct regulation of rhythmic gene expression by feeding–fasting cycles drives a bigger part of oscillating genes in liver than does the core clock alone[60]. They also help to explain the success of increasingly popular diets based on intermittent fasting and other nutritional interventions that are considered a priori to be beneficial for metabolic health. Recently, the ketogenic diet has been shown to work in part through modulation of core clock gene expression in peripheral tissues[190].

transcription of *PER* and *CRY* genes. PER and CRY proteins are imported into the nucleus and repress the transcription of their own gene loci, whereupon a new circadian cycle can begin. A second feedback loop provides additional robustness to the oscillatory mechanism and consists of nuclear receptors of the nuclear receptor subfamily 1 group D (NR1D; more commonly known as REV-ERBA) and RAR-related orphan receptor (ROR) families, which are likewise activated by components of the core clock oscillator and drive rhythmic *BMAL1* expression from ROR response element binding sites (RORE binding sites). These intricate feedback loops generate rhythms with a period of about a day and are accordingly termed circadian (*circa diem*, which is Latin for 'about a day'). Clock factors also bind to cell-specific enhancers and establish complexes with various epigenetic regulators to drive rhythmic expression of target genes outside the core clock mechanism, so-called clock-controlled genes (FIG. 1b; see also below). Overall, the circadian oscillator is a highly complex molecular machinery comprising many components, whose exhaustive description is beyond the scope of this Review and can be found elsewhere[14].

In recent years, several intriguing studies have broadened our view on the core clock mechanism. Metabolic oscillations autonomous of the circadian clock seem to exist in cells of higher organisms, implicating peroxiredoxins[15] and magnesium[16] and potassium[17] channels in the generation of cellular rhythmicity (FIG. 1c–e). However, the molecular make-up of these transcription-independent oscillators, their relation to the core clock and their biological importance are unresolved to date.

A master clock controls peripheral clocks. Molecular oscillators as described above are present in all cells of the body. They are commonly referred to as peripheral oscillators, and they confer circadian rhythmicity to a plethora of tissue-specific functions, such as detoxification in gastrointestinal organs, urine production in the kidney and heart rate regulation. Genomic and proteomic expression studies revealed that they are driven by rhythmic mRNA and protein levels of rate-limiting enzymes and regulators and that oscillations of up to 40% of all genes in peripheral tissues exhibit circadian rhythmicity[18].

Peripheral clocks are under the control of the master clock in the brain, which employs the same molecular machinery made of clock genes to generate rhythmicity. The master clock is composed of 10,000–20,000 neurons located in the suprachiasmatic nucleus (SCN) in the hypothalamus and is synchronized with the external time by light through melanopsin-containing retinal ganglion cells and the retinohypothalamic tract[19]. The SCN transduces daytime information to all other clocks in the body via a variety of communication routes, including, among others, endocrine and neuronal pathways. These signals also synchronize surrounding brain regions in the hypothalamus that control a plethora of behavioural and vegetative body functions. Through control of these potent pleiotropic axes, the SCN reaches all organs and cells in the body[20].

Signals with the capacity to permanently change the phase of a circadian oscillator are called Zeitgeber (German for 'time giver') signals, and the whole process is referred to as entrainment. The major output pathway of the SCN for entrainment of the periphery is the regulation of the sleep–wake cycle. Cycles of activity inevitably determine the pattern of feeding and fasting, and such rhythmic feeding cues appear to be a dominant Zeitgeber, at least in metabolically active tissues such as liver and adipose tissue (BOX 1; FIG. 2a). Accordingly, changes in feeding patterns (switching food intake to the normally inactive phase) desynchronize the peripheral clocks with respect to the SCN[21] (FIG. 2b). Other rhythms with the potential to synchronize peripheral oscillators, including cycles of melatonin secretion (repressed by light), core body temperature regulation (with trough levels during the early morning and peak levels during the day), oxygen delivery to peripheral tissues (high during activity and low at rest) or cyclic glucocorticoid production (with peak

Photic responses
Light-induced molecular changes in cells that contribute to photoentrainment in the suprachiasmatic nucleus.

Arcuate nucleus
A hypothalamic nucleus that contains neuroendocrine and centrally projecting neurons and that has pre-eminent roles in central homeostatic processes, such as energy metabolism.

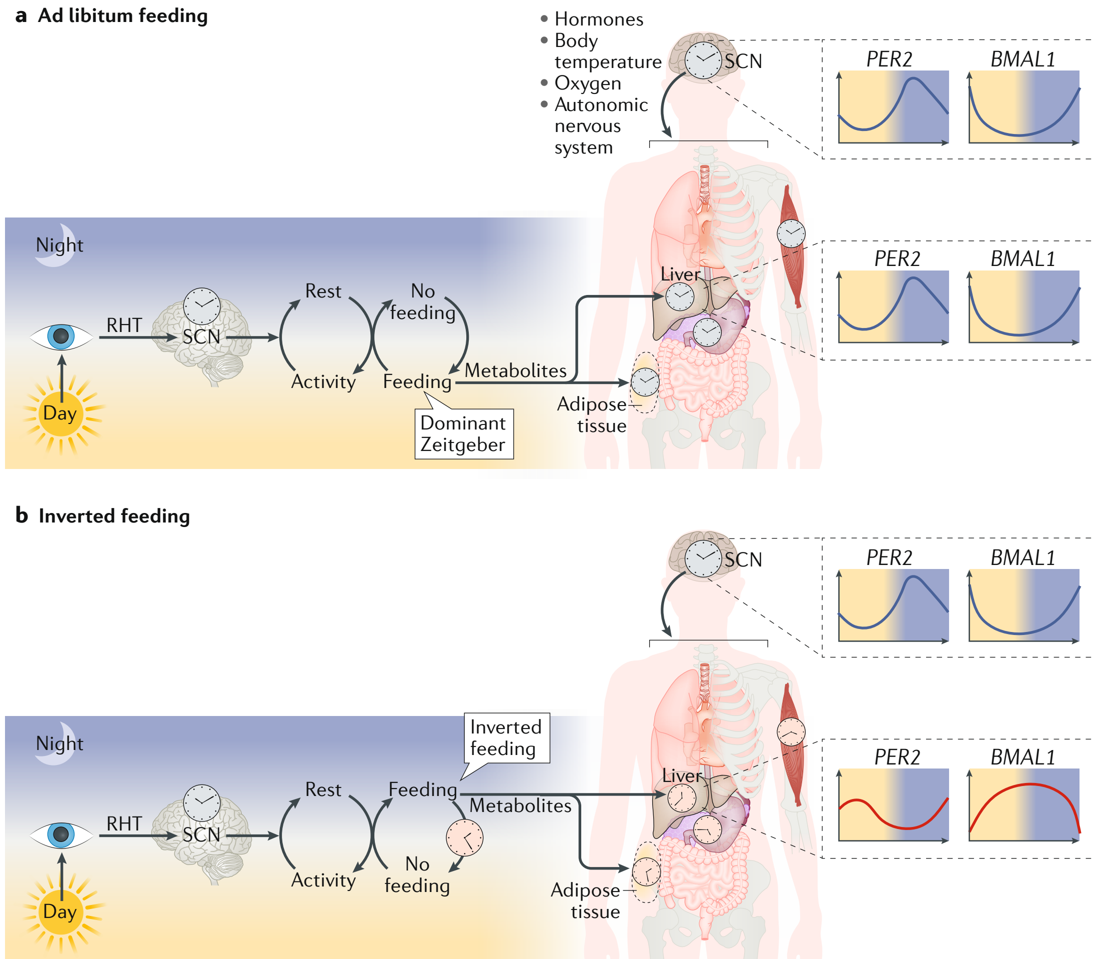


Fig. 2 | **Entrainment of the clock by light and food. a** | Light entrains the central clock in the suprachiasmatic nucleus (SCN) in the brain through the retinohypothalamic tract (RHT). The SCN drives diurnal rhythms of rest and activity that determine feeding–fasting cycles. Metabolic signals induced by rhythmic food consumption together with hormonal and neuronal signals from the SCN synchronize clocks in peripheral organs with the clock in the SCN. **b** | Feeding rhythms are dominant Zeitgebers (at least for the metabolic tissues, for example, the adipose tissue and liver) and can uncouple peripheral organ clocks from the SCN and behavioural rhythms. This can be observed, for example, when feeding patterns are inverted (feeding during the inactive phase). The graphs on the right schematically represent mRNA expression of *PER2* and *BMAL1* in the SCN and in liver under ad libitum (synchronized clocks) or inverted feeding (desynchronized clocks) conditions.

levels at the start of the activity phase), are also driven by the SCN[22]. The capacity for entrainment by a specific Zeitgeber can be tissue-specific, or Zeitgeber signals may act on many tissues, as might be the case for activation of serum response factor (SRF), which is broadly inducible by serum-borne proteins[23]. The mechanisms underlying the metabolic entrainment of peripheral clocks have been partially resolved and are discussed below.

Cellular clocks

According to current knowledge, the minimal unit for autonomous circadian rhythmicity in eukaryotes is a single cell. Cellular clocks can generate rhythmicity of most metabolic functions independently of rhythmic systemic signals generated elsewhere in the body, which are, nevertheless, important for whole-body synchronization of clocks. There is also emerging evidence that the composition of cellular building blocks (such as proteins and lipids) and organelle function follow circadian rhythmicity. Here, we specifically discuss recent studies showing clock-dependent regulation of mitochondrial function and composition.

Cell-autonomous metabolic rhythms. The discovery of self-sustained circadian oscillations in cultured cells has opened the door for experiments addressing circadian clock function independent of the influence of the extracellular milieu, surrounding tissues or other organs[24]. A recent analysis of the circadian transcriptome and metabolome in cultured cells revealed that a substantial fraction (up to 28%) of all quantified metabolites are rhythmic, clock-controlled and cell type-specific and include amino acids and intermediary metabolites of NAD biosynthetic pathways[25]. Moreover, genetic perturbation of the core clock oscillator can alter metabolite rhythmicity and vice versa, supporting a model in which metabolic rhythmicity and transcriptional oscillations are coupled in cultured cells. Likewise, experiments performed with human

Ketogenic diet
A high-fat, low-carbohydrate diet that results in elevated levels of ketone bodies in the circulation by promoting the metabolism of lipids over the use of carbohydrates for energy generation.

RORE binding sites
DNA elements (consensus sequence AGGTCA preceded by a 5 bp AT-rich sequence) bound by transcription factors from the RAR-related orphan receptor (ROR) and nuclear receptor subfamily 1 group D (REV-ERBA) nuclear receptor families.

Peroxiredoxins
Ubiquitous, small (20–30 kDa) antioxidant enzymes that catalyse the reduction of hydroperoxides, toxic by-products of aerobic respiration, to alcohols.

Melanopsin
Opsin of intrinsically photoactive retinal ganglion cells involved in non-image-forming visual functions including light-entrainment of the suprachiasmatic nucleus.

Retinal ganglion cells
Neurons in the ganglion cell layer of the retina; their axons form the optic nerve and the retinohypothalamic tract.

myotubes cultured in vitro revealed that the fraction of oscillating lipid metabolites is comparable between muscle tissue and cultured myotubes. In addition, temporal lipid profiles correlated with transcript profiles of genes implicated in their biosynthesis and were strongly attenuated upon core clock disruption[26], providing evidence that metabolic rhythmicity is a clock-driven process that is sustained after extraction from the tissue. Apart from metabolites, autophagy, which is a catabolic process that breaks down macromolecules, for example, in response to starvation, and thereby provides new building blocks for anabolic pathways and supports cell survival, has been shown to be a rhythmic cell-autonomous process also controlled by the circadian clock[27].

Circadian control of mitochondria. Mitochondria are the powerhouses of eukaryotic cells. In addition to their role in the generation of ATP, mitochondria are involved in elementary cellular processes such as lipid biosynthesis, calcium homeostasis and apoptosis. Because of the widespread role of mitochondria in growth and homeostasis, mitochondrial dysfunction is linked to serious diseases including myopathies, neuropathies and cardiovascular disorders[28]. Although various aspects of circadian metabolic regulation have been extensively studied at the cellular level, our knowledge regarding subcellular clock-dependent processes including circadian mitochondrial regulation is just emerging. However, recent evidence supports the existence of daily rhythms in mitochondrial content, dynamics and function, such as respiration and ATP production, and thereby indicates widespread interplay between the circadian clock and organellar metabolism (FIG. 3).

Loss-of-function studies revealed that clock genes regulate a variety of mitochondrial functions. Oxygen consumption rate (OCR) of whole cells or isolated mitochondria can be used to assess mitochondrial nutrient utilization and respiration and was compared between wild-type and clock gene-deficient mice in various experimental paradigms. Mitochondria isolated from the livers of *Bmal1*-knockout mice[29], liver-specific *Bmal1*-knockout mice[30] and *Per1* and *Per2* double-knockout mice[8] exhibited lower OCRs than the respective wild-type strains (FIG. 3). Fatty acid oxidation is reduced in *Bmal1*-knockout mice[29], and the mitochondrial fatty acid composition as well as its metabolism is dependent on *Bmal1* (REF.[29]) (FIG. 3). Likewise, hepatocytes isolated from mice throughout the day exhibit an increased respiration rate during the dark phase compared with the light phase that is dependent on *Bmal1* (REF.[30]). Additionally, mitochondria of liver-specific *Bmal1*-knockout mice are bigger and more rounded, and they maintain a similar morphology throughout the day, whereas wild-type mitochondria show cyclical changes in morphology between day and night[30]. Given that fission and fusion regulate mitochondrial function and that respiration is less efficient in fragmented mitochondria, the morphological changes in mitochondria of *Bmal1*-knockout animals support the observed functional changes in mitochondrial respiration[31]. The dependency of mitochondrial morphology on clock genes was corroborated in mouse skeletal muscle[32] and heart[33] and is linked to impaired mitochondrial function in these organs upon clock perturbation. These cycles of fission and fusion and, consequently, the rhythms of energy production are regulated by dynamin-related protein 1 (DRP1), which is a key mediator of fission (FIG. 3). Notably, activity of DRP1 is induced by phosphorylation, which occurs in a circadian manner to drive mitochondrial network fragmentation. In turn, suppression of mitochondrial fission eliminates circadian ATP production and feeds back to the core circadian oscillator to regulate circadian rhythmicity[34]. Notably, the overall number of mitochondria is constant and independent of clock genes[8,30,33,35].

Further evidence that mitochondrial function is regulated by the circadian clock stems from measurements of synchronized muscle cells in culture, which exhibit cyclic changes in OCR within an approximately 24 h period[29]. Moreover, mitochondria isolated from mice around the clock use nutrients differently depending on the time of day. In the presence of certain fatty

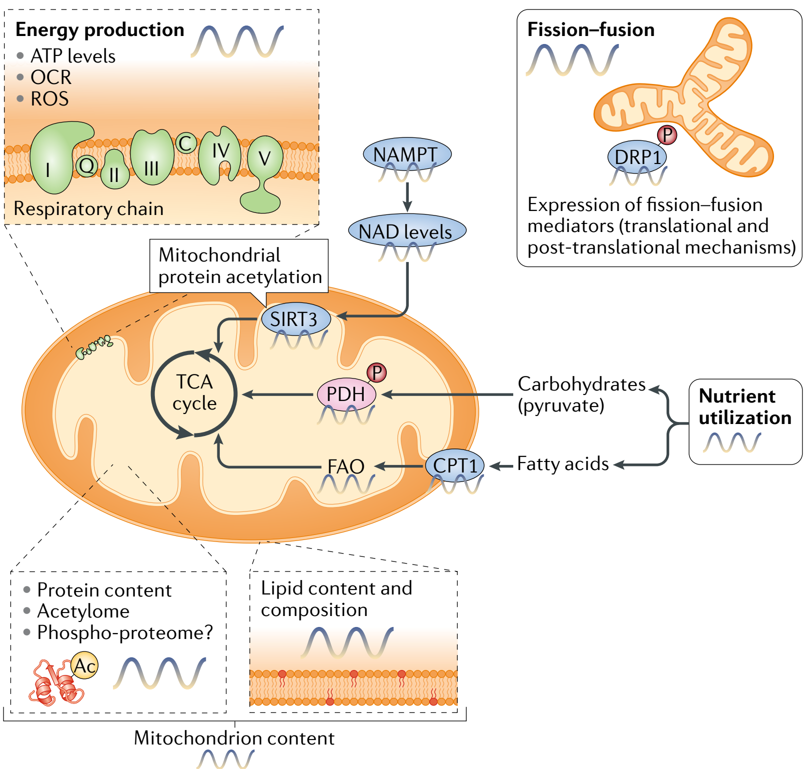


Fig. 3 | **Circadian control of mitochondria.** Mitochondrial composition and function show circadian rhythmicity. ATP production, oxygen consumption rate (OCR) and reactive oxygen species (ROS) production have been shown to oscillate. Furthermore, mitochondrial fission–fusion cycles, which regulate various aspects of mitochondrial biology, show circadian rhythmicity. Rhythmic fission–fusion dynamics are due to circadian regulation of phosphorylation (P) and activity of dynamin-related protein 1 (DRP1), which is a key mediator of fission, and also by circadian regulation of expression of other regulators of mitochondrial dynamics. In addition, utilization of nutrients by mitochondria follows a circadian pattern, which is due to circadian control of the NAD-dependent deacetylase sirtuin 3 (SIRT3) and rate limiting enzymes such as carnitine palmitoyltransferase 1 (CPT1) for lipids and pyruvate dehydrogenase (PDH) for carbohydrates. Moreover, mitochondrial lipidome and proteome show circadian oscillations. Finally, patterns of specific protein post-translational modifications such as acetylation (but likely also others) show circadian oscillations. Rhythmic acetylation could be directly regulated by circadian locomotor output cycles kaput (CLOCK), which has acetyltransferase activity. FAO, fatty acid oxidation; NAMPT, nicotinamide phosphoribosyltransferase; TCA, tricarboxylic acid.

Melatonin
A pineal hormone that regulates circadian rhythms and wakefulness; its synthesis is suppressed by light.

acids, for example, palmitoylcarnitine, mitochondria exhibit rhythmic respiration with peak levels during the transition from the active phase to the rest phase, likely owing to the synchronous rhythmicity of carnitine palmitoyltransferase 1 (CPT1), the rate-limiting enzyme in mitochondrial fatty acid uptake (FIG. 3). Pyruvate utilization is also rhythmic but peaks later during the rest phase, likely through rhythmic pyruvate dehydrogenase (PDH) levels and/or phosphorylation, which regulates its activity[8] (FIG. 3).

Remarkably, the type of nutrition (for example, regular chow versus high-fat diet) and the timing of food consumption control mitochondrial respiratory rhythms, and each parameter differentially affects the oscillations of key mitochondrial enzymes and their respective substrate utilization[8]. In synopsis, these studies indicate that mitochondrial respiration exhibits circadian rhythmicity, which is fine-tuned by the molecular oscillator, feeding time and diet composition.

Lipids define the physical qualities of mitochondrial membranes and determine their protein composition[36]. In addition, they serve as a major energy source for mitochondrial respiration, and several lipids are synthesized in mitochondria. High-throughput lipidomic analyses on isolated mitochondria from mouse liver revealed that approximately one-third of all lipids present in mitochondria accumulate in a circadian manner[37]. Similar to respiratory rhythmicity, both the composition and phase of rhythmic lipids depend on the circadian clock and feeding time. In mice fed ad libitum, the majority of mitochondrial lipids reach their peak levels at the transition between the light phase and the dark phase (transition between the inactive phase and the active phase in mice), whereas limiting food consumption exclusively to the dark phase inverts the phase of these rhythms. Moreover, in animals deficient in PER1 and PER2, mitochondria-associated oscillating lipids exhibit a wide range of peak times without an overt phase, corroborating the role of the circadian clock in the coordination of mitochondrial lipid accumulation[37] (FIG. 3).

Whole liver proteomics[38,39] and proteomic analyses of isolated mitochondria[8] revealed extensive circadian changes in the mitochondrial proteome. More than one-third of mitochondrial proteins exhibit daily rhythmicity, and the majority of them reach their peak levels at dawn[8]. Several rate-limiting enzymes in catabolic and oxidative functions of mitochondria are rhythmic and enable mitochondria to use various nutrients (for example, carbohydrates and fatty acids) in a daytime-dependent manner[8]. While the transcript levels of some nuclear-encoded mitochondrial proteins are deregulated in clock gene mutant mice[33,40], and BMAL1 binds to their promoters[30,41], a global analysis showed only a weak correlation between the phase of the mitochondrial proteome and its respective transcriptome, pointing towards the important involvement of post-transcriptional mechanisms in circadian control of mitochondrial content[8]. Indeed, it has been shown that post-translational modifications heavily influence mitochondrial dynamics and functions. A global acetylome analysis of mouse liver revealed daily changes in the acetylation status of many mitochondrial proteins, with enrichment for CLOCK-dependent acetylation sites within the Krebs cycle and glutathione metabolism enzymes[42]. Furthermore, the acetylation status of many mitochondrial proteins is changed in *Bmal1*-knockout mice[29,43,44]. It appears, therefore, that mitochondrial protein acetylation is under circadian clock control, which raises the question of whether additional post-translational modifications, such as phosphorylation, are likewise rhythmic (FIG. 3).

Circadian metabolism in organs

As the circadian clock ticks in almost every cell of the body, it is not surprising that rhythmic regulation of metabolism has been found in most organs. Almost 40% of all genes in kidney are rhythmically expressed, a proportion that is rivalled only by genes in the liver as the most rhythmic tissue to date (see more detailed discussion below)[18]. Generally, the circadian clock regulates some of the most prominent physiological functions in each organ. For example, in kidney, the circadian clock modulates blood flow, glomerular filtration rate and ion and water excretion, which is controlled, to a large degree, by the rhythmic expression of membrane transport proteins[45]. In the pancreas, excretion of both insulin and glucagon is under circadian control[46], whereas in the muscle, respiration and autophagy — processes that enable robust energy production and maintenance of organellar homeostasis and proteostasis, respectively, in highly metabolic tissue — follow circadian rhythmicity[47]. Equally important is the rhythmic synthesis and excretion of hormones and other active molecules in peripheral tissues, which provide inter-organ crosstalk by driving metabolic programmes at distant locations in the body. Examples of clock-controlled hormones and their main functions are shown in TABLE 1. Additionally, the gastrointestinal tract exerts all metabolic and endocrine functions in close collaboration with the microbiome, the composition of which also follows diurnal rhythmicity in synchrony with the clock of the host (BOX 2).

A central organ with a large repertoire of diverse physiological functions, and probably the best studied model organ to date in circadian biology, is the liver[48]. Among its many functions are the metabolism of carbohydrates, amino acids, lipids and bile acids and the synthesis of detoxification enzymes, hormones, bile components and blood coagulation factors[6]. At the molecular level, the pervasive circadian control of liver metabolic function is achieved through both transcriptional and post-transcriptional mechanisms. Global mRNA expression analyses alongside chromatin immunoprecipitation followed by deep sequencing (ChIP–seq) studies revealed that the central regulatory nodes of the core metabolic pathways of liver are under circadian control[41,49–52]. Subsequent proteomic, metabolomic and lipidomic analyses complemented these studies and revealed a widespread influence of post-translational mechanisms on circadian liver metabolism[18,39,41,53–56].

Glucose metabolism is a good example to illustrate the intricate circadian regulation of peripheral metabolism, and the liver is critically involved in glucose homeostasis, in which its main role seems to be the

Table 1 | **Circadian systemic regulation by hormones and blood gases**

Origin	Circadian-regulated hormone or blood gas	Main function	Refs
Muscle	Myokines	Exercise-associated metabolic regulation	204
Pancreas	Glucagon	Glucose and lipid metabolism	46
	Insulin	Glucose and lipid metabolism	46
Thyroid	Triiodothyronine	Metabolic rate	205
Brain	TRH and TSH	Thyroid hormone production	205
	FSH and LH	Reproductive system	206
	ACTH	Corticosteroid production	207
	Prolactin	Reproductive system	208
	Melatonin	Circadian clock and sleep	209
Lung	Oxygen	Energy expenditure	89
	Carbon dioxide	Energy expenditure; pH buffering	89
Liver	Bile acids	Lipid absorption	210
	FGF21	Nutrient metabolism	178
Kidney	Renin	Blood pressure; osmoregulation	211
	Angiotensin	Blood pressure; osmoregulation	211
	Corticosteroids	Nutrient metabolism; immune system	207
Adipose tissue	Leptin	Energy expenditure; hunger regulation	160
Stomach	Ghrelin	Energy expenditure; hunger regulation	160

ACTH, adrenocorticotropic hormone; FGF21, fibroblast growth factor 21; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

buffering of blood glucose fluctuations that arise owing to SCN-regulated rhythmic food consumption[9]. Cell-autonomous hepatic clocks and their output pathways heavily influence glucose production and breakdown through different mechanisms. The expression levels of glucose transporters and the glucagon receptor peak at the beginning of the activity phase, which coincides with maximal food consumption[49,50]. The storage of excess carbohydrates in the form of glycogen polymers at this time of the day is anticipated by the circadian system through CLOCK-dependent expression of the liver-specific isoform of glycogen synthase[57]. However, the synthesis of glucose via gluconeogenesis in times of low glucose availability, typically during rest, is stimulated by glucocorticoids, and this process is controlled by CRY proteins through repression of the glucocorticoid receptor by protein–protein interaction[58,59]. Rhythmic gluconeogenesis is further regulated by cryptochromes, which also modulate the signalling outcome from the glucagon receptor. Activation of the glucagon receptor leads to the production of cAMP, which mediates the circadian phosphorylation of cAMP response element-binding protein (CREB) and its transcriptional activity[60], including control of expression of the key gluconeogenic gene phosphoenolpyruvate carboxykinase 1 (*PCK1*)[61]. Cryptochromes inhibit cAMP accumulation by negatively modulating heterotrimeric G proteins, which transduce the signal from the glucocorticoid receptor, thereby lowering the expression of PCK1 (FIG. 4a).

Glucose metabolism is additionally controlled by the nuclear receptor REV-ERBAα[62], which has emerged in recent years as a core clock component and a central regulator at the interface between the circadian clock and metabolic regulation in liver[63]. REV-ERBAα is rhythmically expressed through CLOCK–BMAL1-dependent transcriptional activation in one arm of the core oscillator (FIG. 1a). It feeds back on core clock gene expression by competing with RORα and RORγ for binding to RORE and RevDR2 elements in clock gene promoters[64]. Notably, transcriptional regulation of the metabolic output functions of REV-ERBAα is largely independent of its DNA-binding activity. Rather, rhythmically expressed REV-ERBAα associates with the liver-specific transcription factor hepatocyte nuclear factor 4α (HNF4α) or HNF6 at their respective binding sites in target gene promoters and uses histone deacetylase 3 (HDAC3) and other co-repressors to regulate the transcription of metabolic genes, for example, ELOVL (elongation of very long chain fatty acids) fatty acid elongase 5 (ELOVL5) or the acyl-CoA synthetase short-chain family member 3 (ACSS3)[65]. In lipid metabolism, coordinated binding of REV-ERBAα and HDAC3 to lipid biosynthetic genes is essential to prevent hepatic steatosis by limiting lipid biosynthesis and toxic lipid accumulation during the feeding phase[66]. REV-ERBAα and HDAC3 also regulate the expression of 1-acyl-glycerol-3-phosphate acyltransferase (AGPAT) and phosphatidate phosphatase lipin (LPIN), enzymes of the glycerol 3-phosphate pathway that control glycerol and lipid metabolism and triglyceride accumulation in the liver. Additionally, both REV-ERBAα and insulin-induced LPIN phosphorylation control the activity of sterol regulatory element-binding transcription factor (SREBF; also known as SREBP), a central regulator of cholesterol metabolism[67,68]. REV-ERBAα is also highly expressed in skeletal muscle cells, where it regulates mitochondrial function and autophagy[69]. Interestingly, REV-ERBAα binds to haem[62], and its antagonists, RORα and RORγ, have a high affinity for oxysterols[70] (FIG. 4b), indicating the existence of a wide network of protein–metabolite interactions in the circadian regulation of metabolism. In addition to REV-ERBAα, REV-ERBAβ, RORα and RORγ, which are part of the core clock mechanism, almost one-half of all nuclear receptors are rhythmically expressed in liver[71]. These receptors, for example, peroxisome proliferator-activated receptors (PPARs) and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), shape metabolism through the regulation of gene expression programmes in concert with the core clock transcription factors PER2 (REF. 72), CRY1 and CRY2 (REFS 73,74), in part, through protein–protein interactions with core clock transcription factors and, in part, by co-regulating gene expression programmes (FIG. 4b).

Systemic metabolic rhythmicity

At the systemic level, the supply of cells and tissues with nutrients and oxygen exhibits diurnal fluctuations owing to varying uptake volumes during the activity phase (intense uptake) and the rest phase (reduced or no uptake of nutrients). The circadian system buffers these fluctuations by driving the rhythmic uptake and the release mechanisms of metabolites and blood gases into cells of peripheral organs and concurrently uses

Heterotrimeric G proteins
Membrane-associated GTP-binding and/or GDP-binding signalling proteins consisting of three subunits α, β and γ.

RevDR2 elements
DNA elements (direct repeats of two AGGTCA motifs separated by 2 bp) bound by transcription factors from the RAR-related orphan receptor (ROR) and nuclear receptor subfamily 1 group D (REV-ERBA) nuclear receptor families.

Haem
A porphyrin complex with a central iron atom that can bind and transport diatomic gases and can be used as a redox partner in electron transfer reactions.

Oxysterols
Oxidized derivatives of cholesterol with biological activity, for example, as binding partners for nuclear receptors.

Box 2 | Links between the gut microbiome and the clock

The gut microbiome is now well established to contribute to the regulation of the host's physiology[191]. Notably, Toll-like receptors, which have been shown to integrate nutritional and bacterially derived signals to regulate intestinal metabolism, are under the control of the intestinal circadian clock[192]. Furthermore, approximately 15% of all bacterial operational taxonomic units fluctuate in a diurnal manner[193] with a higher net biomass during the active phase[194]. Diurnal changes of the microbiome depend on a functional circadian clock of the host, probably via the control of rhythmic feeding behaviour[193,194]. Time-restricted feeding can restore the rhythmicity of the microbiome disturbed by clock perturbations (clock gene deletion or jet lag), albeit only partially[193,195]. Strikingly, germ-free mice display defects in metabolism as well as in circadian clock function under normal and high-fat feeding conditions[196,197]. Furthermore, the circadian rhythmicity of the microbiome conveys diurnal fluctuations of physiological processes and disease susceptibility to its host by driving transcriptomic, epigenetic and metabolite oscillations in the intestinal epithelium[198]. Thus, the response of the circadian clock to metabolic challenges is importantly affected by the microbiome, which in turn is regulated by the clock through the timing of food intake.

systemic metabolite and blood gas levels as Zeitgebers for peripheral clocks.

Circadian control of systemic metabolite levels. A direct consequence of diurnal behavioural rhythmicity is that nutrient flux in the bloodstream varies considerably depending on the time of day. For obvious reasons, food is mainly consumed during the activity phase — approximately 80% in mice[75] and (ideally) 100% in humans[76] — whereas internal energy stores are tapped during the rest phase to replenish declining nutrient levels. Consequently, the levels of most intermediary metabolites, including glucose[77], amino acids[78] and lipids[79], oscillate in the blood with peak levels during wakefulness and activity. As these oscillations are synchronous with environmental time, our body uses clock-controlled mechanisms of cellular metabolite uptake and release to align with the environmental inputs and to buffer excessive fluctuations[80], which can have damaging effects on the body. Here, again, the circadian regulation of blood glucose levels highlights the importance of clocks not only for cellular glucose metabolism but also for the intricate mechanisms through which clocks in different organs, mainly in the hypothalamus, liver, pancreas and skeletal muscle, act in concert to control glucose homeostasis.

The SCN is the principal driver of circadian blood glucose fluctuations by scheduling food ingestion to the activity phases (FIG. 2). In these times of relative nutrient abundance, muscle and liver cells take up glucose via the insulin-dependent transporters: GLUT4 (also known as SLC2A4) and GLUT2 (also known as SLC2A2), respectively, whose expression is co-regulated by the clock[81]. Low glucose levels during fasting in the rest phase are replenished via circadian glucose excretion from the liver, which is mediated by clock-dependent expression of GLUT2, which peaks during the rest (fasting) phase[9]. The pancreas supports the transporter-based regulation of glucose uptake and release from tissues by clock-dependent rhythmic insulin and glucagon excretion[46,82,83], which is paralleled by clock-regulated rhythmic insulin sensitivity and insulin signalling in peripheral tissues[84,85] (FIG. 4a). Therefore, it is the interplay of clock-controlled uptake and release processes in different organs that ultimately maintains blood glucose homeostasis throughout the day.

The regulation of blood glucose levels demonstrates the complexity of systemic circadian regulation and the frequently applied principle that multiple clocks in various organs work together to maintain systemic homeostasis. Hence, do these concepts also apply to other blood metabolites, for example, the levels of amino acids and lipids[86]? Much less is known about circadian systemic regulation of metabolites other than glucose, and tissue-specific knockout studies that could assign the role of individual organs in these processes are lacking. Nevertheless, what is known is that the synthesis of glutamine, which is released in considerable amounts from skeletal muscle as systemic fuel for other tissues, seems to be rhythmic owing to circadian activity of glutamine synthetase[87]. Similarly, various extracellular lipases are rhythmically expressed and should facilitate the circadian mobilization of fatty acids from lipoprotein particles for cellular uptake[88].

Rhythmicity of blood gases. Under aerobic conditions. mammalian cells generate energy preferably through mitochondrial respiration, in which carbohydrates, lipids and other intermediary metabolites are broken down and converted into chemical energy equivalents. In this process, oxygen is consumed through oxidative phosphorylation, and carbon dioxide is generated as an end product of the citric acid cycle. Oxygen supply and carbon dioxide clearance require a constant exchange of respiration gases between blood and tissues. Moreover, because pulmonary ventilation rises during the active phase and drops during the rest phase[89], oxygen consumption and blood and tissue oxygenation exhibit daily oscillations[90,91]. Hypoxia-inducible factor 1α (HIF1α), a critical regulator of oxygen homeostasis that accumulates upon hypoxia, has recently emerged as a pre-eminent molecular link between the cellular clock and oxygen. It transcriptionally reprogrammes cells in response to low oxygen levels, and disruption of the circadian clock has been shown to impair HIF1α-mediated oxygen sensing, leading to expression changes of HIF1α target genes involved in glucose metabolism and mitochondrial respiration[92]. Another blood gas, nitric oxide, has been linked to the rhythmicity of endovascular contractions and thereby to circadian variations in blood pressure via cyclic expression of endothelial nitric oxide synthetase[93].

Circadian rhythm in metabolic balance

Sleep is a metabolic master switch, and regulation of the sleep–wake cycle is the most powerful means for the circadian clock to exert metabolic control over the entire body. Clock genes have been shown to regulate the timing, quality and duration of sleep cycles in animal models[94] and in humans[95]. Sleep cycles in mammals are regulated by two components: the homeostatic drive to sleep, which increases with continued wakefulness, and the circadian clock, which aligns the times of falling asleep and waking up with the solar day[10]. Daytime-dependent regulation of sleep defines the temporal ecological niche of the organism. First, the ability to align activity and rest phases to limited and

Toll-like receptors
Transmembrane receptors with homology to the *Drosophila melanogaster* Toll protein that recognize microbial pathogen structures.

Operational taxonomic units
Classifiers for clusters of closely related organisms, in particular used for prokaryotes owing to the lack of a traditional system of biological classification.

favourable time windows optimizes the chances to encounter prey and avoid predators. Second, and more pertinent from a metabolic point of view, energy allocation differs widely between sleep and wakefulness[96]. Studies in humans determined that energy expenditure during sleep is reduced to two-thirds that of wakefulness[97], and although a sleep phase results in a reduction of only ~562 kJ (~134 kcal) of total daily energy expenditure, energy saved globally and mostly on motility processes can be redistributed to protective, regenerative, adaptive and anabolic processes such as immune functions, synaptic plasticity and glycogen storage[96,97]. Finally, the rhythm of sleep and wake inevitably determines the rhythm of feeding and fasting and thereby sets the clock in peripheral tissues through nutrient levels and a variety of connected parameters such as energy availability and redox levels (FIG. 2). The molecular mechanisms of peripheral entrainment are discussed in the following section.

Feedback of metabolism on the clock

There is ample evidence that metabolic regulation is more than an output function of the circadian clock. Metabolic parameters are also potent regulators of circadian oscillators, and the emerging mechanisms of this feedback regulation point towards interactions between small metabolites and their cellular binding partners. These are often called metabolic sensor proteins, which perceive changes in nutrient, energy and redox levels and modify gene expression programmes accordingly.

Systemic metabolite levels as peripheral Zeitgebers. Feedback of systemic metabolites to peripheral oscillators is a hallmark of circadian regulation in organs. A multitude of circulating nutrients and hormones, such as glucose[98], amino acids[99], insulin[100], glucagon[101], glucocorticoids[102] and many more[23,103], possess Zeitgeber capacity. Moreover, two of the most abundant circulating nutrients for cellular energy generation besides glucose, namely, glutamine and lactate, have been linked to feedback signalling pathways for circadian clock control (FIG. 4b). Glucose and glutamine are converted through the hexosamine pathway into uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), the substrate for the covalent modification of proteins by *O*–β-D-*N*-acetylglucosamine (*O*-GlcNAc)[104]. *O*-GlcNAcylation of BMAL1, CLOCK and PER2 regulates their transcriptional activity and stability with effects on entrainment and period length of the circadian clock[105–107]. Lactate is the metabolite with the highest metabolic flux in tissues[108], and lactate addition to cells stimulates NPAS2–BMAL1 activity (FIG. 4b), possibly through fluctuations in the ratio of reduced-to-oxidized NAD cofactors by a yet-to-be determined mechanism[109]. Interestingly, lactate flux increases an additional 2.5-fold during fasting[108], and therefore, lactate is a strong candidate metabolite for mediating the effect of feeding rhythms on peripheral clocks.

It has been further shown that oxygen rhythms within the physiological range synchronize the circadian clock between cells in a HIF1α-dependent manner in cultured cells. In addition, it has been demonstrated that targeted modulation of ambient oxygen levels accelerates the adaptation to jet lag by mice, indicating the role of oxygen sensing in modulating the circadian clock[90]. Activation of HIF1α increases the circadian period length of cultured myotubes[92], and this effect seems, at least in part, to be based on physical interaction between HIF1α and the core clock protein BMAL1 (REF.[110]), which is also reflected by genome-wide synergistic DNA binding of HIF1α and BMAL1 to gene promoters targeted by BMAL1 (REF.[111]) (FIG. 4b). Another blood gas, carbon monoxide, is predominantly generated intracellularly as a by-product of haem degradation. Rhythmic activity of the carbon-monoxide-producing enzyme, haem oxygenase, is required for normal functioning of the circadian clock, whereas its depletion leads to deregulated circadian-controlled gene transcription and disturbances of glucose metabolism[112]. Carbon monoxide modulates the transcriptional activity of BMAL1 by attenuating the binding of CLOCK–BMAL1 complexes to DNA[112] (FIG. 4b). Additionally, haem functions as a prosthetic group for NPAS2, an alternative BMAL1 dimerization partner, and binding of carbon monoxide to haem prevents the formation of transcriptionally competent NPAS2–BMAL1 heterodimers in favour of unproductive BMAL1–BMAL1 homodimers[113] (FIG. 4b). Thus, the crosstalk between gaseous monoxides and the circadian clock is well demonstrated and might even hold therapeutic potential: a recent report showed that carbon monoxide increases PER2 levels as part of its protective effect in ischaemia–reperfusion injury[114].

Metabolites as feedback cues for cellular oscillators. Cells seem to possess a multitude of molecular feedback mechanisms to connect their circadian clock with their metabolic state. A genome-wide RNAi screen for modifiers of the circadian clock in cultured human cells identified various metabolic genes that affect the core clock oscillator, including components of insulin and folate metabolism[115], which demonstrates the deep integration of metabolism in circadian clock control. Cellular metabolites and their related processes that have been demonstrated to provide feedback to the circadian clock are discussed below. Notably, these examples are likely only the tip of the iceberg in metabolite-mediated regulation of the clock, and it is conceivable that additional metabolites and their corresponding metabolic sensor proteins participate in this intricate crosstalk.

Cellular redox levels are reflected by the ratio of reduced-to-oxidized NAD and FAD, which serve as cofactors for a number of proteins that regulate core oscillator function[116,117]. For example, the enzymes sirtuin 1 (SIRT1) and poly(ADP-ribose) polymerase 1 (PARP1) use NAD as a cofactor for deacetylation and poly-ADP-ribosylation in transcriptional regulation, respectively[118–122]. NAD levels are themselves rhythmic owing to circadian activity of the nicotinamide phosphoribosyltransferase (NAMPT)-dependent NAD salvage pathway[116,117], and disruption of redox homeostasis in various clock-gene knockout models has been linked to neurodegeneration and ageing[123]. Whereas SIRT1 expression and/or activity is oscillating[119,122], PARP1 protein

O-GlcNAcylation
Post-translational modification of proteins, whereby *N*-acetylglucosamine is covalently attached via an *O*-glycosidic linkage to serine or threonine residues.

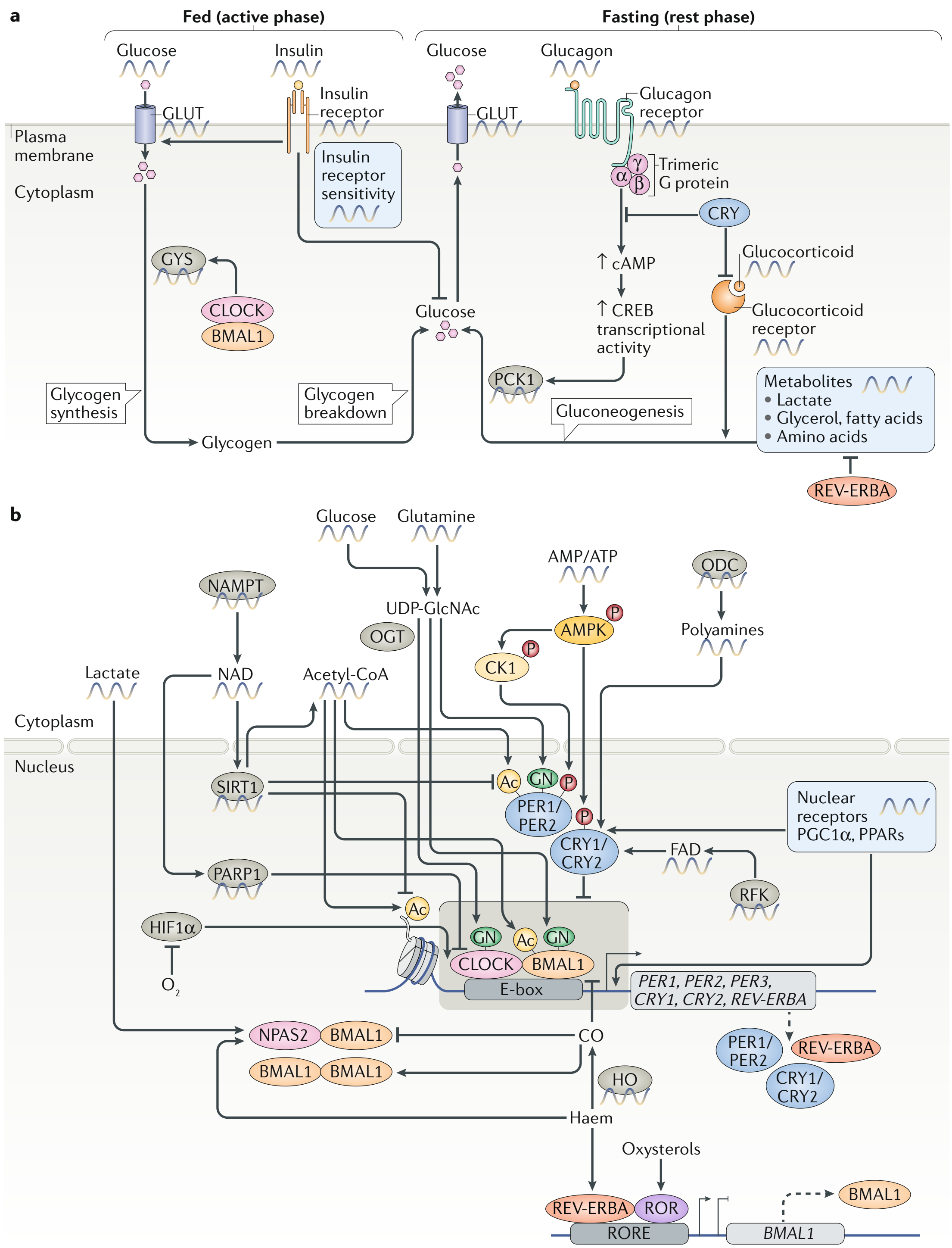
Metabolic flux
Substrate use in a biochemical pathway determined as the turnover rate of a metabolite as opposed to its steady-state levels, which can be constant in different conditions despite widely varying flux rates.

NAD cofactors
NAD molecules that can serve in their reduced form, NADH, as an electron donor and in their oxidized form, NAD^+, as an electron acceptor in biochemical reactions.

Prosthetic group
A small molecule that is covalently bound to a protein and is essential for its function. An example of a prosthetic group is haem bound to haemoglobin.

Ischaemia–reperfusion injury
The tissue damage caused by oxidative stress when cells are resupplied with oxygen and nutrients after a period of anoxia, for example, after a stroke.

Poly-ADP-ribosylation
The post-translational modification by transfer of multiple ADP-ribose units to target proteins.



levels are non-rhythmic, and cell-autonomous rhythmic PARP1 activity is driven by an as-yet-unidentified mechanism[124]. PARP1 modulates phase resetting of the circadian clock via poly-ADP-ribosylation of CLOCK, which weakens binding of CLOCK–BMAL1 to DNA[125] (FIG. 4b). Additionally, PARP1 drives circadian changes in chromatin interactions with the nuclear lamina that regulate circadian gene expression[126]. SIRT1 adjusts the circadian clock by rhythmic deacetylation of histones, BMAL1 (REF. [122]), PER2 (REF. [119]) and the methyltransferase mixed-lineage leukaemia 1 (MLL1; also known as lysine *N*-methyltransferase 2 A), which interacts with CLOCK and facilitates chromatin accessibility at clock-dependent promoters[118] (FIG. 4b). Additionally, SIRT1 drives specific metabolic programmes through interactions with PPARα[127], PGC1α[128] and CREB-regulated transcription co-activator 2 (CRTC2)[129]. Furthermore, the levels of FAD and its biosynthetic enzyme riboflavin kinase (RFK) are rhythmic in the nucleus and regulate CRY protein stability (FIG. 4b). Experimentally induced low levels of FAD change the amount of CRY in the liver and reprogramme metabolic gene expression with

◀ Fig. 4 | **Cellular circadian metabolism of glucose and feedback of metabolism on the cellular clock. a** | Glucose metabolism is an example of circadian regulation of peripheral metabolism. Rhythmic expression (wave pattern) of glucose transporters (GLUTs) enables efficient glucose uptake into cells during nutrient abundance (active phase) or its release (as is the case for glucose-storing hepatocytes) during fasting (rest phase). Glucose uptake is further stimulated by insulin signalling, which depends on cyclic insulin secretion from the pancreas. Insulin-receptor expression and sensitivity are also coordinated by the circadian clock. In hepatocytes and the muscle, glucose is efficiently stored as glycogen, and the rate-limiting enzyme of this reaction, glycogen synthase 2 (GYS2), is under circadian control. Under fasting (rest phase), glycogen is broken down to glucose. Glucose is also synthesized from other metabolites (which are rhythmically regulated by nuclear receptor subfamily 1 group D (REV-ERBA) in the process of gluconeogenesis. A key enzyme of gluconeogenesis, phosphoenolpyruvate carboxykinase 1 (PCK1) is rhythmic and regulated by glucagon signalling (the cycles of which are determined by circadian secretion from the pancreas), which is negatively influenced by cryptochromes (CRYs). Gluconeogenesis is also stimulated by glucocorticoids, which are controlled by CRY proteins through repression of the glucocorticoid receptor. **b** | There are many inputs from metabolism into core clock gene regulation. Glucose and glutamine (which are both cyclic) can be converted into uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), the substrate for protein *O*-GlcNAcylation (GN) by *O*-linked *N*-acetylglucosamine transferase (OGT), which regulates the stability and transcriptional activity of circadian locomotor output cycles kaput (CLOCK), aryl hydrocarbon receptor nuclear translocator-like protein 1 (BMAL1) and period 2 (PER2)[105–107]). Lactate enhances neuronal PAS domain protein 2 (NPAS2)–BMAL1 activity and has been linked to NAD metabolism[109]. NAD is also a cofactor for sirtuin 1 (SIRT1) and poly(ADP-ribose)-polymerase 1 (PARP1), which is required for their enzymatic activity. SIRT1 deacetylates the clock proteins PER2 (REF.[119]) and BMAL1 (REF.[122]) as well as histones to regulate gene expression. Reverse reaction, acetylation (Ac), depends on the presence of acetyl-CoA, which functions as a substrate. This mechanism is wired to the circadian clock through SIRT1-dependent rhythmic deacetylation of acetyl-CoA synthetase, which promotes acetyl-CoA synthesis[140]. PARP1 regulates entrainment of the cellular oscillator through poly-ADP-ribosylation of CLOCK, which weakens binding of CLOCK–BMAL1 to DNA[125]. NAD levels are themselves rhythmic owing to circadian activity of nicotinamide phosphoribosyltransferase (NAMPT)[117]. In addition, the levels of another cofactor, FAD, and its biosynthetic enzyme riboflavin kinase (RFK) are rhythmic and regulate CRY protein stability. AMP-activated protein kinase (AMPK) senses the cellular ATP:AMP ratio and negatively modulates PER and CRY protein stability by phosphorylation (P)[132,133]. AMPK also promotes the activity of SIRT1 (REF.[134]) (not shown). Circadian ornithine decarboxylase (ODC) drives rhythmic accumulation of polyamines, which affect the interaction between PER and CRY proteins[143]. Nuclear receptors (including peroxisome proliferator-activated receptors (PPARs) and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α)) interact with clock proteins and shape circadian expression programmes[72,73,202]. Physiological oxygen rhythms entrain cellular clocks through the interaction of hypoxia-inducible factor 1α (HIF1α) with BMAL1 (REFS[90,92,110,111]). Haem is degraded by haem oxygenase (HO) in a reaction that also generates carbon monoxide (CO), which attenuates CLOCK–BMAL1 binding to DNA[112] and leads to the formation of transcriptionally incompetent BMAL1–BMAL1 homodimers[113]. Haem is also a prosthetic group for NPAS2 (REF.[113]) and a cofactor for REV-ERBA[62], while the antagonistically functioning RAR-related orphan receptor (ROR) proteins interact with oxysterols[203]. CREB, cAMP response element-binding protein.

consequent changes in glucose homeostasis[130]. Cellular redox levels are also influenced by the pentose phosphate pathway, which generates NADPH, an important electron carrier for anabolic reactions. Inhibition of the pentose phosphate pathway lengthens the period of circadian oscillations through changes in CLOCK–BMAL1 binding to target genes and p300-dependent histone acetylation[131].

Cellular energy is mainly stored in the form of nucleoside triphosphates. AMP-activated protein kinase (AMPK), a potent inhibitor of anabolic pathways, measures the ATP-to-AMP ratio and adapts circadian clock function to changing energy levels by inducing phosphorylation and the degradation of PER and CRY proteins[132,133]. CRY phosphorylation by AMPK occurs directly, whereas PER phosphorylation is mediated by casein kinase 1 (CK1δ and CK1ε), activity of which is induced by AMPK (FIG. 4b). AMPK also promotes the activity of SIRT1 (REF.[134]). Moreover, AMPK senses glucose levels directly[135], which are also a rhythmic measure of energy status[119,136].

The metabolite acetyl-CoA, which connects carbohydrate, lipid and amino acid metabolism, is another evolutionarily conserved energy sensor. In mammals, gene expression is strongly influenced by histone acetylation levels, and the required acetyl-CoA stems from cytoplasmic and nuclear pools of the acetyl-CoA-generating enzymes ATP-citrate lyase[137] and acetyl-CoA synthetase[138]. Acetyl-CoA regulates transcription and post-transcriptional events as a substrate for the acetylation of histone and non-histone proteins, and this post-translational modification regulates enzyme levels and activity[139]. This mechanism is wired to the circadian clock through SIRT1-dependent rhythmic deacetylation of acetyl-CoA synthetase, which promotes acetyl-CoA synthesis[140]. Importantly, essential steps of the core oscillator mechanisms are regulated by acetylation and deacetylation of histones[141] and clock proteins[119,122] (FIG. 4b): cycles of acetylation and deacetylation of histones regulate the expression of *PER* and *CRY* genes, whereas at the level of clock proteins, acetylation can promote their activity (as in the case of BMAL1 (REF.[122])) or stability (as shown for PER2 (REF.[119])). Interestingly, CLOCK itself has acetyltransferase activity, which is stimulated by BMAL1 (REF.[142]), further highlighting the importance of acetylation in the regulation of circadian oscillations in gene expression.

For a final example, we highlight the clock-regulating role of polyamines. The small multifunctional molecule class of polyamines can be ingested in the diet and synthesized de novo or by the microbiome in the gut. Polyamine levels exhibit circadian rhythmicity and in turn alter the circadian period in cultured cells and mice, most likely by modulating the interaction between the repressors PER2 and CRY1 (FIG. 4b). Strikingly, supplementation of polyamines (such as spermidine) in the diet of aged mice is capable of rejuvenating their circadian clocks and shortening the circadian period to the length of young mice, demonstrating the possibility of nutritional intervention in age-related clock decline[143].

Disturbed clock in metabolic pathology

When functioning properly, the aforementioned regulatory mechanisms considerably contribute to mammalian homeostasis and health. Consequently, chronodisruption, the disturbance of behavioural rhythmicity as it occurs, for example, during shift work or chronic jet lag, is associated with the development of various metabolic diseases. Likewise, mutations in clock genes have been shown in animal models to cause a plethora of metabolic phenotypes.

Perturbations of the clock cause nutrient mishandling and metabolic disease. Disruption of the circadian system in different organs leads to a multitude of phenotypes related to disturbed nutrient metabolism.

SCN-lesioned mice completely lack daily rhythms of plasma glucose and insulin[136], and mice with a liver-specific disruption of the circadian clock fail to properly buffer circadian glucose fluctuations that originate from SCN-mediated food consumption cycles[9]. Muscle-specific knockout of *Bmal1* leads to impaired insulin-stimulated glucose uptake[81], and targeted deletion of the pancreas clock causes fasting hyperglycaemia and glucose intolerance[82]. Loss of clock function in the whole body (for example, in *Clock* or *Bmal1* knockout animals) leads to hyperglycaemia, glucose intolerance and ultimately obesity and metabolic syndrome[9,144,145]. Shift work in humans, which is inevitably associated with decoupling of the clock from the natural day–night cycles, has multiple detrimental effects on nutrient metabolism. It triggers changes of the lipid profile including elevated levels of cholesterol and low-density lipoprotein[146] and leads to higher postprandial increases in insulin, glucose and lipid levels[147]. Accordingly, shift workers are at a significantly higher risk of developing type 2 diabetes[148] and cardiovascular diseases[149]. Furthermore, all parts of the gastrointestinal tract are under pervasive control of the circadian clock, and perturbances of circadian rhythmicity through jet lag or shift work acutely manifest themselves predominantly as gastrointestinal discomfort[150]. Long-term disruption of the circadian clock in these tissues may lead to serious diseases such as colorectal cancer[151] or metabolic syndrome[152].

Perturbation of sleep and metabolic disease. Decreased and increased duration of sleep and poor sleep quality all disrupt the endocrine system and are strongly linked to insulin resistance, type 2 diabetes and obesity[153,154]. Sleep deprivation in combination with circadian misalignment (meaning difference between outside time and circadian time, for example, during shift work) has been experimentally linked to a decreased resting metabolic rate and increased postprandial plasma glucose concentrations[155]. Sleep deprivation also reduces leptin[156] and increases ghrelin and promotes the feeling of hunger[157] despite the lower energy demands during sleep[158]. Leptin and ghrelin are strong modifiers of metabolism[159] and are themselves under circadian clock control[160,161] (TABLE 1). Not surprisingly, environmentally induced circadian disruptions, such as chronic jet lag, lead to obesity, and various links converge on leptin resistance[162]. Moreover, a congenital sleep rhythm disorder, familial advanced sleep phase syndrome, is caused by mutations in the clock genes encoding CK1δ, PER2 (REF. 163) or CRY2 (REF. 164) and makes affected individuals want to go to bed in the late afternoon and get up in the middle of the night, almost incapacitating them for a normal social life. Similarly, a specific mutation of CRY1 can have the opposite effect and cause delayed sleep phase disorder[165]. More importantly, forced to adjust to a normal rhythm, the circadian system of people with these and other circadian sleep–wake cycle disorders is in constant asynchrony with the environment. Such a persistent, genetic-based misalignment of the inner and external time has been dubbed 'social jet lag' and is linked to metabolic diseases and obesity[166,167].

Leptin
An adipocyte-derived hormone that can cross the blood–brain barrier and inhibit hunger by regulating the production of other satiety-controlling hormones in the hypothalamus. The name comes from a Greek word meaning thin.

Ghrelin
(Growth hormone release inducing). A gastrointestinal peptide hormone that stimulates growth hormone secretion from the anterior pituitary and can cross the blood–brain barrier to increase hunger in the hypothalamus antagonistically to leptin.

Conclusions and perspective

Research on circadian metabolic crosstalk is rapidly expanding and covers almost every aspect of metabolism. In fact, the substantial advance of the field in recent years has already permitted the development of small-molecule drugs that target the circadian system with the goal of reverting adverse metabolic changes or modulating the activity of existing drugs whose activities and stabilities are often heavily influenced by the circadian system[168]. Future studies are expected to further consolidate the mutual integration of metabolic pathways and the circadian clock by working out the underlying molecular mechanisms of crosstalk, expanding the repertoire of metabolic pathways and processes intersecting with circadian rhythms, and employing novel genetic, pharmacological and environmental models to address pathophysiological consequences of circadian metabolic disruption.

It is a well-established fact that disruption of mammalian clock genes leads to increased metabolic-related morbidity and mortality. Unfortunately, however, the vast majority of studies only demonstrated the importance of individual clock genes for the phenotypes under investigation and did not address the question of whether disease states were induced by a perturbation of general circadian clock function. Thus, it is likely that many processes controlled by clock-related transcription factors merely depend specifically upon these regulators but not upon their function within the core clock circuitry. In several cases, the use of more than a single clock-deficient animal model, namely, a model for loss of function in both the positive (for example, *Bmal1* or *Clock*) and the negative (for example, *Cry1*, *Cry 2*, *Per1*, *Per2*) circadian circuitry, supports a role for the circadian clock per se rather than a role for a specific transcription factor. Nevertheless, even such an approach cannot always discriminate unequivocally between these possibilities because core clock transcription factor levels are strongly dependent on each other. The best strategy to tackle this endeavour appears to be resonance experiments. If circadian oscillators had evolved to anticipate the metabolic needs of an organism during the daily light–dark cycle, their period-length τ should be roughly in accordance with the period of the light–dark cycle — the so-called 'T cycle'. If discordance of τ and T affects the fitness of an organism, then it must be the interaction of the clock with environmental rhythms rather than the mutation of the clock gene itself that is responsible for the phenotype. Such analyses have been successfully conducted with different cyanobacteria strains[169,170] and plants[171], and the time is ripe to expand similar studies to animals, including mammals[172]. Such experiments might also provide evidence for the prevailing concept that the temporal orchestration of metabolism is advantageous for cells, given that they have limited resources and that maintaining all physiological mechanisms at a constant high level would be energetically wasteful[173].

As discussed extensively in this article, many systemic physiological functions are not carried out by a single organ but are rather the result of different inputs and outputs of various tissues. Hence, dissecting the contributions of the circadian clocks in all individual organs is mandatory for understanding the global control of metabolic pathways by the circadian system. The establishment of novel tissue-specific, clock-deficient mouse models is an imperative step towards achieving this goal.

Last but not least, a circadian viewpoint on metabolic control is valuable not only for chronobiologists but also for researchers with a core competence in metabolism and physiology, because taking this unique standpoint might expose many aspects of metabolic regulation that have been overlooked so far. It is highly likely that many studies of metabolic pathways that did not take the circadian element into account will reveal much new information once they are conducted in a time-of-day-dependent manner.

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1. Teleman, A. A. Metabolism meets development at Wiston House. *Development* **143**, 3045–3049 (2016).
2. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
3. Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194–1217 (2013).
4. DeBerardinis, R. J. & Thompson, C. B. Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* **148**, 1132–1144 (2012).
5. Le Loir, Y., Baron, F. & Gautier, M. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* **2**, 63–76 (2003).
6. Reinke, H. & Asher, G. Circadian clock control of liver metabolic functions. *Gastroenterology* **150**, 574–580 (2016).
7. Pittendrigh, C. S. & Bruce, V. G. in *Rhythmic and Synthetic Processes in Growth* (eds Pittendrigh, C. S. et al.) 75–110 (Princeton Univ. Press, 1957).
8. Neufeld-Cohen, A. et al. Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins. *Proc. Natl Acad. Sci. USA* **113**, E1673–E1682 (2016).
9. Lamia, K. A., Storch, K. F. & Weitz, C. J. Physiological significance of a peripheral tissue circadian clock. *Proc. Natl Acad. Sci. USA* **105**, 15172–15177 (2008).
Demonstration of clock-orchestrated interplay of multiple organs in glucose homeostasis.
10. Borbely, A. A., Daan, S., Wirz-Justice, A. & Deboer, T. The two-process model of sleep regulation: a reappraisal. *J. Sleep Res.* **25**, 131–143 (2016).
11. Greenham, K. & McClung, C. R. Integrating circadian dynamics with physiological processes in plants. *Nat. Rev. Genet.* **16**, 598–610 (2015).
12. Allada, R. & Chung, B. Y. Circadian organization of behavior and physiology in. *Drosophila. Annu. Rev. Physiol.* **72**, 605–624 (2010).
13. Shultzaberger, R. K., Boyd, J. S., Diamond, S., Greenspan, R. J. & Golden, S. S. Giving time purpose: the *Synechococcus elongatus* clock in a broader network context. *Annu. Rev. Genet.* **49**, 485–505 (2015).
14. Takahashi, J. S. Transcriptional architecture of the mammalian circadian clock. *Nat. Rev. Genet.* **18**, 164–179 (2017).
15. O'Neill, J. S. & Reddy, A. B. Circadian clocks in human red blood cells. *Nature* **469**, 498–503 (2011).
Discovery of non-transcriptional circadian rhythms in mammals.
16. Feeney, K. A. et al. Daily magnesium fluxes regulate cellular timekeeping and energy balance. *Nature* **532**, 375–379 (2016).
17. Henslee, E. A. et al. Rhythmic potassium transport regulates the circadian clock in human red blood cells. *Nat. Commun.* **8**, 1978 (2017).
18. Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E. & Hogenesch, J. B. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl Acad. Sci. USA* **111**, 16219–16224 (2014).
19. Hatori, M. & Panda, S. The emerging roles of melanopsin in behavioral adaptation to light. *Trends Mol. Med.* **16**, 435–446 (2010).
20. Buijs, F. N. et al. The circadian system: a regulatory feedback network of periphery and brain. *Physiology* **31**, 170–181 (2016).
21. Damiola, F. et al. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* **14**, 2950–2961 (2000).
Demonstration that daytime feeding in mice uncouples organ clocks from the SCN.
22. Kalsbeek, A. et al. Mammalian clock output mechanisms. *Essays Biochem.* **49**, 137–151 (2011).
23. Gerber, A. et al. Blood-borne circadian signal stimulates daily oscillations in actin dynamics and SRF activity. *Cell* **152**, 492–503 (2013).
24. Balsalobre, A., Damiola, F. & Schibler, U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* **93**, 929–937 (1998).
25. Krishnaiah, S. Y. et al. Clock regulation of metabolites reveals coupling between transcription and metabolism. *Cell Metab.* **25**, 961–974 (2017).
26. Loizides-Mangold, U. et al. Lipidomics reveals diurnal lipid oscillations in human skeletal muscle persisting in cellular myotubes cultured in vitro. *Proc. Natl Acad. Sci. USA* **114**, E8565–E8574 (2017).
27. Kalfalah, F. et al. Crosstalk of clock gene expression and autophagy in aging. *Aging* **8**, 1876–1895 (2016).
28. Nunnari, J. & Suomalainen, A. Mitochondria: in sickness and in health. *Cell* **148**, 1145–1159 (2012).
29. Peek, C. B. et al. Circadian clock NAD+cycle drives mitochondrial oxidative metabolism in mice. *Science* **342**, 1243417 (2013).
Demonstration of the circadian rhythmicity of mitochondrial functions.
30. Jacobi, D. et al. Hepatic Bmal1 regulates rhythmic mitochondrial dynamics and promotes metabolic fitness. *Cell Metab.* **22**, 709–720 (2015).
31. Wai, T. & Langer, T. Mitochondrial dynamics and metabolic regulation. *Trends Endocrinol. Metab.* **27**, 105–117 (2016).
32. Andrews, J. L. et al. CLOCK and BMAL1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. *Proc. Natl Acad. Sci. USA* **107**, 19090–19095 (2010).
33. Kohsaka, A. et al. The circadian clock maintains cardiac function by regulating mitochondrial metabolism in mice. *PLOS ONE* **9**, e112811 (2014).
34. Schmitt, K. et al. Circadian control of DRP1 activity regulates mitochondrial dynamics and bioenergetics. *Cell Metab.* **27**, 657–666 (2018).
35. Magnone, M. C. et al. The mammalian circadian clock gene *per2* modulates cell death in response to oxidative stress. *Front. Neurol.* **5**, 289 (2014).
36. Osman, C., Voelker, D. R. & Langer, T. Making heads or tails of phospholipids in mitochondria. *J. Cell Biol.* **192**, 7–16 (2011).
37. Aviram, R. et al. Lipidomics analyses reveal temporal and spatial lipid organization and uncover daily oscillations in intracellular organelles. *Mol. Cell* **62**, 636–648 (2016).
38. Robles, M. S., Cox, J. & Mann, M. In-vivo quantitative proteomics reveals a key contribution of post-transcriptional mechanisms to the circadian regulation of liver metabolism. *PLOS Genet.* **10**, e1004047 (2014).
39. Mauvoisin, D. et al. Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. *Proc. Natl Acad. Sci. USA* **111**, 167–172 (2014).
40. Gong, C. et al. The daily rhythms of mitochondrial gene expression and oxidative stress regulation are altered by aging in the mouse liver. *Chronobiol. Int.* **32**, 1254–1263 (2015).
41. Koike, N. et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science* **338**, 349–354 (2012).
42. Masri, S. et al. Circadian acetylome reveals regulation of mitochondrial metabolic pathways. *Proc. Natl Acad. Sci. USA* **110**, 3339–3344 (2013).
43. Cela, O. et al. Clock genes-dependent acetylation of complex I sets rhythmic activity of mitochondrial OxPhos. *Biochim. Biophys. Acta* **1863**, 596–606 (2016).
44. Mauvoisin, D. et al. Circadian and feeding rhythms orchestrate the diurnal liver acetylome. *Cell Rep.* **20**, 1729–1743 (2017).
45. Solocinski, K. & Gumz, M. L. The circadian clock in the regulation of renal rhythms. *J. Biol. Rhythms* **30**, 470–486 (2015).
46. Petrenko, V. et al. Pancreatic α- and β-cellular clocks have distinct molecular properties and impact on islet hormone secretion and gene expression. *Genes Dev.* **31**, 383–398 (2017).
47. Mayeuf-Louchart, A., Staels, B. & Duez, H. Skeletal muscle functions around the clock. *Diabetes Obes. Metab.* **17** (Suppl. 1), 39–46 (2015).
48. Zwighaft, Z., Reinke, H. & Asher, G. The liver in the eyes of a chronobiologist. *J. Biol. Rhythms* **31**, 115–124 (2016).
49. Storch, K. F. et al. Extensive and divergent circadian gene expression in liver and heart. *Nature* **417**, 78–83 (2002).
50. Panda, S. et al. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* **109**, 307–320 (2002).
51. Akhtar, R. A. et al. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr. Biol.* **12**, 540–550 (2002).
52. Rey, G. et al. Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver. *PLOS Biol.* **9**, e1000595 (2011).
53. Dyar, K. A. et al. Atlas of circadian metabolism reveals system-wide coordination and communication between clocks. *Cell* **174**, 1571–1585 (2018).
54. Eckel-Mahan, K. L. et al. Coordination of the transcriptome and metabolome by the circadian clock. *Proc. Natl Acad. Sci. USA* **109**, 5541–5546 (2012).
55. Adamovich, Y. et al. Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. *Cell Metab.* **19**, 319–330 (2014).
56. Atger, F. et al. Circadian and feeding rhythms differentially affect rhythmic mRNA transcription and translation in mouse liver. *Proc. Natl Acad. Sci. USA* **112**, E6579–E6588 (2015).
57. Doi, R., Oishi, K. & Ishida, N. CLOCK regulates circadian rhythms of hepatic glycogen synthesis through transcriptional activation of Gys2. *J. Biol. Chem.* **285**, 22114–22121 (2010).
58. Lamia, K. A. et al. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* **480**, 552–556 (2011).
59. So, A. Y., Bernal, T. U., Pillsbury, M. L., Yamamoto, K. R. & Feldman, B. J. Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. *Proc. Natl Acad. Sci. USA* **106**, 17582–17587 (2009).
60. Vollmers, C. et al. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc. Natl Acad. Sci. USA* **106**, 21453–21458 (2009).
61. Zhang, E. E. et al. Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. *Nat. Med.* **16**, 1152–1156 (2010).
62. Yin, L. et al. Rev-erbα, a heme sensor that coordinates metabolic and circadian pathways. *Science* **318**, 1786–1789 (2007).
63. Yin, L., Wu, N. & Lazar, M. A. Nuclear receptor Rev-erbα: a heme receptor that coordinates circadian rhythm and metabolism. *Nucl. Recept. Signal.* **8**, e001 (2010).
64. Preitner, N. et al. Orphan nuclear receptors, molecular clockwork, and the entrainment of peripheral oscillators. *Novartis Found. Symp.* **253**, 89–99; discussion 99–109 (2003).
65. Zhang, Y. et al. Discrete functions of nuclear receptor Rev-erbα couple metabolism to the clock. *Science* **348**, 1488–1492 (2015).
Report of the finding that REV-ERBAα has major metabolic functions outside the core clock mechanism.
66. Feng, D. et al. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. *Science* **331**, 1315–1319 (2011).
67. Peterson, T. R. et al. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell* **146**, 408–420 (2011).
68. Le Martelot, G. et al. REV-ERBα participates in circadian SREBP signaling and bile acid homeostasis. *PLOS Biol.* **7**, e1000181 (2009).

69. Woldt, E. et al. Rev-erb-α modulates skeletal muscle oxidative capacity by regulating mitochondrial biogenesis and autophagy. *Nat. Med.* **19**, 1039–1046 (2013).
70. Kallen, J. A. et al. X-ray structure of the hRORα LBD at 1.63Å: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of RORα. *Structure* **10**, 1697–1707 (2002).
71. Yang, X. et al. Nuclear receptor expression links the circadian clock to metabolism. *Cell* **126**, 801–810 (2006).
72. Schmutz, I., Ripperger, J. A., Baeriswyl-Aebischer, S. & Albrecht, U. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. *Genes Dev.* **24**, 345–357 (2010).
73. Kriebs, A. et al. Circadian repressors CRY1 and CRY2 broadly interact with nuclear receptors and modulate transcriptional activity. *Proc. Natl Acad. Sci. USA* **114**, 8776–8781 (2017).
74. Liu, C., Li, S., Liu, T., Borjigin, J. & Lin, J. D. Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. *Nature* **447**, 477–481 (2007).
75. Kohsaka, A. et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab.* **6**, 414–421 (2007).
Demonstration of the effect of high-fat diet on circadian clocks.
76. Gill, S. & Panda, S. A. Smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits. *Cell Metab.* **22**, 789–798 (2015).
77. Bolli, G. B. et al. Demonstration of a dawn phenomenon in normal human volunteers. *Diabetes* **33**, 1150–1153 (1984).
78. Feigin, R. D., Klainer, A. S. & Beisel, W. R. Circadian periodicity of blood amino-acids in adult men. *Nature* **215**, 512–514 (1967).
79. Rivera-Coll, A., Fuentes-Arderiu, X. & Diez-Noguera, A. Circadian rhythmic variations in serum concentrations of clinically important lipids. *Clin. Chem.* **40**, 1549–1553 (1994).
80. Van Cauter, E., Polonsky, K. S. & Scheen, A. J. Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocr. Rev.* **18**, 716–738 (1997).
81. Dyar, K. A. et al. Muscle insulin sensitivity and glucose metabolism are controlled by the intrinsic muscle clock. *Mol. Metab.* **3**, 29–41 (2014).
82. Marcheva, B. et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* **466**, 627–631 (2010).
The discovery of the role of the pancreatic circadian clock in insulin and glucose homeostasis.
83. Perelis, M. et al. Pancreatic beta cell enhancers regulate rhythmic transcription of genes controlling insulin secretion. *Science* **350**, aac4250 (2015).
84. Shi, S. Q., Ansari, T. S., McGuinness, O. P., Wasserman, D. H. & Johnson, C. H. Circadian disruption leads to insulin resistance and obesity. *Curr. Biol.* **23**, 372–381 (2013).
85. Zhou, B. et al. CLOCK/BMAL1 regulates circadian change of mouse hepatic insulin sensitivity by SIRT1. *Hepatology* **59**, 2196–2206 (2014).
86. Dyar, K. A. et al. Transcriptional programming of lipid and amino acid metabolism by the skeletal muscle circadian clock. *PLOS Biol.* **16**, e2005886 (2018).
87. Yao, Z., DuBois, D. C., Almon, R. R. & Jusko, W. J. Modeling circadian rhythms of glucocorticoid receptor and glutamine synthetase expression in rat skeletal muscle. *Pharm. Res.* **23**, 670–679 (2006).
88. Benavides, A., Siches, M. & Llobera, M. Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat. *Am. J. Physiol.* **275**, R811–R817 (1998).
89. Mortola, J. P. Breathing around the clock: an overview of the circadian pattern of respiration. *Eur. J. Appl. Physiol.* **91**, 119–129 (2004).
90. Adamovich, Y., Ladeuix, B., Golik, M., Koeners, M. P. & Asher, G. Rhythmic oxygen levels reset circadian clocks through HIF1α. *Cell Metab.* **25**, 93–101 (2017).
91. Emans, T. W., Janssen, B. J., Joles, J. A. & Krediet, C. T. P. Circadian rhythm in kidney tissue oxygenation in the rat. *Front. Physiol.* **8**, 205 (2017).
92. Peek, C. B. et al. Circadian clock interaction with HIF1α mediates oxygenic metabolism and anaerobic glycolysis in skeletal muscle. *Cell Metab.* **25**, 86–92 (2017).
93. Rodrigo, G. C. & Herbert, K. E. Regulation of vascular function and blood pressure by circadian variation in redox signalling. *Free Radic. Biol. Med.* **119**, 115–120 (2017).
94. Franken, P. A role for clock genes in sleep homeostasis. *Curr. Opin. Neurobiol.* **23**, 864–872 (2013).
95. Viola, A. U. et al. PER3 polymorphism predicts sleep structure and waking performance. *Curr. Biol.* **17**, 613–618 (2007).
96. Schmidt, M. H. The energy allocation function of sleep: a unifying theory of sleep, torpor, and continuous wakefulness. *Neurosci. Biobehav. Rev.* **47**, 122–153 (2014).
97. Jung, C. M. et al. Energy expenditure during sleep, sleep deprivation and sleep following sleep deprivation in adult humans. *J. Physiol.* **589**, 235–244 (2011).
98. Hirota, T. et al. Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. *J. Biol. Chem.* **277**, 44244–44251 (2002).
99. Oike, H., Nagai, K., Fukushima, T., Ishida, N. & Kobori, M. Feeding cues and injected nutrients induce acute expression of multiple clock genes in the mouse liver. *PLOS ONE* **6**, e23709 (2011).
100. Dang, F. et al. Insulin post-transcriptionally modulates Bmal1 protein to affect the hepatic circadian clock. *Nat. Commun.* **7**, 12696 (2016).
101. Petrenko, V. & Dibner, C. Cell-specific resetting of mouse islet cellular clocks by glucagon, glucagon-like peptide 1 and somatostatin. *Acta Physiol* **222**, e13021 (2017).
102. Balsalobre, A. et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* **289**, 2344–2347 (2000).
103. Balsalobre, A., Marcacci, L. & Schibler, U. Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts. *Curr. Biol.* **10**, 1291–1294 (2000).
104. Hart, G. W., Housley, M. P. & Slawson, C. Cycling of O-linked β-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature* **446**, 1017–1022 (2007).
105. Ma, Y. et al. O-GlcNAcylation of BMAL1 regulates circadian rhythms in NIH3T3 fibroblasts. *Biochem. Biophys. Res. Commun.* **431**, 382–387 (2013).
106. Li, M.-D. et al. O-GlcNAc signaling entrains the circadian clock by inhibiting BMAL1/CLOCK ubiquitination. *Cell Metab.* **17**, 303–310 (2013).
107. Kaasik, K. et al. Glucose sensor O-GlcNAcylation coordinates with phosphorylation to regulate circadian clock. *Cell Metab.* **17**, 291–302 (2013).
108. Hui, S. et al. Glucose feeds the TCA cycle via circulating lactate. *Nature* **551**, 115–118 (2017).
109. Rutter, J., Reick, M., Wu, L. C. & McKnight, S. L. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* **293**, 510–514 (2001).
110. Hogenesch, J. B. et al. Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. *J. Biol. Chem.* **272**, 8581–8593 (1997).
111. Wu, Y. et al. Reciprocal regulation between the circadian clock and hypoxia signaling at the genome level in mammals. *Cell Metab.* **25**, 73–85 (2017).
Refs 90, 92 and 111 are three related publications showing the interplay between oxygen, HIF and circadian clocks.
112. Klemz, R. et al. Reciprocal regulation of carbon monoxide metabolism and the circadian clock. *Nat. Struct. Mol. Biol.* **24**, 15–22 (2017).
113. Dioum, E. M. et al. NPAS2: a gas-responsive transcription factor. *Science* **298**, 2385–2387 (2002).
114. Correa-Costa, M. et al. Carbon monoxide protects the kidney through the central circadian clock and CD39. *Proc. Natl Acad. Sci. USA* **115**, E2302–E2310 (2018).
115. Zhang, E. E. et al. A genome-wide RNAi screen for modifiers of the circadian clock in human cells. *Cell* **139**, 199–210 (2009).
116. Ramsey, K. M. et al. Circadian clock feedback cycle through NAMPT-mediated NAD^+ biosynthesis. *Science* **324**, 651–654 (2009).
117. Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M. & Sassone-Corsi, P. Circadian control of the NAD^+ salvage pathway by CLOCK-SIRT1. *Science* **324**, 654–657 (2009).
118. Aguilar-Arnal, L., Katada, S., Orozco-Solis, R. & Sassone-Corsi, P. NAD^+-SIRT1 control of H3K4 trimethylation through circadian deacetylation of MLL1. *Nat. Struct. Mol. Biol.* **22**, 312–318 (2015).
119. Asher, G. et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* **134**, 317–328 (2008).
120. Chang, H. C. & Guarente, L. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. *Cell* **153**, 1448–1460 (2013).
121. Masri, S. et al. Partitioning circadian transcription by SIRT6 leads to segregated control of cellular metabolism. *Cell* **158**, 659–672 (2014).
122. Nakahata, Y. et al. The NAD^+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* **134**, 329–340 (2008).
Refs 119 and 122 are two related publications reporting that SIRT1 regulates circadian rhythmicity.
123. Musiek, E. S. et al. Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *J. Clin. Invest.* **123**, 5389–5400 (2013).
124. Choi, J. Y. et al. Non-thermal plasma-induced apoptosis is modulated by ATR- and PARP1-mediated DNA damage responses and circadian clock. *Oncotarget* **7**, 32980–32989 (2016).
125. Asher, G. et al. Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. *Cell* **142**, 943–953 (2010).
126. Zhao, H. et al. PARP1- and CTCF-mediated interactions between active and repressed chromatin at the lamina promote oscillating transcription. *Mol. Cell* **59**, 984–997 (2015).
127. Purushotham, A. et al. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.* **9**, 327–338 (2009).
128. Rodgers, J. T. et al. Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. *Nature* **434**, 113–118 (2005).
129. Liu, Y. et al. A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. *Nature* **456**, 269–273 (2008).
130. Hirano, A., Braas, D., Fu, Y.-H. & Ptacek, L. J. FAD regulates CRYPTOCHROME protein stability and circadian clock in mice. *Cell Rep.* **19**, 255–266 (2017).
131. Rey, G. et al. The pentose phosphate pathway regulates the circadian clock. *Cell Metab.* **24**, 462–473 (2016).
132. Lamia, K. A. et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* **326**, 437–440 (2009).
Discovery that AMPK regulates circadian rhythmicity.
133. Um, J. H. et al. Activation of 5′-AMP-activated kinase with diabetes drug metformin induces casein kinase Iepsilon (CKIepsilon)-dependent degradation of clock protein mPer2. *J. Biol. Chem.* **282**, 20794–20798 (2007).
134. Canto, C. et al. AMPK regulates energy expenditure by modulating NAD^+ metabolism and SIRT1 activity. *Nature* **458**, 1056–1060 (2009).
135. Zhang, C.-S. et al. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature* **548**, 112–116 (2017).
136. Yamamoto, H., Nagai, K. & Nakagawa, H. Role of SCN in daily rhythms of plasma glucose, FFA, insulin and glucagon. *Chronobiol. Int.* **4**, 483–491 (1987).
137. Wellen, K. E. et al. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* **324**, 1076–1080 (2009).
138. Ariyannur, P. S. et al. Nuclear-cytoplasmic localization of acetyl coenzyme a synthetase-1 in the rat brain. *J. Comp. Neurol.* **518**, 2952–2977 (2010).
139. Zhao, S. et al. Regulation of cellular metabolism by protein lysine acetylation. *Science* **327**, 1000–1004 (2010).
140. Sahar, S. et al. Circadian control of fatty acid elongation by SIRT1 protein-mediated deacetylation of acetyl-coenzyme A synthetase 1. *J. Biol. Chem.* **289**, 6091–6097 (2014).
141. Etchegaray, J. P., Lee, C., Wade, P. A. & Reppert, S. M. Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. *Nature* **421**, 177–182 (2003).
142. Doi, M., Hirayama, J. & Sassone-Corsi, P. Circadian regulator CLOCK is a histone acetyltransferase. *Cell* **125**, 497–508 (2006).
143. Zwighaft, Z. et al. Circadian clock control by polyamine levels through a mechanism that declines with age. *Cell Metab.* **22**, 874–885 (2015).
144. Turek, F. W. et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* **308**, 1043–1045 (2005).
145. Rudic, R. D. et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLOS Biol.* **2**, e377 (2004).
146. Ghiasvand, M. et al. Shift working and risk of lipid disorders: a cross-sectional study. *Lipids Health Dis.* **5**, 9 (2006).
147. Morgan, L., Hampton, S., Gibbs, M. & Arendt, J. Circadian aspects of postprandial metabolism. *Chronobiol. Int.* **20**, 795–808 (2003).

148. Pan, A., Schernhammer, E. S., Sun, Q. & Hu, F. B. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. *PLOS Med.* **8**, e1001141 (2011).
149. Thosar, S. S., Butler, M. P. & Shea, S. A. Role of the circadian system in cardiovascular disease. *J. Clin. Invest.* **128**, 2157–2167 (2018).
150. Konturek, P. C., Brzozowski, T. & Konturek, S. J. Gut clock: implication of circadian rhythms in the gastrointestinal tract. *J. Physiol. Pharmacol.* **62**, 139–150 (2011).
151. Schernhammer, E. S. et al. Night-shift work and risk of colorectal cancer in the nurses' health study. *J. Natl Cancer Inst.* **95**, 825–828 (2003).
152. De Bacquer, D. et al. Rotating shift work and the metabolic syndrome: a prospective study. *Int. J. Epidemiol.* **38**, 848–854 (2009).
153. Zizi, F. et al. Sleep duration and the risk of diabetes mellitus: epidemiologic evidence and pathophysiologic insights. *Curr. Diabetes Rep.* **10**, 43–47 (2010).
154. Lucassen, E. A., Rother, K. I. & Cizza, G. Interacting epidemics? Sleep curtailment, insulin resistance, and obesity. *Ann. NY Acad. Sci.* **1264**, 110–134 (2012).
155. Buxton, O. M. et al. Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci. Transl Med.* **4**, 129ra143 (2012).
156. Mullington, J. M. et al. Sleep loss reduces diurnal rhythm amplitude of leptin in healthy men. *J. Neuroendocrinol.* **15**, 851–854 (2003).
157. Schmid, S. M., Hallschmid, M., Jauch-Chara, K., Born, J. & Schultes, B. A single night of sleep deprivation increases ghrelin levels and feelings of hunger in normal-weight healthy men. *J. Sleep Res.* **17**, 331–334 (2008).
158. Beccuti, G. & Pannain, S. Sleep and obesity. *Curr. Opin. Clin. Nutr. Metab. Care* **14**, 402–412 (2011).
159. Cui, H., Lopez, M. & Rahmouni, K. The cellular and molecular bases of leptin and ghrelin resistance in obesity. *Nat. Rev. Endocrinol.* **13**, 338–351 (2017).
160. Kalsbeek, A. et al. The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* **142**, 2677–2685 (2001).
161. Laermans, J., Vancleef, L., Tack, J. & Depoortere, I. Role of the clock gene Bmal1 and the gastric ghrelin-secreting cell in the circadian regulation of the ghrelin-GOAT system. *Sci. Rep.* **5**, 16748 (2015).
162. Kettner, N. M. et al. Circadian dysfunction induces leptin resistance in mice. *Cell Metab.* **22**, 448–459 (2015).
163. Xu, Y. et al. Functional consequences of a CKIδ mutation causing familial advanced sleep phase syndrome. *Nature* **434**, 640–644 (2005).
164. Hirano, A. et al. A cryptochrome 2 mutation yields advanced sleep phase in humans. *eLife* **5**, e16695 (2016).
165. Patke, A. et al. Mutation of the human circadian clock gene CRY1 in familial delayed sleep phase disorder. *Cell* **169**, 203–215 (2017).
166. Parsons, M. J. et al. Social jetlag, obesity and metabolic disorder: investigation in a cohort study. *Int. J. Obes.* **39**, 842–848 (2015).
167. Roenneberg, T., Allebrandt, K. V., Merrow, M. & Vetter, C. Social jetlag and obesity. *Curr. Biol.* **22**, 939–943 (2012).
168. Chen, Z., Yoo, S.-H. & Takahashi, J. S. Development and therapeutic potential of small-molecule modulators of circadian systems. *Annu. Rev. Pharmacol. Toxicol.* **58**, 231–252 (2018).
169. Ouyang, Y., Andersson, C. R., Kondo, T., Golden, S. S. & Johnson, C. H. Resonating circadian clocks enhance fitness in cyanobacteria. *Proc. Natl Acad. Sci. USA* **95**, 8660–8664 (1998).
170. Woelfle, M. A., Ouyang, Y., Phanvijhitsiri, K. & Johnson, C. H. The adaptive value of circadian clocks: an experimental assessment in cyanobacteria. *Curr. Biol.* **14**, 1481–1486 (2004).
171. Dodd, A. N. et al. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* **309**, 630–633 (2005).
172. Martino, T. A. et al. Circadian rhythm disorganization produces profound cardiovascular and renal disease in hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R1675–R1683 (2008).
173. Wang, G.-Z. et al. Cycling transcriptional networks optimize energy utilization on a genome scale. *Cell Rep.* **13**, 1868–1880 (2015).
174. Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y. & Menaker, M. Entrainment of the circadian clock in the liver by feeding. *Science* **291**, 490–493 (2001). **Demonstration that daytime feeding uncouples organ clocks from the SCN**.
175. Mendoza, J., Graff, C., Dardente, H., Pevet, P. & Challet, E. Feeding cues alter clock gene oscillations and photic responses in the suprachiasmatic nuclei of mice exposed to a light/dark cycle. *J. Neurosci.* **25**, 1514–1522 (2005).
176. Acosta-Rodriguez, V. A., de Groot, M. H. M., Rijo-Ferreira, F., Green, C. B. & Takahashi, J. S. Mice under caloric restriction self-impose a temporal restriction of food intake as revealed by an automated feeder system. *Cell Metab.* **26**, 267–277 (2017).
177. Potthoff, M. J., Kliewer, S. A. & Mangelsdorf, D. J. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. *Genes Dev.* **26**, 312–324 (2012).
178. Bookout, A. L. et al. FGF21 regulates metabolism and circadian behavior by acting on the nervous system. *Nat. Med.* **19**, 1147–1152 (2013).
179. Hakansson, M. L., Brown, H., Ghilardi, N., Skoda, R. C. & Meister, B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *J. Neurosci.* **18**, 559–572 (1998).
180. Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B. & Elmquist, J. K. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* **494**, 528–548 (2006).
181. Grosbellet, E. et al. Circadian phenotyping of obese and diabetic db/db mice. *Biochimie* **124**, 198–206 (2016).
182. Mistlberger, R. E. Food-anticipatory circadian rhythms: concepts and methods. *Eur. J. Neurosci.* **30**, 1718–1729 (2009).
183. Orozco-Solis, R. et al. The circadian clock in the ventromedial hypothalamus controls cyclic energy expenditure. *Cell Metab.* **23**, 467–478 (2016).
184. Eckel-Mahan, K. L. et al. Reprogramming of the circadian clock by nutritional challenge. *Cell* **155**, 1464–1478 (2013).
185. Abbondante, S., Eckel-Mahan, K. L., Ceglia, N. J., Baldi, P. & Sassone-Corsi, P. Comparative circadian metabolomics reveal differential effects of nutritional challenge in the serum and liver. *J. Biol. Chem.* **291**, 2812–2828 (2016).
186. Hatori, M. et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab.* **15**, 848–860 (2012).
187. Sherman, H. et al. Timed high-fat diet resets circadian metabolism and prevents obesity. *FASEB J.* **26**, 3493–3502 (2012).
188. Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R. M. & Escobar, C. Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. *Endocrinology* **151**, 1019–1029 (2010).
189. Branecky, K. L., Niswender, K. D. & Pendergast, J. S. Disruption of daily rhythms by high-fat diet is reversible. *PLOS ONE* **10**, e0137970 (2015).
190. Tognini, P. et al. Distinct circadian signatures in liver and gut clocks revealed by ketogenic diet. *Cell Metab.* **26**, 523–538 (2017).
191. Lynch, S. V. & Pedersen, O. The human intestinal microbiome in health and disease. *N. Engl. J. Med.* **375**, 2369–2379 (2016).
192. Mukherji, A., Kobiita, A., Ye, T. & Chambon, P. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell* **153**, 812–827 (2013).
193. Thaiss, C. A. et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* **159**, 514–529 (2014). **Refs 186, 187 and 193 report the discovery of the metabolic benefits of time-restricted feeding**.
194. Liang, X., Bushman, F. D. & FitzGerald, G. A. Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. *Proc. Natl Acad. Sci. USA* **112**, 10479–10484 (2015).
195. Zarrinpar, A., Chaix, A., Yooseph, S. & Panda, S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metab.* **20**, 1006–1017 (2014).
196. Leone, V. et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* **17**, 681–689 (2015).
197. Murakami, M. et al. Gut microbiota directs PPARgamma-driven reprogramming of the liver circadian clock by nutritional challenge. *EMBO Rep.* **17**, 1292–1303 (2016).
198. Thaiss, C. A. et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* **167**, 1495–1510 (2016).
199. Kil, I. S. et al. Circadian oscillation of sulfiredoxin in the mitochondria. *Mol. Cell* **59**, 651–663 (2015).
200. Edgar, R. S. et al. Peroxiredoxins are conserved markers of circadian rhythms. *Nature* **485**, 459–464 (2012).
201. O'Neill, J. S. et al. Circadian rhythms persist without transcription in a eukaryote. *Nature* **469**, 554–558 (2011).
202. Grimaldi, B. et al. PER2 controls lipid metabolism by direct regulation of PPARgamma. *Cell Metab.* **12**, 509–520 (2010).
203. Wang, Y. et al. Modulation of retinoic acid receptor-related orphan receptor alpha and gamma activity by 7-oxygenated sterol ligands. *J. Biol. Chem.* **285**, 5013–5025 (2010).
204. Perrin, L. et al. Human skeletal myotubes display a cell-autonomous circadian clock implicated in basal myokine secretion. *Mol. Metab.* **4**, 834–845 (2015).
205. Philippe, J. & Dibner, C. Thyroid circadian timing: roles in physiology and thyroid malignancies. *J. Biol. Rhythms* **30**, 76–83 (2015).
206. Mortola, J. F., Laughlin, G. A. & Yen, S. S. A circadian rhythm of serum follicle-stimulating hormone in women. *J. Clin. Endocrinol. Metab.* **75**, 861–864 (1992).
207. Chung, S., Son, G. H. & Kim, K. Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. *Biochim. Biophys. Acta* **1812**, 581–591 (2011).
208. Waldstreicher, J. et al. Gender differences in the temporal organization of proclactin (PRL) secretion: evidence for a sleep-independent circadian rhythm of circulating PRL levels- a clinical research center study. *J. Clin. Endocrinol. Metab.* **81**, 1483–1487 (1996).
209. Lynch, H. J., Wurtman, R. J., Moskowitz, M. A., Archer, M. C. & Ho, M. H. Daily rhythm in human urinary melatonin. *Science* **187**, 169–171 (1975).
210. Eggink, H. M. et al. Complex interaction between circadian rhythm and diet on bile acid homeostasis in male rats. *Chronobiol. Int.* **34**, 1339–1353 (2017).
211. Ohashi, N., Isobe, S., Ishigaki, S. & Yasuda, H. Circadian rhythm of blood pressure and the renin-angiotensin system in the kidney. *Hypertens. Res.* **40**, 413–422 (2017).

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