A CLOCKWORK WEB: CIRCADIAN TIMING IN BRAIN AND PERIPHERY, IN HEALTH AND DISEASE

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The hypothalamic suprachiasmatic nuclei (SCN) are our principal circadian oscillator, coordinating daily cycles of physiology and behaviour that adapt us to the world. Local versions of the SCN clockwork are also active in peripheral, non-neural tissues, driving the tissue-specific cycles of gene expression that underpin circadian organization. These local oscillators are tuned to each other, and to solar time, by neuroendocrine and metabolic cues that depend on the SCN. The discovery of these local circadian clocks forces a re-appraisal of established models of circadian biology. It also presents new avenues for therapeutic intervention in conditions where disturbance of circadian gene expression is an important cause of morbidity.

CIRCADIAN DAY/CIRCADIAN NIGHT
A notation of biological time, applied when organisms are in temporal isolation, devoid of external timing cues. The full circadian cycle is divided into 24 circadian hours, with circadian time (CT) 0 corresponding to subjective dawn, and CT12 subjective dusk. For nocturnal rodents, therefore, the onset of locomotor activity at CT12 marks the start of circadian night.

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In his *Herbal Treatise* of 1632, John Wren described the daily flows of the four humours — fluids that were considered to govern human nature. Whilst blood '…..reyneth for 6 hours from 9 o'clock in the night 'till 3 in the morning….' cholic, melancholy and phlegm followed on in series, each being in the ascendant for six hours (FIG. 1a). Although the biochemical validity of the humours might be doubted, Wren's view of an inexorable, repetitive order to our internal lives governing daily cycles of mood, intellect and physical ability chimes with modern concepts of circadian neurobiology. Circadian rhythms are daily cycles of physiology and behaviour that are driven by an endogenous oscillator with a period of approximately (*circa*-) one day (*diem*)[1,2]. Their expression continues (free-runs) when subjects are temporally isolated, with the oscillator defining predicted CIRCADIAN DAY AND NIGHT and organizing our biology appropriately to prepare for their contrasting demands. In humans, the most obvious circadian rhythm is the cycle of sleep and wakefulness[3]. In circadian day our physiology is given over to catabolic processes to facilitate engagement with the world, whereas at night anabolic functions of growth, repair and consolidation predominate. These intermeshed programmes establish an internal temporal order that optimizes our biological machinery. For example, as evening progresses, our body temperature falls and melatonin is secreted to facilitate sleep (FIG. 1b). Sleep onset is accompanied by increased secretion of growth hormone and prolactin, whereas before dawn, circadian activation of the adrenocorticotropic axis prepares us for the physical and mental demands of awakening[4].

Clearly, Wren was not too far off the mark, and he also recognized that circumstances that interfere with this smooth cyclical operation carry a severe cost. In lower organisms, competitive growth experiments show that genotypes with circadian periods that match the light–dark cycle enjoy a fitness gain relative to individuals with faster or slower clocks that cannot 'tune in' so well to the temporal environment[5]. In our modern '24/7' society, the social and commercial pressures that oppose internal temporal order are a growing source of circadian stress and are implicated in the aetiologies of chronic illnesses such as cardiovascular disease (CVD) and cancer[6,7]. Unravelling the clockwork and the mechanisms by which it governs circadian physiology will add to our understanding of a fundamental aspect of cellular organization. It will also facilitate the development of strategies to ameliorate conditions where the severity of illness and/or therapeutic efficacy carry a strong circadian bias.

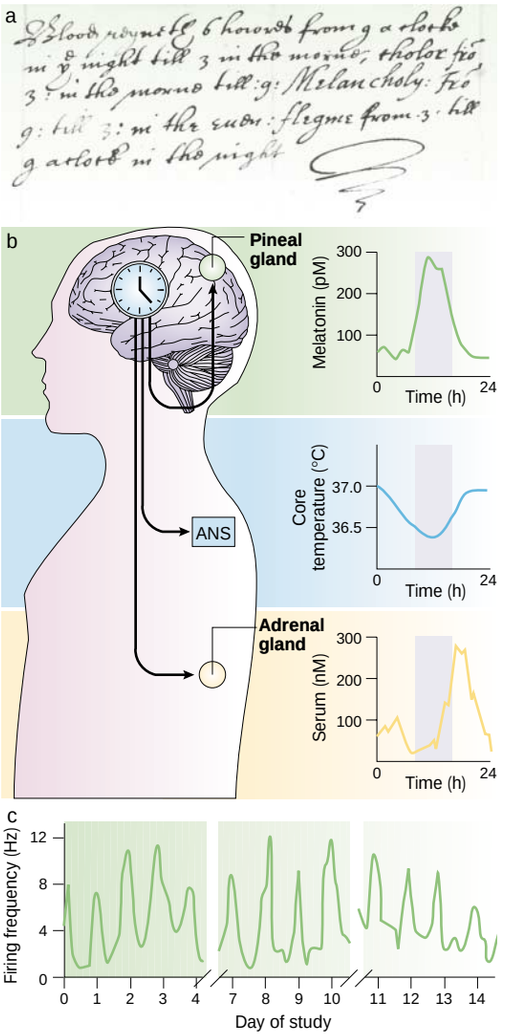


Figure 1 | **A brief history of circadian time. a** | Detail from John Wren's *Herbal Treatise* (1632) describing the daily ebbs and flows of the four humours that we would now recognize as circadian (courtesy of P. Redfern, University of Bath, UK and M. Hansen). **b** | A contemporary view of circadian organization in which a hypothalamic pacemaker, in the suprachiasmatic nuclei (SCN), communicates through various neural and endocrine links to drive and/or synchronize rhythms in peripheral physiology and behaviour. This ensures that as individuals progress through the regular 24-h cycle of sleep and wakefulness (grey shading), their metabolism is adjusted accordingly to anticipate the demands and opportunities of the solar day. ANS, autonomic nervous system. Modified, with permission, from REF. 151 © (1998) BMJ Publishing Group Ltd. **c** | Cell-autonomous circadian time-keeping reflected in the spontaneous firing rate of an isolated SCN neuron in culture. Modified, with permission, from REF. 16 © (2000) Cell Press.

The SCN as our body clock

The SCN are the 'head ganglion' of the circadian timing system[8]. They are remarkable structures, able to express *in vitro* sustained circadian cycles of electrical firing, cytosolic Ca^{2+} concentrations[9] and gene expression[10] that underpin the circadian organization of the individual. Recordings from single dispersed SCN neurons reveal that the circadian timing mechanism is not an emergent property of the SCN neuronal network. It is cell autonomous, with individual SCN neurons able to define and express their own circadian time (FIG. 1c). The γ-aminobutyric acid (GABA)-containing neurons of the SCN are segregated into a dorsal 'shell', characterized by vasopressin (AVP) expression, and a ventral 'core' where the neurons contain vasoactive intestinal polypeptide (VIP)[11]. Both divisions innervate a range of targets. Multi-synaptic links through the hypothalamic paraventricular nucleus carry circadian outflow to the adrenocorticotropic axis and to autonomic ganglia that innervate the viscera[12], whereas innervation of the dorsomedial hypothalamus contributes to circadian control of the orexin/hypocretin system, which consolidates wakefulness[13]. This circadian control of rest/activity cycles involves paracrine signalling: secretion of transforming growth factor-α (TGFα)[14] and prokineticin-2 (PK2)[15] by the SCN is implicated in activity regulation. Other circadian outputs, such as melatonin and corticosteroid secretion, depend on the integrity of 'hard-wired' neural connections[12].

In the SCN, GABA acts as a primary synchronizing signal among SCN neurons[16]. In addition, the VIP neurons of the core act through the VIP receptor subtype 2 (VPAC2), which is highly expressed across the SCN. In VPAC2-deficient mice, circadian activity/rest cycles are severely disrupted, and circadian cycles of gene expression, including AVP, are attentuated across the SCN[17]. This is accompanied by a reduction in spontaneous firing in SCN slices, and loss of the circadian rhythm of electrical activity[18]. So, although isolated individual SCN neurons can act as autonomous circadian clocks, if the balance between VIP, GABA and other signalling pathways is disturbed, the core oscillator is disabled.

Clock genes and circadian oscillation

How might an individual neuron sustain a 24-h oscillation? The identification of mammalian homologues of genes encoding the circadian clockwork of *Drosophila* supports a model of interlinked transcriptional and post-translational feedback loops in which complexes of the protein products of 'clock' genes enter the nucleus and suppress transcription of their cognate genes[8] (FIG. 2). The negative components of the loop are the products of three *Period* (*Per*) and two *Cryptochrome* (*Cry*) genes. *Per* and *Cry* messenger RNAs peak in the SCN in mid to late circadian day, regardless of whether an animal is nocturnal or diurnal[19]. Per and Cry proteins peak about 4 h later, when they form stabilizing complexes that allow them to enter the nucleus and suppress gene expression. The engine of the clock, against which Per/Cry complexes act, is transcriptional activation of *Per* and *Cry* expression by heterodimers consisting of the basic helix–loop–helix (bHLH)-PAS PROTEINS Clock and Bmal1 (MOP3). *Clock* mRNA and protein are constitutively expressed in the SCN[20], whereas *Bmal1* mRNA expression peaks in the middle of the circadian night. This delayed expression of *Bmal1* depends on *Rev-erbα*, another gene that is controlled by Clock/Bmal1 (REFS 21,22) (FIG. 1d). The Rev-erbα protein peaks in late subjective day, and

bHLH-PAS PROTEINS
Transcription factors characterized by a basic helix–loop–helix (bHLH) motif that facilitates DNA-binding and dimerization, and PAS protein–protein interaction domains that facilitate formation of heterodimeric complexes. They are characteristically involved in developmental events and adaptation to the environment.

RORE DNA SEQUENCES
Regulatory DNA sequences that are a target for retinoic acid receptor-related orphan receptors (ROR) — nuclear proteins with homology to retinoic acid receptors. The typical ROR element in the circadian system has the nucleotide sequence AGGTCA.

DNA E-BOX SEQUENCES
Regulatory DNA sequences that enhance transcription by providing a target for transcription factors, including bHLH-PAS proteins. They are involved in cell division, differentiation and apoptosis. The typical E-box in the circadian system has the nucleotide sequence CACGTG.

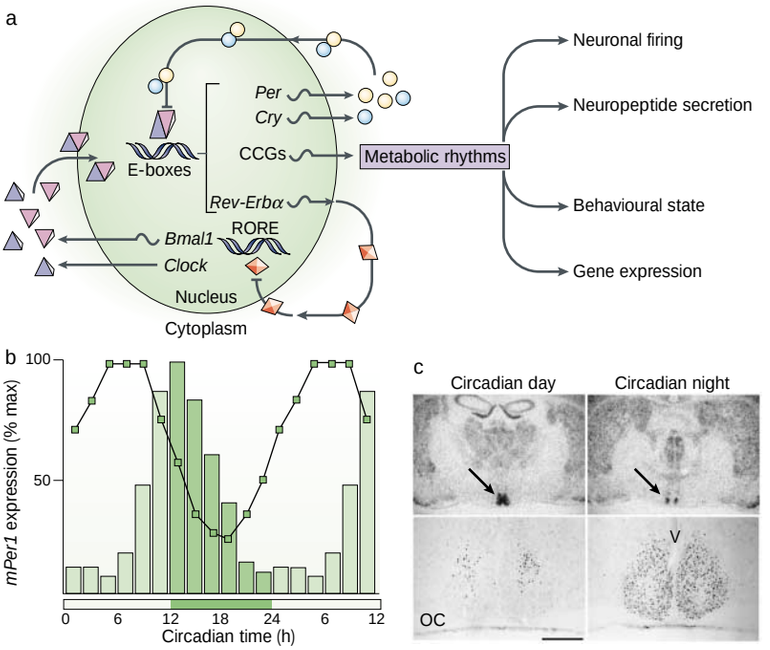


Figure 2 | **The molecular feedback loops that define circadian time. a** | In neurons of the suprachiasmatic nuclei (SCN), three interlocked streams of rhythmic gene expression sustain circadian timing. In the core oscillation, E-box-mediated activation of genes (including *Per* and *Cry*) by Clock/Bmal heterodimers in early circadian day is inhibited in late circadian day by nuclear accumulation of Per/Cry complexes, thereby closing an oscillatory negative feedback loop. The subsequent circadian cycle of expression is initiated when Per/Cry levels decline, the rate of which is sensitive to the phosphorylation status of Per. *Rev-erbα* is a negative regulator of *Bmal1* and is expressed in phase with *Per* and *Cry*. It establishes a positive feedforward loop that, through disinhibition, drives expression of *Bmal1* in antiphase to the negative factors. This contributes to the initiation of the new cycle of gene expression and enhances the core oscillation by segregating the intervals of peak transcriptional drive by Clock/Bmal and peak negative feedback by Per/Cry. The third molecular stream is the circadian expression of clock-controlled genes (CCGs), the ultimate arbiters of metabolic rhythms throughout the organism. One characteristic phase group of CCGs are regulated through E-boxes and are sensitive to the alternating balance of Clock/Bmal and Per/Cry activity, so they are driven in phase with *Per*. A second cluster of genes (not shown) are driven by RORE sequences, are sensitive to negative regulation by Rev-erbα and so are expressed in phase with *Bmal1*. Downstream cascading effects of some of these CCGs will direct the expression of further gene clusters to appropriate circadian phases, thereby completing the temporal programme. **b** | Circadian expression of *mPer1* mRNA (line) and nuclear immunoreactivity (bars) in mouse SCN. Expression of mRNA peaks in mid circadian day when protein levels are low, and declines as protein levels peak and initiate negative feedback at the end of circadian day. Only when the nuclear protein is cleared at the end of circadian night does mRNA expression start again. Circadian time (CT) 0, indicates start of circadian day; CT12, start of circadian night. Shaded bars represent previous light/dark cycle. Data for CT0–CT10 are double-plotted for clarity. Modified, with permission, from REF. 20 © (2003) Blackwell Science. **c** | Representative coronal *in situ* autoradiographs of mouse forebrain (scale bar 2 mm) depicting *mPer1* mRNA signal in SCN (arrow), and corresponding high-power images (scale bar 200 μm) of SCN immunostained for mPer1. Circadian day is characterized by high mRNA expression and low protein levels, whereas at night high nuclear protein levels are associated with attenuated gene expression. V, third ventricle; OC, optic chiasm.

acts through RORE DNA SEQUENCES in the *Bmal1* promoter to delay *Bmal1* expression until late circadian night. Although Clock/Bmal1 complexes bind to target DNA E-BOX SEQUENCES at all phases of the cycle, activation of gene expression is associated with circadian-gated chromatin remodelling by histone acetylation[23]. During the negative feedback phase, Per/Cry complexes associate with Clock/Bmal1, histone acetylation is blocked and gene expression is suspended.

The feedforward regulation of *Bmal1* expression interlocks the positive and negative phases of the molecular cycle, ensuring that the interval of maximal transcriptional drive, mediated by a surge in Clock/Bmal1 activity, coincides with the low point of negative feedback. Equally, as negative feedback by Per/Cry begins, transcriptional drive is starting to wane. This alternation provides the contrast enhancement that is necessary to sustain robust, high-amplitude oscillations[24]. Although other oscillatory systems, such as somite development[25], use cycles of gene expression to trigger periodic events, such cycles are rarely longer than a few hours. In the SCN circadian loop, the component parts, such as protein accumulation and degradation, are drawn out to produce a 24-h cycle that can sustain oscillation indefinitely. Identification of the key features of this temporal tuning remains an important goal.

Mutations of core clock genes are associated with altered stability, amplitude and/or period length of activity cycles, and, where tested, with altered circadian rhythms of electrical firing of the SCN *in vitro*[8] and sleep patterning in humans[26–29]. Mutations of factors outside the loop that affect stability of the proteins also alter clock speed. For example, hypophosphorylation of Per2, either because of a mutation of the casein kinase (CK1ε) enzyme in the *tau* mutant hamster[30] or because of a mutation of the phospho-acceptor site of human PER2 in subjects suffering from familial advanced sleep phase syndrome (FASPS)[26], shortens circadian period by 2–4 h. In FASPS, this is accompanied by advances of sleep onset and wakening, core body temperature minimum and onset of melatonin secretion[31]. Phosphorylated Per is degraded by the ubiquitin–proteasome system[32], and in *Drosophila* mutations of *slimb*, a gene that encodes a component of the ubiquitin ligase complex, disrupt circadian control of activity cycles by perturbing Per clearance[33]. As the influence of cellular mechanisms on the properties of the core loop is better defined, the boundaries between clock genes and non-clock genes are becoming less clear.

Clock input and output

Circadian time in the SCN is entrained to the solar day by glutamatergic innervation from the retina, principally to the SCN core[11]. Conventional retinal signalling through rods and cones might be sufficient but is not necessary for entrainment, and another putative photoreceptive system based on melanopsin, a homologue of a photoreceptor in amphibian skin, has been implicated[34,35]. Melanopsin is expressed in a sub-population of retinal ganglion cells (RGCs) that project directly to the SCN and other sites, mediating non-image-forming photoreception[36]. When isolated in culture, these RGCs depolarize in direct response to light. Knock-out of the melanopsin gene in mice attenuates circadian and other subliminal responses to light *in vivo*[37], and in the absence of melanopsin the 'circadian' RGCs lose their intrinsic photosensitivity[38]. This establishes these RGCs as an important input to the clock and implicates melanopsin in the circadian phototransduction cascade.

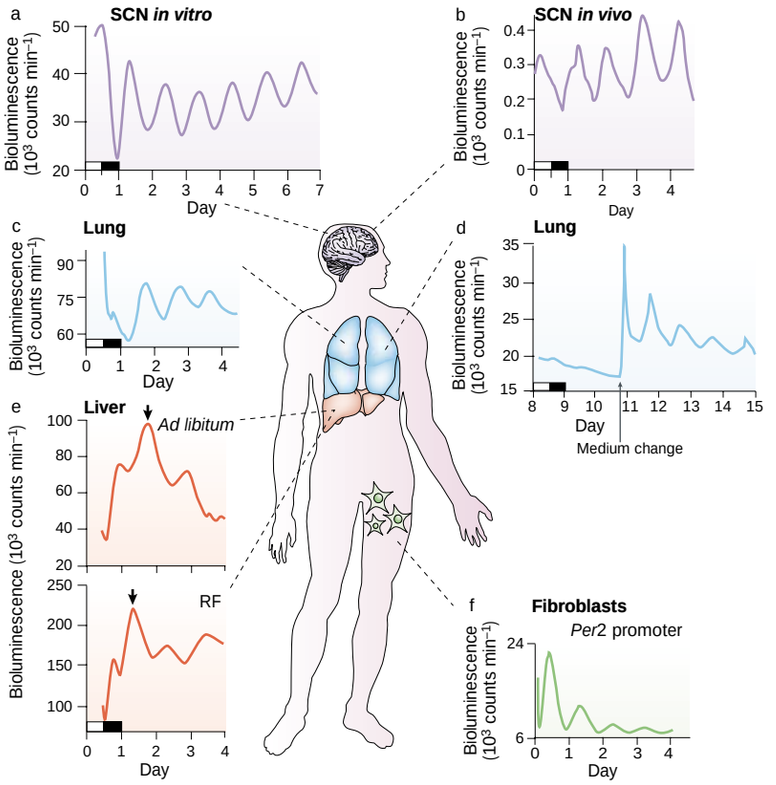


Figure 3 | ***Per::luciferase* transgenes reveal a diversity of tissue based circadian oscillators.** **a** | Circadian *Per1*-driven luciferase activity in rat suprachiasmatic nuclei (SCN) recorded *in vitro* is sustained for weeks[75]. **b** | This rhythm can also be monitored *in situ* in a transgenic mouse through a fibre-optic probe directed at the SCN[152]. **c** | Spontaneous cycling of *Per::luc* in culture also reveals a local oscillation in rat lung[10]. **d** | Having dampened after a few cycles *in vitro*, the lung oscillator can be reactivated by a change of medium (arrow)[79]. **e** | The phase of the liver circadian oscillation is set by feeding schedule: the peak in the liver of animals subject to restricted feeding (RF) is advanced relative to that of rats fed *ad libitum*. The bars on day 0 denote light schedule before the animal is killed and the tissue removed[79]. **f** | Fibroblast cell lines also contain a circadian oscillator based on rhythmic activity of *Per2* that can be activated by serum shock[22].

Exposure to light during circadian night increases the firing rates of SCN core neurons[39]. Simultaneously, gene expression in the core SCN is activated through a Ca^{2+}-dependent kinase cascade, ultimately inducing genes carrying Ca^{2+}/cyclic AMP response elements (CRE), including *Per1* and *Per2*, accompanied, as in the spontaneous cycle of *Per* induction, by chromatin remodelling by histone acetylation[8,40,41]. Several hours after light exposure, the core neurons show a sustained elevation of spike frequency, and electrical and/or paracrine signals spread to the SCN shell where further *Per* induction occurs[42]. With prolonged light exposure, *Cry* is also induced[43]. Consequently, the spontaneous cycle of gene expression across the SCN is regularly adjusted as dusk light delays the loop by inducing *Per* as it spontaneously falls, whereas dawn light prematurely activates *Per* expression, advancing the clock[44]. These adjustments maintain periodicity at exactly 24 h. They also match the circadian cycle to seasonal changes in daylength because as dawn and dusk move apart in spring, the duration of *Per* expression in the SCN lengthens[45–47], thereby incorporating a calendar into the clock. The oscillator is also sensitive to imposed changes in the rest/activity cycle, as manifested in shift-workers. This non-photic resetting is mediated by neuropeptide Y (NPY) innervation of the SCN from the thalamus, and a serotonergic input from the raphe nuclei. In contrast to the effect of nocturnal light, which elevates *Per* levels, these non-photic pathways suppress *Per* expression in the circadian day, advancing the molecular oscillation to a more nocturnal phase[48–50].

The entrained oscillator imposes temporal order within and beyond the SCN through the regulated expression of clock-controlled genes (CCGs). These sit outside the core loop but undergo periodic transcriptional activation and repression by Per/Cry complexes. The clock-controlled secretory peptides AVP, PK2 and TGFα contribute to extracellular signalling, controlling behavioural and neuroendocrine cycles. Other CCGs encode transcription factors, such as albumin D element-binding protein (Dbp) and the basic helix–loop–helix proteins Dec1 and Dec2, which coordinate downstream cascades of circadian gene expression[8,51]. *Pk2* and *Dec1* are also acutely upregulated by nocturnal light and so might contribute to the entrainment of the clockwork or mediate the direct effects of nocturnal light on physiology, such as acute suppression of melatonin biosynthesis. *Nocturnin* is a CCG that encodes a deadenylase enzyme[52]. It is expressed rhythmically in the SCN, eye and many peripheral tissues of mouse[53], and might facilitate mRNA degradation in the core loop or in output pathways, thereby contributing to rhythmic gene expression at a post-transcriptional level.

Autonomous peripheral oscillators

Recognition of the SCN as the primary circadian oscillator led to a model in which the periphery was totally dependent on the SCN to define circadian intervals and establish internal temporal order. The retina seemed to be the only other structure capable of intrinsic circadian oscillation in the adult mammal, but this simply involved local housekeeping functions. However, the identification of the clock genes was followed by observations that have challenged this hierarchical view. In *Drosophila*, *per* is expressed with a circadian rhythm in the excretory tissue of the Malpighian tubules, and rhythmic accumulation of nuclear Per persists in tubules cultured *in vitro* where it can also be entrained by light–dark cycles. This shows that the tubules contain an intrinsically light-sensitive circadian clock[54]. Experiments in which a luciferase reporter gene was used to monitor *Per* expression in isolated organ explants showed that many other components of the fly's body also contain autonomous, light-sensitive circadian clocks, assembled from the same gene products as the 'master' oscillator[55]. These local clocks are functional, for example driving a circadian rhythm of olfactory sensitivity in the antennae that probably mediates aspects of social entrainment in flies[56,57]. Lower vertebrates also show dispersed organization of the circadian timing system: explants of zebrafish heart and liver tissue, and even zebrafish cell lines, show circadian clock gene expression that is directly entrainable by light acting through a local photoreceptor[58].

Mammalian clock genes are also expressed rhythmically in peripheral tissues, although sustained circadian expression is lost in SCN-lesioned animals[59], and the period of circadian activity/rest cycles restored by SCN grafts is determined solely by SCN genotype, rather than by peripheral genotype[60,61]. This indicates that the SCN are the sole origin of circadian structure. Surprisingly, though, when a mammalian fibroblast cell line is grown to confluence, halting cell division, and is stimulated with a high concentration of serum, the acute induction of *Per1* and *Per2* is followed by several circadian cycles of expression of these clock genes and known CCGs[62]. The fundamental circadian properties of the neural oscillator of the SCN — acute induction of *Per* and sustained circadian gene expression — are apparently shared with a 'simple' cell line. It remains unclear whether the measurable rhythms of gene expression represent *de novo* induction of a previously quiescent oscillator in every cell, or synchronization of previously active oscillators across the population of cells[62]. Nevertheless, the relevance of this observation was demonstrated by using tissue from rats carrying a mouse *Per1::luc* transgene (FIG. 3). As in *Drosophila*, there were spontaneous circadian rhythms of luciferase activity in various organs in culture including liver, lung, kidney and several brain regions outside the SCN. The cycles dampened after 2–7 days, but could be reinitiated by serum shock or medium change[10], and although not light entrainable, these local oscillators are essentially a replication of the SCN machinery. For example, cultures of embryonic fibroblasts from mutant mice show rhythmic phenotypes *in vitro* equivalent to the rest/activity cycle of their donor[63,64]: knocking out *Per1*, *Cry1* or *Cry2* alters the circadian period of the cells, whereas knocking out both *Cry* genes renders the cells arrhythmic.

Local versions of the SCN clockwork based on rhythmic *Per* expression are therefore present in many peripheral tissues. A crucial difference from the peripheral clocks of lower species is that mammalian tissue clocks are not intrinsically light sensitive and so, *in vivo*, will rely on the SCN for entrainment to the solar day and perhaps to each other. These peripheral oscillators also contain additional, tissue-specific factors. Circadian expression of *Per2* in the forebrain depends on Npas2 (MOP4), a paralogue of Clock that can also dimerize with Bmal1 and drive gene expression through E-box sequences[65]. In the vasculature, complexes in which Bmal1 dimerizes with either Clock or Npas2 drive circadian gene expression[66], whereas in other peripheral tissues, Bmal2 (MOP9 or CLIF) is expressed in up to nine tissue-specific isoforms, and dimerizes with Clock to drive CCGs[67]. Precise details of the internal sequence of the core loop also vary between tissues. In the periphery, clock genes tend to peak about 4–8 h later than in the SCN, and in the liver, peak expression of *Cry1* relative to the other clock genes is further delayed because of a local action of Rev-erbα on the *Cry1* promoter[23]. In liver from *Rev-erbα*-knockout mice, the peak of *Cry1* expression is advanced to the phase that it occupies in other tisues. In the pituitary *pars tuberalis*, *Per* and *Cry* expression are even more divergent, being linked to circadian dawn and dusk, respectively[68]. These local variations on the core loop offer scope for local fine-tuning of the clockwork, and present specific targets for therapeutic management.

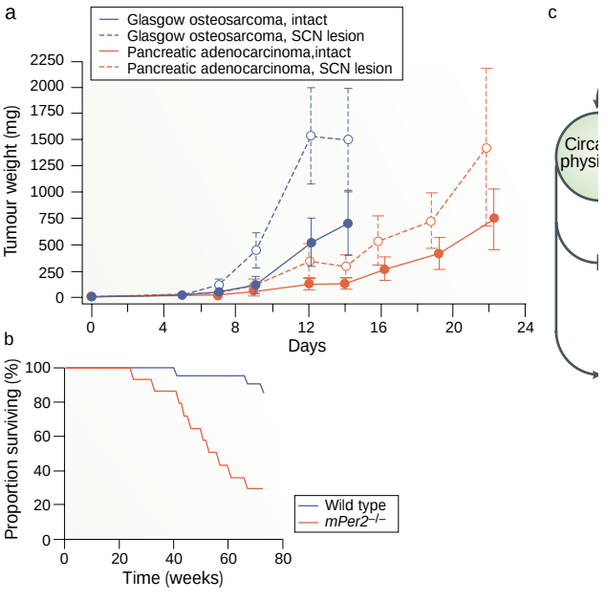
Tissue-specific circadian programming

Given that the core oscillator seems to be active in many tissues and is 'hard-wired' into cellular function, it is important to know how many genes it drives, and how much peripheral circadian expression varies. DNA microarrays indicate that beyond the core loop, about 5–9% of the transcriptome is under circadian control in the liver, with a similar proportion in the heart and in the SCN[22,59,69,70]. The complement of rhythmic genes differs, though, with less than 10% likely to be common to any two tissues; and where genes are common, they are genes close to the core loop, such as *Dbp*, and at the top of a transcriptional cascade with local, tissue-specific targets. The common, *Per*-based oscillatory mechanism is used to drive tissue-specific output programmes that are matched to local needs. Consequently, across the body, a large proportion of the genome is probably subject to circadian regulation.

In any single tissue, rhythmic genes are clustered into phase-typical groups, notably CCGs activated in phase with *Per* and an anti-phasic cluster that peaks in phase with *Bmal1* (REF. 22). These common phases reflect shared regulatory mechanisms: for example, the genes expressed in the liver in phase with *Bmal1* are enriched for RORE sequences and regulated by Rev-erbα. Clusters of CCGs that are activated by Clock/Bmal acting on E-box sequences have been identified in fibroblasts[71] and mouse liver, and their expression is downregulated in *Clock*-mutant mice[69]. Surprisingly, many non-rhythmic genes are also up- or downregulated by the *Clock* mutation, indicating either that Clock has non-circadian roles or that the circadian oscillator is necessary for the regulation of non-rhythmic genes. A complete decoding of the circadian programme for a particular tissue has not yet been achieved, but it is likely to involve both generic and tissue-specific regulatory factors and DNA sequences.

The genome-wide programmes of circadian gene expression provide a template with which to understand the circadian physiology of a particular tissue. The transcriptional clock regulates key, often rate-limiting, enzymes. In the liver, these include proteins that are involved in the metabolism of glucose, proteins and lipids, and in vesicular trafficking. Interestingly, circadian transcription of numerous cytochrome P450 (Cyp) enzymes, which are important in steroid metabolism and detoxification, has been observed in both mammals and *Drosophila*, indicating that temporal aspects of their regulation are highly conserved and, by implication, important for their function. In the liver the same clock output protein, Dbp, drives expression of both Cyp7a, a key factor in bile acid production, and Cyp2a4, which is involved in steroid metabolism. Through a single output, therefore, the core loop orchestrates circadian timing in different metabolic pathways. Glutathione-*S*-transferases (GSTs) are another family of enzymes that

Box 1 | Peripheral circadian clocks, cell cycle and cancer



Cell growth and division in peripheral tissues follow a tissue-specific circadian programme. In rodents, DNA synthesis occurs predominantly at the start of circadian night, and mitosis at the beginning of circadian day[138], and microarray analyses indicate that the expression of cell-cycle regulators is under circadian control, regardless of whether tissues are mitotically active[59,69,71]. In human oral mucosa, both cell proliferation and the expression of cell-cycle regulatory proteins (such as p53 and cyclins) is circadian, cycling in parallel with the rhythmic expression of core clock genes[139,140], and topoisomerase II-α — an essential factor in mitosis and an important target for chemotherapeutic agents — shows a pronounced circadian cycle in human rectal epithelium[141]. This gating of the cell division cycle by local circadian oscillators has considerable importance for cancer therapy, not least in defining optimum daily intervals for intervention[142,143]. For example, circadian-modulated chemotherapy is more effective than constant infusion, enhancing the efficacy of surgical intervention and improving survival of patients with metastatic colorectal carcinoma[144]. Circadian regulation of the cell cycle is also important for tumour progression because tumours have their own endogenous circadian clock that rhythmically expresses core clock genes that are synchronized to the host[129]. Destruction of peripheral circadian organization in the host and tumour, either by ablation of the suprachiasmatic nuclei (SCN)[145] or by exposure to irregular light/dark cycles[129], accelerates tumour growth (part a). For example, growth of transplanted Glasgow osteosarcoma (blue) and pancreatic adenocarcinoma (yellow) tumours is accelerated in mice bearing lesions of the SCN compared with intact mice (part a modified, with permission, from REF. 145 © (2002) Oxford University Press).

Equally, disruption of the core clockwork by mutation of *Per2* increases the susceptibility of mice to spontaneous and irradiation-induced tumours[146] (part b, modified, with permission, from REF. 146 © (2002) Cell Press), and impaired circadian function is associated with a poor prognosis in cancer patients[142]. The proto-oncogene *c-Myc* might be one route through which circadian (dis)order affects tumour cell division. It is a clock-controlled gene that is downregulated by Npas2/Bmal1 through E-box sequences in its promoter, and is released from this inhibition by Cry. *c-Myc* is de-repressed in the liver of *Per2* mutant mice, and expression of its target, the tumour suppressor *Gadd45a*, is markedly attenuated[146], leading to increased genomic instability. Therefore, disruption of both the core loop and circadian physiology might contribute to the increased incidence of tumour initiation and progression in *Per2* mutant mice[147]. The disruptive effect of circadian disorder — disabling physiological checkpoints in tumour progression — is highlighted by epidemiological studies of breast cancer, in which long-term shift work is associated with an increased risk of 10–60% (REFS 148–150).

The putative relationships between circadian timing, cell cycle and tumour progression are shown in part c. In normal tissues, cellular proliferation is gated by circadian factors, arising from local tissue-based clocks, and more general circadian physiology, synchronized by signals from the SCN. The cell cycle in tumours is also subject to negative regulation by circadian factors, derived from autonomous clocks in the tumour, which are in turn entrained by the host circadian system, and by peripheral clocks in healthy local tissues. Disturbance of circadian structure either locally or systemically across the organism will relieve this gate and accelerate tumour progression. Identification of factors mediating circadian suppression to tumour growth will facilitate development of novel therapeutic approaches.

are involved in detoxification, and Gstt2 transcription is also circadian. Reduced levels of GSTs are correlated with an increase in some solid tumours, and it is possible that chronic disruption of the circadian transcription of these enzymes in the liver could increase susceptibility to environmental carcinogens (BOX 1). Equally, circadian cycles of liver and kidney metabolism might contribute to the established circadian patterns of toxicity for agents such as antibiotics and cytotoxic factors[72], and in other clinical contexts, for example in treating narcotic overdose, or for long-term survival of haemodialysis patients[73,74].

A resonant network of peripheral clocks

The emerging view is that peripheral physiology is tightly regulated in time as a consequence of recurrent daily waves of differential gene expression that underpin a tissue-specific metabolic programme. The ability of

peripheral tissues to sustain autonomous cycles emphasizes the universality of circadian programming to cellular physiology, but raises another important issue. The old view had the periphery as a blank sheet, awaiting signals from the driving pacemaker of the SCN to oscillate and establish temporal organization. But is the SCN principally a coordinator, a time signal to otherwise independent, self-sustaining peripheral clocks? In both cases, ablation of the SCN would lead to a loss of circadian organization at the level of the individual's physiology, but for different reasons: either because in the absence of the SCN the clock mechanisms wind down and become inactive, or because the component oscillators lose coordination and temporal definition is lost. Equally, the reported loss or attenuated amplitude of circadian patterning to gene expression in the liver and other peripheral tissues of SCN-lesioned animals might reflect an averaging effect of pooling samples from a population of rhythmic but desynchronized individuals[59], rather than a real loss of circadian cycles in the periphery of each individual.

In favour of the 'SCN-as-driver' view, *Per1::luc* rhythms that are reported by the transgene from peripheral tissues dampen rapidly *in vitro* over 3 to 6 days[10], whereas cultures of the SCN continue to show a high-amplitude rhythm over that interval, consistent with electrophysiological evidence of sustained SCN oscillation[75]. On the face of it, therefore, the SCN is a more robust clock, and the dampening of the peripheral oscillator highlights its inadequacy once deprived of SCN-defined circadian time. However, the dampening in peripheral tissues might in part reflect uncoupling among individual oscillatory cells rather than a loss of individual cellular rhythms within the tissue. In this case one special feature of the SCN might lie in strong coupling between its component cellular clocks (possibly mediated by GABA and VIP), rather than in particular properties of the core molecular loop *per se*. By extension, the *in vivo* role of the SCN would be to ensure that multiple tissue-based oscillators retained synchrony, rather than to sustain them. Real-time analysis of *in vitro* circadian activity in individual SCN and peripheral cells will resolve this issue. In addition, the progressive dampening observed *in vitro* might be a feature of the transgenic construct, which uses mouse sequences in the environment of rat peripheral tissue. More representative homotypical reporters, embedded in and driven by endogenous regulatory sequences, should reveal new features of peripheral cyclicity, and perhaps a more robust circadian mechanism.

But *in vitro* studies alone will not clarify the role of the SCN in circadian physiology, not least because establishing tissue cultures from SCN-lesioned animals might initiate oscillation in otherwise quiescent tissues. Real-time *in vivo* analysis of tissue-specific luciferase or other reporter activity, similar to that currently used to monitor tumour growth, will be necessary to resolve this issue. Is it possible to observe sustained circadian gene expression in the peripheral tissues of SCN-lesioned animals that are held in temporal isolation and, if so, do these rhythms run independently, tissue by tissue, or is there evidence of synchronization in the absence of SCN cues? It is entirely possible that the consequences of SCN lesions vary for different tissues, depending on whether they are 'hard-wired' into the SCN, or regulated indirectly: rhythms such as activity/rest cycles, core body temperature and pineal melatonin secretion, which can be monitored in individual animals, do not survive SCN ablation. Tissue oscillators that are regulated by more indirect routes, for example the food-entrainable oscillator (see later discussion), might have far greater autonomous properties.

Whatever the answer, in the intact animal the peripheral oscillations clearly depend on the SCN to set their phase and maintain their amplitude, but the autonomous ability of the periphery to establish its own circadian structure argues against a simple master–slave hierarchy, and more in favour of a resonant network. This resonant network holds tissues reciprocally in a tight temporal order, enabling local circadian time to be defined continuously and precisely, maintaining local rhythmic functions and anticipating physiological demands with only intermittent input from the SCN. Local clocks will allow individual tissues to respond differently to generic SCN signals, and to filter and modify temporal cues that are embedded in SCN signals for transmission onwards to other structures. By analogy with the SCN, where the response to light is enhanced at night, the tissue-specific clocks probably upregulate entrainment pathways in anticipation of SCN-dependent signals, and the acute induction of *Per* by serum shock or medium change in peripheral tissues might represent the same resetting event as retinally-mediated, photic induction of *Per* in the SCN.

Synchronization of peripheral clocks

Potential routes for internal synchronization are varied, but one over-riding factor is feeding. Spontaneous feeding in nocturnal rodents reflects the SCN-determined rest/activity cycle, but when access to food is experimentally restricted to a few hours during circadian day, nocturnal animals become active in anticipation of, and during, that interval. This behaviour and its accompanying physiology have many characteristics of a circadian oscillation, but are independent of the SCN. Restricted feeding does not alter the circadian molecular cycle of the SCN, which remains faithful to solar time, and food-anticipatory behaviour survives SCN ablation[76,77]. However, restricted feeding does advance the *in vivo* circadian rhythms of gene expression in the liver, kidney, heart and other tissues, uncoupling them from control by the SCN even on a test day when food is not presented[78]. Moreover, when liver tissue from animals that have been subject to restricted feeding is cultured, the spontaneous circadian cycle of *Per1::luc* activity is advanced to match the behaviour and is sustained *in vitro* for several cycles[79]. The rate of this advance is tissue-specific: for example, the lung is slower to respond than the liver, and it is possible that the elusive food-entrainable oscillator reflects intrinsic circadian properties of the liver, and/or activity in neural centres that receive hepatic sensory information, such as the ventromedial hypothalamus. Intriguingly, homozygous *Clock*-mutant mice exhibit food-entrainable activity

rhythms and cycles of clock gene expression in the heart, indicating that factors other than Clock can, at least for one or two cycles, sustain the oscillator[80,81].

What type of feeding-dependent signal might synchronize the peripheral circadian network? Restricted feeding perturbs the circadian rhythm of glucocorticoid secretion, which shows an accessory peak associated with anticipatory arousal[76,82]. Systemic injection of the glucocorticoid receptor agonist dexamethasone acutely shifts the cycle of liver gene expression *in vivo*, advancing or delaying expression of *Dbp* and *Rev-erbα*, depending on when it is administered. Glucocorticoids can also induce circadian *Per* expression in cell cultures[83], but these actions alone cannot explain resetting by restricted feeding, not least because injections designed to mimic the induced glucocorticoid peak and delivered *in vivo* fail to reset the liver *Per1::luc* cycle subsequently measured *in vitro*[79]. Moreover, restricted feeding, but not injection of dexamethasone, can entrain the liver gene expression rhythm in mice carrying a liver-specific deletion of the glucocorticoid receptor[83,84]. In fact, entrainment to restricted feeding is faster in mice with the liver-specific receptor knockout. It seems likely, therefore, that the endogenous glucocorticoid rhythm that is driven by the SCN *in vivo* opposes rather than facilitates the response to restricted feeding. The ability of restricted feeding to shift peripheral oscillators depends on factors that can over-ride this glucocorticoid-mediated inertia.

Restricted feeding causes periodic availability of circulating macronutrients, and consequent activation of many signalling pathways. In cultured fibroblasts, the ability of serum shock to induce *Per* and set the oscillator running is shared by activators of many intracellular kinases, including protein kinase A, protein kinase C and mitogen-activated protein kinase (MAPK)[82,85,86], many of which act ultimately through the CRE sequences in *Per* genes[40]. There is considerable redundancy of these pathways, and blockade of individual pathways does not compromise the overall response to serum shock. Glucose can also initiate circadian gene expression in fibroblast culture, although in an echo of NPY-mediated non-photic resetting of the SCN, it does so after acutely suppressing *Per* (possibly through glucose-induced immediate-early genes)[87]. Insulin can also induce *Per* and initiate the oscillator, although the rise in insulin that is associated with feeding does not seem to be necessary to drive liver gene expression *in vivo,* as diabetic rats can entrain both anticipatory behaviour and *Per1::luc* rhythms to restricted feeding[88]. In vasculature smooth muscle, the oscillator can be initiated by angiotensin II and reset by retinoic acid, possibly through interactions of retinoic acid nuclear receptors with Clock/Bmal and Npas2/Bmal complexes[66,89]. In the *pars tuberalis* of the pituitary gland, circadian *Per* expression is phased to dawn[45], driven by an interaction between nocturnal melatonin secretion and adenosine[90].

Many endocrine and paracrine cues, therefore, can influence peripheral clocks in a tissue-specific manner. Rhythmic cultures of immortalized SCN cells can establish circadian gene expression in co-cultured fibroblasts[91], indicating that internal entrainment mechanisms might be conserved and generalized. One common factor might be changes to the metabolic status of a tissue that affect its redox state[92]. For example, increasing levels of the reduced metabolic co-factors NADPH and NADH increase the affinity of Clock/Bmal and Npas2/Bmal complexes for their target DNA *in vitro*, and in neuroblastoma cells, transcriptional activation by Npas2/Bmal is enhanced by treatment with lactate, which increases NADH levels. This 'redox' model also indicates a mechanism for transcriptional repression by Cry in which Cry-bound FAD oxidizes the Clock and Npas2/Bmal complexes, reducing their DNA-binding affinity[92]. Although many tissues undergo pronounced circadian cycles in redox state, reflecting a metabolic output of the loop, it remains to be determined how much these changes feedback to reset the core loop *in vivo*.

Restricted feeding also depresses nocturnal core body temperature[78], and fibroblast cultures that are incubated in a temperature cycle that reproduces this modification sustain circadian gene expression for longer than those incubated at a constant temperature[93]. A larger amplitude temperature cycle of 4°C can initiate circadian gene expression *de novo* in fibroblasts, and exposing mice to hot nights resets the phase of their liver gene expression cycles *in vivo*, without affecting the phase of the SCN clockwork. Although the mechanism by which both physiological and non-physiological temperature cycles might entrain peripheral clocks is unknown, metabolic status and/or the autonomic nervous system might be involved. The latter has the potential to drive or reset peripheral oscillators; for example, local circadian *Per* gene expression can be abolished by sympathetic denervation of the pineal gland or liver *in vivo*, and it can be acutely induced by adrenergic agonists[94,95]. Circadian regulation of autonomic tone might therefore contribute to the phasing and amplitude of peripheral clocks.

These findings not only highlight the potential complexity of maintaining circadian order across peripheral functions, but they also emphasize the problems posed by environmental regimes that provide conflicting signals to the circadian system, such as those experienced by shift workers and during jet-lag. Altered behavioural patterns, meal schedules, lighting regimes or environmental temperature will perturb the synchrony of the resonant network, with potentially serious physiological consequences. Equally, ageing undermines internal temporal order. Although the circadian rhythm of *Per1::luc* in the SCN is not affected by age, old rats show marked changes in the robustness and relative phasing of peripheral oscillations[75]. Disturbances of the 'phase map' could arise from alterations in the ability of the SCN to communicate with peripheral targets or from changes in clock function in individual tissues. In humans, a progressive advance in timing and loss of precision of the sleep/wake cycle accompanies ageing[96,97], and is particularly pronounced in Alzheimer's disease[98–100]. These abnormalities of circadian behaviour are accompanied by changes in the phase, amplitude and precision of the core body temperature rhythm, and it is likely that ageing and

particularly dementia are accompanied by a global disturbance of circadian metabolic order. Disturbances of the sleep/wake cycle are the primary cause of institutionalization in dementia[97,101]. Management regimes that minimize or restrict these disturbances, such as regular light/dark cycles, exercise and dietary programmes, and timed administration of circadian cues such as melatonin, might ameliorate these disturbances and delay institutionalization[97]. In the longer term, identification of the tissue-specific molecular pathways that sustain the phase map will support the development of more precise therapies.

Circadian networks and disease

Circadian control of normal physiology will inevitably lead to circadian variation in disease. For example, acute cardiovascular and cerebrovascular episodes, such as angina pectoris[102] and intracerebral haemorrhagic stroke[103], show a pronounced morning peak associated with circadian changes in blood pressure, cardiac output and so on (FIG. 4). The relative risk for initiation of acute myocardial infarction (AMI) is 40% higher in the morning, and relative to other causes, about 9% of all AMI can be attributed to this circadian wave[104]. For sudden cardiac death, meta-analysis indicates an increased risk of 30% in the morning, and the circadian surge contributes about 7% of total causes[104]. The hours immediately after awakening are therefore a crucial period for those suffering from CVD[105]. Therapies designed to alleviate this circadian load would have an important impact on morbidity and mortality across the developed world. For example, beta-blockers that attenuate the circadian surge in autonomic activation also stop the morning peak of CVD[106,107], and timed-release formulations of anti-hypertensive agents that can be taken at bedtime have been designed to target the morning 'crisis-point'[108–110].

The identification of local circadian clocks provides new ways to address this problem. First, it implicates local tissue-specific events as potential contributors to temporal patterning of CVD, which might be amenable to local modulation. Second, it reveals a molecular mechanism, the core loop, as a source of circadian prevalence and therefore a target for local, tissue-specific therapy, independent of the SCN and other clock mechanisms. Third, factors responsible for entraining or sustaining the local circadian loop are avenues for management. Fourth, it emphasizes the importance of local CCGs as direct or indirect mediators of circadian prevalence. Finally, the contribution of a resonant network of tissue-based oscillators means that crucial processes will behave in non-linear and time-sensitive ways.

Peripheral oscillators contribute to the circadian prevalence of CVD in many ways (FIG. 4). First, the heart has an intrinsic clock that enables it to anticipate, prepare for and adapt to circadian changes in physiological demand[111]. *In vitro*, the rat heart shows pronounced circadian variation in contractile function, which is sustained by circadian variation in oxidative metabolism. This is associated with circadian rhythms in the expression of a range of CCGs that are involved in carbohydrate utilization, mitochondrial function and fatty acid metabolism, and are driven by the core loop of Per/Cry-mediated negative feedback[111]. In experimentally induced cardiac hypertrophy, the core molecular oscillator continues to cycle, but circadian expression of clock-controlled transcription factors, including *Dbp*, is blunted[112]. Furthermore, the circadian cycle of metabolic gene expression is lost, and many of the genes remain at or close to their circadian nadir throughout the 24 h. Under these conditions of attenuated output, the tissue will be less able to prepare for, and cope with, routine increases in physiological demand, predisposing it to metabolic crisis. In a different model of contractile dysfunction, streptozotocin-induced diabetes in rats, the molecular cycles retain their normal amplitude, but show a phase advance of about 3 h (REF. 113), whereas spontaneously hypertensive rats show a marked increase in the amplitude of daily rhythms of mRNA encoding components of the renin-angiotensin system[114]. Clearly, one aim of treatments for cardiovascular dysfunction should be to facilitate or restore circadian control of cardiac gene expression.

Circadian variation in blood pressure also contributes to periodic CVD — the morning peak of blood pressure being retained in hypertension[110]. The peak depends on several factors, including circadian changes in cardiac output and autonomic tone, and changes in the tone of vascular smooth muscle (VSM). The VSM contains an intrinsic oscillator that can be initiated in culture by serum stimulation[66] and angiotensin[89], both of which acutely induce *Per* expression (the latter through MAPK signalling), and can sustain circadian cycles of gene expression driven by Clock, Npas2 and Bmal1. Once running, this oscillator can be reset by retinoic acid or glucocorticoids. Retinoic acid receptors interfere with gene expression that depends on Npas2/Clock/Bmal1 complexes. Retinoic acid can also reset circadian gene expression in the aorta and heart *in vivo*. The transcription factor *Dbp* is controlled by this local oscillator, although it is not clear how rhythmic gene activity in the VSM is coupled to circadian changes in vascular tone. The endothelium also contributes to rhythms in vascular tone *in vivo*, maintaining circadian changes in flow-mediated dilatation of the arterial wall[115,116] and vasodilatation in the skin[117], both of which show a morning trough that is associated with the highest risk of CVD. Circadian release by the endothelium of endothelins, which are potent vasoconstrictors, might also contribute to the rhythm in vascular tone[117]; in fibroblast cultures, endothelin-1 induces *Per* and initiates the circadian oscillator[63]. Circadian regulation of vascular tone might therefore involve coordination of molecular oscillators in both endothelium and VSM, synchronized by endothelins and other entraining factors acting on the core loop.

Circadian incidence of CVD is also caused by variation in haematogenous factors. The tendency of platelets to aggregate is increased after waking[118], thrombosis is more likely in the morning[119] and the efficacy of tissue plasminogen activator (tPA) and other thrombolytic agents in breaking down clots is lowest in the

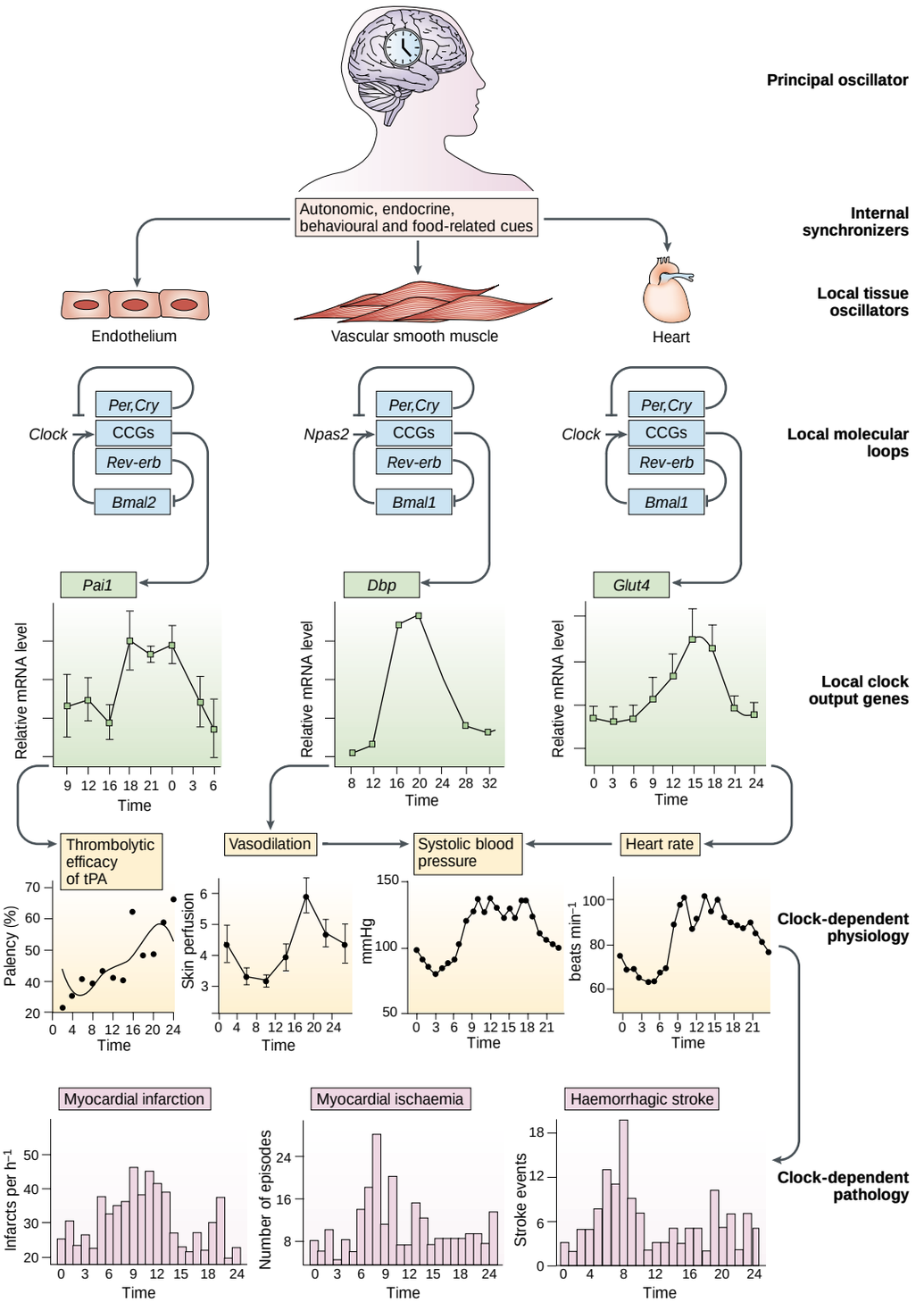


Figure 4 | **Peripheral circadian clocks in cardiovascular disease.** Local circadian oscillators in the vascular endothelium, smooth muscle and myocardium, based on clock gene feedback loops, are synchronized by signals from the suprachiasmatic nuclei (SCN). They drive tissue-specific circadian patterns of clock-controlled gene (CCG) expression, represented here by *Pai1*, *Dbp* and *Glut4* (glucose transporter 4)[89,111,125]. In turn, these molecular cycles sustain local clock-dependent physiologies, including circadian cycles in thrombolytic activity of tissue plasminogen activator (tPA)[121], vasodilatation[117], blood pressure and heart rate[153]. These fluctuating physiological states interact with each, further maintaining synchrony between the peripheral oscillators. Clock-dependent cardiovascular pathologies, for example infarction, ischaemia[106,153] and stroke[103], are a consequence of the actions of the network of local circadian oscillators in vascular tissues. Because the local tissue oscillators show regional differences in their molecular components, their cellular outputs and the synchronizing factors to which they respond, they present new avenues for therapeutic intervention that can be effected locally, and without impact on the principal oscillator of the SCN.

morning[120–123]. These changes in the likelihood of clotting and the resistance of thrombi once formed arise from circadian changes in endothelial and blood cell function[124], including a daily rhythm in the activity of plasminogen activator inhibitor (Pai1), which counteracts the thrombolytic effect of endogenous tPA[124]. *Pai1* is a typical CCG of vascular endothelium. Its expression is driven by Clock/Bmal2 complexes acting through circadian E-box sequences, and is opposed by Per/Cry complexes[67,125]. In the rat heart, the peak of Pai1 enzyme activity is synchronized to the onset of nocturnal locomotor activity and corresponds to a circadian peak in mRNA expression[125]. This circadian mRNA peak is lost in the *Clock* mutant mouse[126], whereas in the wild-type mouse, restricted feeding can phase-shift the *Pai1* rhythm in the heart, just as it shifts rhythmic *Per* expression[126]. Although the factors that mediate this shift of the *Pai1* rhythm are unknown, they are independent of Clock insofar as they can drive rhythmicity of *Pai1* in the heart of *Clock* mutants, an echo of the ability of light to drive *Per* in the SCN of these mutants. *Pai1* mRNA is also rhythmically expressed in the endothelium of other tissues, including the kidneys[125], and it can be upregulated by glucocorticoids — an additional rhythmic cue and mediator of stress[127].

Circadian prevalence of CVD arises from a complex interplay among local oscillators in the heart, endothelium and VSM, their endocrine interactions, and their regulation by SCN-dependent changes in autonomic tone, feeding, stress and energetic demands. The involvement of this and other resonant circadian networks in healthy physiology helps to explain the severe cost of circadian desynchronization. In transgenic rats subjected to 6- or 9-h shifts of the light/dark cycle, the rhythm of *Per1::luc* activity in the SCN adjusts rapidly. However, the circadian cycles of the viscera take several days to readjust, and they do so at different rates, so the phase map of the circadian network is 'scrambled'[10]. In hamsters with pre-existing cardiomyopathy, such circadian desynchronization decreases survival by about 11% (REF. 128), and in mice it accelerates the growth of transplanted tumours[129]. In the *tau* mutant hamster, the 20-h circadian period is associated with enhanced metabolic rate and reduced growth[130,131]. In modern society, shift-work is the most prevalent cause of circadian dyschrony[6]. It causes marked alterations in the cardiac autonomic profile[132] and is strongly associated with CVD and other chronic illnesses, including gastrointestinal disease[7]. One direct circadian link between CVD and gastrointestinal dysfunction lies in the physiological response to meals. High circulating levels of triacylglycerol (TAG) are a known risk factor in CVD[133], promoting cholesterol deposition in the arterial wall. The post-prandial surge in TAG levels is considerably higher after a meal taken at night than during the day, owing to circadian differences in gut physiology[134]. Furthermore, post-prandial TAG levels are higher and the elevation more prolonged in subjects following a shift-work schedule[135–137], presumably because gastrointestinal function is compromised during adjustment to the altered work schedule. A working life that is spent with such sub-optimal cardiovascular and gastrointestinal physiology will impose a progressive burden on health that underlies the chronic morbidity seen in long-term shift-workers[7].

Conclusions

Analysis of clock gene expression has revealed that a local version of the SCN clockwork is active in peripheral, non-neural tissues. This unanticipated and primitive molecular oscillation is a fundamental component of cellular physiology, and drives the tissue-specific gene expression cycles that underpin the circadian organization of the organism. The identities and functions of cells and tissues are defined not simply by what genes they express, but also by when they are expressed and in what order. The tissue-based oscillators are tuned to each other, and to solar time, by endocrine, neural and metabolic cues that are ultimately dependent on the SCN. Disturbance of these temporal relationships of gene expression within a tissue or among tissues is increasingly recognized as an important cause of morbidity. This new understanding of the interplay between the SCN master clock and its dependent resonant network of peripheral oscillators establishes the dimension of time at the forefront of our appreciation of health and disease. Future goals include characterization of the factors that are needed to sustain cell-autonomous oscillations and to couple populations of oscillatory cells both within and between tissues. In SCN neurons, a key aim is to define the reciprocal interactions between the molecular loop and the excitable cell membranes that sustain rhythms in the long term. In the periphery it is necessary to identify the extent to which particular tissues rely on the SCN for maintenance, rather than synchronization, of their clockwork, and to characterize the mechanisms that serve these functions. An important goal is to develop a better appreciation of the interactions among different timing mechanisms, including circadian clocks and the cell cycle, which might shed new light on the mechanisms of tumorigenesis and neoplastic change in multiple organ systems. Finally, a global aim is to apply this knowledge to develop therapies for diseases with a strong circadian component, including CVD and cancer.

1. Aschoff, J. Circadian timing. *Ann. NY Acad. Sci.* **423**, 442–468 (1984).
2. Pittendrigh, C. S. Temporal organization: reflections of a Darwinian clock-watcher. *Annu. Rev. Physiol.* **55**, 16–54 (1993).
References 1 and 2 summarize the basic biology of circadian timing, from two workers who established the field.
3. Pace-Schott, E. F. & Hobson, J. A. The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nature Rev. Neurosci.* **3**, 591–605 (2002).
An extensive review that places the circadian system into the broader context of sleep regulation.
4. Hastings, M. H. Neuroendocrine rhythms. *Pharmacol. Ther.* **50**, 35–71 (1991).
5. Ouyang, Y. *et al.* Resonating circadian clocks enhance fitness in cyanobacteria. *Proc. Natl Acad. Sci. USA* **95**, 8660–8664 (1998).
A definitive experimental demonstration of the selective advantage conferred by a circadian clock.
6. Rajaratnam, S. M. & Arendt, J. Health in a 24-h society. *Lancet* **358**, 999–1005 (2001).

7. Knutsson, A. Health disorders of shift workers. *Occup. Med. (Lond.)* **53**, 103–108 (2003).
8. Reppert, S. M. & Weaver, D. R. Coordination of circadian timing in mammals. *Nature* **418**, 935–941 (2002).
An in-depth, authoritative review of molecular events in the core oscillator.
9. Ikeda, M. *et al.* Circadian dynamics of cytosolic and nuclear Ca^{2+} in single suprachiasmatic nucleus neurons. *Neuron* **38**, 253–263 (2003).
10. Yamazaki, S. *et al.* Resetting central and peripheral circadian oscillators in transgenic rats. *Science*. **288**, 682–685 (2000).
The first demonstration of autonomous circadian timing in rat peripheral tissues, revealed at the molecular level by the *Per::luc* transgene.
11. Abrahamson, E. E. & Moore, R. Y. Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res.* **916**, 172–191 (2001).
12. Buijs, R. M. & Kalsbeek, A. Hypothalamic integration of central and peripheral clocks. *Nature Rev. Neurosci.* **2**, 521–526 (2001).
13. Abrahamson, E. E., Leak, R. K. & Moore, R. Y. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. *Neuroreport* **12**, 435–440 (2001).
14. Kramer, A. *et al.* Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science* **294**, 2511–2515 (2001).
15. Cheng, M. Y. *et al.* Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* **417**, 405–410 (2002).
16. Liu, C. & Reppert, S. M. GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron* **25**, 123–128 (2000).
17. Harmar, A. J. *et al.* The $VPAC_2$ receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell* **109**, 497–508 (2002).
18. Cutler, D. J. *et al.* The mouse $VPAC_2$ receptor confers suprachiasmatic nuclei cellular rhythmicity and responsiveness to vasoactive intestinal polypeptide *in vitro*. *Eur. J. Neurosci.* **17**, 197–204 (2003).
References 17 and 18 show that extracellular activation through VPAC2 receptors is necessary to sustain the core molecular oscillation of SCN neurons.
19. Mrosovsky, N. *et al.* Cycle of period gene expression in a diurnal mammal (*Spermophilus tridecemlineatus*): implications for nonphotic phase shifting. *J. Biol. Rhythms* **16**, 471–478 (2001).
20. Maywood, E. S., O'Brien, J. A. & Hastings, M. H. Expression of mCLOCK and other circadian clock-relevant proteins in the mouse suprachiasmatic nuclei. *J. Neuroendocrinol.* **15**, 329–334 (2003).
21. Preitner, N. *et al.* The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* **110**, 251–260 (2002).
22. Ueda, H. R. *et al.* A transcription factor response element for gene expression during circadian night. *Nature* **418**, 534–539 (2002).
References 21 and 22 revealed the contribution of Rev-erbα in establishing appropriate phasing of circadian gene expression, both within the core loop and for clock-controlled genes.
23. Etchegaray, J. P. *et al.* Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. *Nature* **421**, 177–182 (2003).
24. Hastings, M. H. Circadian clockwork: two loops are better than one. *Nature Rev. Neurosci.* **1**, 143–146 (2000).
25. Hirata, H. *et al.* Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop. *Science* **298**, 840–843 (2002).
26. Toh, K. L. *et al.* An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* **291**, 1040–1043 (2001).
Shows a clear biochemical linkage between a human clock gene mutation and a marked sleep disorder.
27. Ebisawa, T. *et al.* Association of structural polymorphisms in the human *period3* gene with delayed sleep phase syndrome. *EMBO Rep.* **2**, 342–346 (2001).
28. Katzenberg, D. *et al.* A CLOCK polymorphism associated with human diurnal preference. *Sleep* **21**, 569–576 (1998).
29. Archer, S. N. *et al.* A length polymorphism in the circadian clock gene *Per3* is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* **26**, 413–415 (2003).
30. Lowrey, P. L. *et al.* Positional syntenic cloning and functional characterization of the mammalian circadian mutation tau. *Science* **288**, 483–492 (2000).
A landmark piece of work defining the molecular genetic basis of the first described mutation of circadian periodicity in a mammal.
31. Jones, C. R. *et al.* Familial advanced sleep-phase syndrome: a short-period circadian rhythm variant in humans. *Nature Med*. **5**, 1062–1065 (1999).
32. Yagita, K. *et al.* Nucleocytoplasmic shuttling and mCRY-dependent inhibition of ubiquitylation of the mPER2 clock protein. *EMBO J.* **21**, 1301–1314 (2002).
33. Grima, B. *et al.* The F-box protein slimb controls the levels of clock proteins period and timeless. *Nature* **420**, 178–182 (2002).
34. Menaker, M. Circadian rhythms. Circadian photoreception. *Science* **299**, 213–214 (2003).
A contemporary mini-review of the breakthrough findings that melanopsin and melanopsin-containing retinal ganglion cells are components of the circadian photoreceptive pathway in mammals.
35. Provencio, I. *et al.* A novel human opsin in the inner retina. *J. Neurosci.* **20**, 600–605 (2000).
36. Hattar, S. *et al.* Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* **295**, 1065–1070 (2002).
37. Panda, S. *et al.* Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* **298**, 2213–2216 (2002).
38. Lucas, R. J. *et al.* Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* **299**, 245–247 (2003).
39. Kuhlman, S. J. *et al.* Phase resetting light pulses induce *Per1* and persistent spike activity in a subpopulation of biological clock neurons. *J. Neurosci.* **23**, 1441–1450 (2003).
40. Travnickova-Bendova, Z. *et al.* Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. *Proc. Natl Acad. Sci. USA* **99**, 7728–7733 (2002).
41. Crosio, C. *et al.* Light induces chromatin modification in cells of the mammalian circadian clock. *Nature Neurosci*. **3**, 1241–1247 (2000).
42. Field, M. D. *et al.* Analysis of clock proteins in mouse SCN demonstrates phylogenetic divergence of the circadian clockwork and resetting mechanisms. *Neuron* **25**, 437–447 (2000).
43. Reddy, A. B. *et al.* Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. *J. Neurosci.* **22**, 7326–7330 (2002).
44. Shigeyoshi, Y. *et al.* Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the *mPer1* transcript. *Cell* **91**, 1043–1053 (1997).
45. Messager, S. *et al.* Decoding photoperiodic time through *Per1* and *ICER* gene amplitude. *Proc. Natl Acad. Sci. USA* **96**, 9938–9943 (1999).
46. Hastings, M. H. & Follett, B. K. Toward a molecular biological calendar? *J. Biol. Rhythms* **16**, 424–430 (2001).
47. Nuesslein-Hildesheim, B. *et al.* The circadian cycle of *mPER* clock gene products in the suprachiasmatic nucleus of the siberian hamster encodes both daily and seasonal time. *Eur. J. Neurosci.* **12**, 2856–2864 (2000).
48. Maywood, E. S. *et al.* Rapid down-regulation of mammalian period genes during behavioral resetting of the circadian clock. *Proc. Natl Acad. Sci. USA* **96**, 15211–15216 (1999).
49. Maywood, E. S., Okamura, H. & Hastings, M. H. Opposing actions of neuropeptide Y and light on the expression of circadian clock genes in the mouse suprachiasmatic nuclei. *Eur. J. Neurosci.* **15**, 216–220 (2002).
50. Horikawa, K. *et al.* Nonphotic entrainment by $5\text{-HT}_{1A/7}$ receptor agonists accompanied by reduced *Per1* and *Per2* mRNA levels in the suprachiasmatic nuclei. *J. Neurosci.* **20**, 5867–5873 (2000).
51. Honma, S. *et al. Dec1* and *Dec2* are regulators of the mammalian molecular clock. *Nature* **419**, 841–844 (2002).
52. Baggs, J. E. & Green, C. B. Nocturnin, a deadenylase in *Xenopus laevis* retina. A mechanism for posttranscriptional control of circadian-related mRNA. *Curr. Biol.* **13**, 189–198 (2003).
53. Wang, Y. *et al.* Rhythmic expression of *Nocturnin* mRNA in multiple tissues of the mouse. *BMC Dev. Biol.* **1**, 9 (2001).
54. Giebultowicz, J. M. & Hege, D. M. Circadian clock in Malpighian tubules. *Nature* **386**, 664 (1997).
55. Plautz, J. D. *et al.* Independent photoreceptive circadian clocks throughout *Drosophila*. *Science* **278**, 1632–1635 (1997).
References 54 and 55 are the original reports of self-sustaining, *Per*-based peripheral circadian oscillators in *Drosophila*.
56. Krishnan, B., Dryer, S. E. & Hardin, P. E. Circadian rhythms in olfactory responses of *Drosophila melanogaster*. *Nature* **400**, 375–378 (1999).
57. Levine, J. D. *et al.* Resetting the circadian clock by social experience in *Drosophila melanogaster*. *Science* **298**, 2010–2012 (2002).
58. Whitmore, D., Foulkes, N. S. & Sassone-Corsi, P. Light acts directly on organs and cells in culture to set the vertebrate circadian clock. *Nature* **404**, 87–91 (2000).
59. 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).
The demonstration that circadian control of the mouse liver transcriptome is dependent on the SCN.
60. King, V. M. *et al.* A *hVIPR* transgene as a novel tool for the analysis of circadian function in the mouse suprachiasmatic nucleus. *Eur. J. Neurosci.* **17**, 822–832 (2003).
61. Sujino, M. *et al.* Suprachiasmatic nucleus grafts restore circadian behavioral rhythms of genetically arrhythmic mice. *Curr. Biol.* **13**, 664–668 (2003).
62. Balsalobre, A., Damiola, F. & Schibler, U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* **93**, 929–937 (1998).
A breakthrough paper revealing the capacity of cell cultures to express circadian molecular oscillations.
63. Yagita, K. *et al.* Molecular mechanisms of the biological clock in cultured fibroblasts. *Science* **292**, 278–281 (2001).
64. Pando, M. P. *et al.* Phenotypic rescue of a peripheral clock genetic defect via SCN hierarchical dominance. *Cell* **110**, 107–117 (2002).
65. Reick, M. *et al.* NPAS2: an analog of clock operative in the mammalian forebrain. *Science* **293**, 506–509 (2001).
66. McNamara, P. *et al.* Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell* **105**, 877–889 (2001).
67. Schoenhard, J. A. *et al.* Alternative splicing yields novel BMAL2 variants: tissue distribution and functional characterization. *Am. J. Physiol. Cell. Physiol.* **283**, C103–114 (2002).
68. Lincoln, G. *et al.* Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. *Proc. Natl Acad. Sci. USA* **99**, 13890–13895 (2002).
69. Panda, S. *et al.* Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* **109**, 307–320 (2002).
70. Storch, K. F. *et al.* Extensive and divergent circadian gene expression in liver and heart. *Nature* **417**, 78–83 (2002).
71. Duffield, G. E. *et al.* Circadian programs of transcriptional activation, signaling, and protein turnover revealed by microarray analysis of mammalian cells. *Curr. Biol.* **12**, 551–557 (2002).
72. Redfern, P. H. & Lemmer, B. *Handbook of Experimental Pharmacology* Vol. 125 (Springer, Berlin, 1997).
73. Gallerani, M. *et al.* Circadian differences in the individual sensitivity to opiate overdose. *Crit. Care Med.* **29**, 96–101 (2001).
74. Bliwise, D. L. *et al.* Survival by time of day of hemodialysis in an elderly cohort. *JAMA* **286**, 2690–2694 (2001).
75. Yamazaki, S. *et al.* Effects of aging on central and peripheral mammalian clocks. *Proc. Natl Acad. Sci. USA* **99**, 10801–10806 (2002).
76. Mistlberger, R. E. Circadian food-anticipatory activity: formal models and physiological mechanisms. *Neurosci. Biobehav. Rev.* **18**, 171–195 (1994).
77. Davidson, A. J. & Stephan, F. K. Feeding-entrained circadian rhythms in hypophysectomized rats with suprachiasmatic nucleus lesions. *Am. J. Physiol.* **277**, R1376–1384 (1999).
78. 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).
79. Stokkan, K. A. *et al.* Entrainment of the circadian clock in the liver by feeding. *Science* **291**, 490–493 (2001).
References 78 and 79 showed that restricted feeding can entrain the autonomous circadian clockwork of the liver.
80. Pitts, S., Perone, E. & Silver, R. Food-entrained circadian rhythms are sustained in arrhythmic *Clk/Clk* mutant mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R57–67 (2003).
81. Oishi, K., Miyazaki, K. & Ishida, N. Functional CLOCK is not involved in the entrainment of peripheral clocks to the restricted feeding: entrainable expression of *mPer2* and *BMAL1* mRNAs in the heart of *Clock* mutant mice on Jcl:ICR background. *Biochem. Biophys. Res. Commun.* **298**, 198–202 (2002).
82. Balsalobre, A., Marcacci, L. & Schibler, U. Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts. *Curr. Biol.* **10**, 1291–1294 (2000).
83. Balsalobre, A. *et al.* Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* **289**, 2344–2347 (2000).
84. Le Minh, N. *et al.* Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *EMBO J.* **20**, 7128–7136 (2001).
85. Akashi, M. & Nishida, E. Involvement of the MAP kinase cascade in resetting of the mammalian circadian clock. *Genes Dev*. **14**, 645–649 (2000).
86. Yagita, K. & Okamura, H. Forskolin induces circadian gene expression of *rPer1*, *rPer2* and *dbp* in mammalian rat-1 fibroblasts. *FEBS Lett*. **465**, 79–82 (2000).

87. 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).
88. Davidson, A. J. *et al.* Food-anticipatory activity and liver per1-luc activity in diabetic transgenic rats. *Physiol. Behav.* **76**, 21–26 (2002).
89. Nonaka, H. *et al.* Angiotensin II induces circadian gene expression of clock genes in cultured vascular smooth muscle cells. *Circulation* **104**, 1746–1748 (2001).
90. von Gall, C. *et al.* Rhythmic gene expression in pituitary depends on heterologous sensitization by the neurohormone melatonin. *Nature Neurosci.* **5**, 234–238 (2002).
91. Earnest, D. J. *et al.* Immortal time: circadian clock properties of rat suprachiasmatic cell lines. *Science* **283**, 693–695 (1999).
92. Rutter, J., Reick, M. & McKnight, S. L. Metabolism and the control of circadian rhythms. *Annu. Rev. Biochem.* **71**, 307–331 (2002).
93. Brown, S. A. *et al.* Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr. Biol.* **12**, 1574–1583 (2002).
94. Takekida, S. *et al.* Differential adrenergic regulation of the circadian expression of the clock genes *Period1* and *Period2* in the rat pineal gland. *Eur. J. Neurosci.* **12**, 4557–4561 (2000).
95. Terazono, H. *et al.* Adrenergic regulation of clock gene expression in the mouse liver. *Proc. Natl Acad. Sci. USA* **100**, 6795–6800 (2003).
96. Duffy, J. F. *et al.* Later endogenous circadian temperature nadir relative to an earlier wake time in older people. *Am. J. Physiol.* **275**, R1478–1487 (1998).
97. van Someren, E. J. Circadian and sleep disturbances in the elderly. *Exp. Gerontol.* **35**, 1229–1237 (2000).
A contemporary review of the effects of ageing on sleep patterns.
98. Satlin, A. *et al.* Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease. *Neurobiol. Aging* **16**, 765–771 (1995).
99. Volicer, L. *et al.* Sundowning and circadian rhythms in Alzheimer's disease. *Am. J. Psychiatry* **158**, 704–711 (2001).
100. Harper, D. G. *et al.* Differential circadian rhythm disturbances in men with Alzheimer disease and frontotemporal degeneration. *Arch. Gen. Psychiatry* **58**, 353–360 (2001).
The demonstration of the impact of Alzheimer's disease on circadian function.
101. Bliwise, D. L. in *Regulation of Sleep and Circadian Rhythms* (eds Turek, F. & Zee, P.) 752 (Marcel-Dekker, New York, 1999).
102. Cannon, C. P. *et al.* Circadian variation in the onset of unstable angina and non-Q-wave acute myocardial infarction (the TIMI III Registry and TIMI IIIB). *Am. J. Cardiol.* **79**, 253–258 (1997).
103. Casetta, I. *et al.* Circadian variability in hemorrhagic stroke. *JAMA* **287**, 1266–1267 (2002).
104. Cohen, M. C. *et al.* Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. *Am. J. Cardiol.* **79**, 1512–1516 (1997).
A comprehensive meta-analysis of circadian prevalence in acute cardiovascular disease.
105. Goldberg, R. J. *et al.* Time of onset of symptoms of acute myocardial infarction. *Am. J. Cardiol.* **66**, 140–144 (1990).
106. Muller, J. E. *et al.* Circadian variation in the frequency of onset of acute myocardial infarction. *N. Engl. J. Med.* **313**, 1315–1322 (1985).
107. Onaka, H. *et al.* Circadian variation of myocardial ischemia in patients with unstable angina pectoris secondary to fixed and/or spastic coronary narrowing. *Am. J. Cardiol.* **81**, 629–632 (1998).
108. White, W. B. *et al.* Preventing increases in early-morning blood pressure, heart rate, and the rate-pressure product with controlled onset extended release verapamil at bedtime versus enalapril, losartan, and placebo on arising. *Am. Heart J.* **144**, 657–665 (2002).
109. Smith, D. H., Neutel, J. M. & Weber, M. A. A new chronotherapeutic oral drug absorption system for verapamil optimizes blood pressure control in the morning. *Am. J. Hypertens.* **14**, 14–19 (2001).
110. Lemmer, B. Chronopharmacology of hypertension. *Ann. NY Acad. Sci.* **783**, 242–253 (1996).
111. Young, M. E. *et al.* Intrinsic diurnal variations in cardiac metabolism and contractile function. *Circ. Res.* **89**, 1199–1208 (2001).
112. Young, M. E., Razeghi, P. & Taegtmeyer, H. Clock genes in the heart: characterization and attenuation with hypertrophy. *Circ. Res.* **88**, 1142–1150 (2001).
113. Young, M. E. *et al.* Alterations of the circadian clock in the heart by streptozotocin-induced diabetes. *J. Mol. Cell. Cardiol.* **34**, 223–231 (2002).
114. Naito, Y. *et al.* Augmented diurnal variations of the cardiac renin-angiotensin system in hypertensive rats. *Hypertension* **40**, 827–833 (2002).
115. Gaenzer, H. *et al.* Circadian variation of endothelium-dependent vasodilatation of the brachial artery as a confounding factor in the evaluation of endothelial function. *Atherosclerosis* **149**, 227–228 (2000).
116. Etsuda, H. *et al.* Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? *Clin. Cardiol.* **22**, 417–421 (1999).
117. Elherik, K. *et al.* Circadian variation in vascular tone and endothelial cell function in normal males. *Clin. Sci. (Lond.)* **102**, 547–552 (2002).
118. Tofler, G. H. *et al.* Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N. Engl. J. Med.* **316**, 1514–1518 (1987).
119. Kurnik, P. B. Practical implications of circadian variations in thrombolytic and antithrombotic activities. *Cardiol. Clin.* **14**, 251–262 (1996).
120. Braunwald, E. Morning resistance to thrombolytic therapy. *Circulation* **91**, 1604–1606 (1995).
121. Kurnik, P. B. Circadian variation in the efficacy of tissue-type plasminogen activator. *Circulation* **91**, 1341–1346 (1995).
122. Decousus, H. A. *et al.* Circadian changes in anticoagulant effect of heparin infused at a constant rate. *Br. Med. J. Clin. Res. Ed.* **290**, 341–344 (1985).
123. Krulder, J. W. *et al.* Diurnal changes in heparin effect during continuous constant-rate infusion. A study in nine patients with venous thromboembolism. *J. Intern. Med.* **235**, 411–417 (1994).
124. Bridges, A. B. *et al.* Circadian variation of endothelial cell function, red blood cell deformability and dehydro-thromboxane B2 in healthy volunteers. *Blood Coagul. Fibrinolysis* **2**, 447–452 (1991).
125. Maemura, K. *et al.* CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression. *J. Biol. Chem.* **275**, 36847–36851 (2000).
A key study linking the core molecular clockwork to a mechanism that contributes to circadian prevalence of acute cardiovascular disease.
126. Minami, Y. *et al.* Restricted feeding induces daily expression of clock genes and *Pai-1* mRNA in the heart of Clock mutant mice. *FEBS Lett.* **526**, 115–118 (2002).
127. Bruzdzinski, C. J. *et al.* Mechanism of glucocorticoid induction of the rat plasminogen activator inhibitor-1 gene in HTC rat hepatoma cells: identification of *cis*-acting regulatory elements. *Mol. Endocrinol.* **7**, 1169–1177 (1993).
128. Penev, P. D. *et al.* Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. *Am. J. Physiol.* **275**, H2334–2337 (1998).
129. Filipski, E. *et al.* Chronic jet-lag alters circadian gene expression and accelerates malignant growth in tumor-bearing mice. *Am. Assoc. Cancer Res*. Abstr. 2463 (2003).
130. Oklejewicz, M. *et al.* Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamsters. *J. Biol. Rhythms* **12**, 413–422 (1997).
131. Lucas, R. J. *et al.* Postnatal growth rate and gonadal development in circadian tau mutant hamsters reared in constant dim red light. *J. Reprod. Fertil.* **118**, 327–330 (2000).
132. Furlan, R. *et al.* Modifications of cardiac autonomic profile associated with a shift schedule of work. *Circulation* **102**, 1912–1916 (2000).
133. Hokanson, J. E. & Austin, M. A. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk* **3**, 213–219 (1996).
134. Sopowski, M. J. *et al.* Postprandial triacylglycerol responses in simulated night and day shift: gender differences. *J. Biol. Rhythms* **16**, 272–276 (2001).
135. Ribeiro, D. C. *et al.* Altered postprandial hormone and metabolic responses in a simulated shift work environment. *J. Endocrinol.* **158**, 305–310 (1998).
136. Hampton, S. M. *et al.* Postprandial hormone and metabolic responses in simulated shift work. *J. Endocrinol.* **151**, 259–267 (1996).
137. Lund, J. *et al.* Postprandial hormone and metabolic responses amongst shift workers in Antarctica. *J. Endocrinol.* **171**, 557–564 (2001).
138. Rensing, L. & Goedeke, K. Circadian rhythm and cell cycle: possible entraining mechanisms. *Chronobiologia* **3**, 853–865 (1976).
139. Bjarnason, G. A., Jordan, R. C. & Sothern, R. B. Circadian variation in the expression of cell-cycle proteins in human oral epithelium. *Am. J. Pathol.* **154**, 613–622 (1999).
140. Bjarnason, G. A. *et al.* Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. *Am. J. Pathol.* **158**, 1793–1801 (2001).
141. Clayton, F. *et al.* Circadian variation of topoisomerase II-α in human rectal crypt epithelium: implications for reduction of toxicity of chemotherapy. *Mod. Pathol.* **15**, 1191–1196 (2002).
142. Levi, F. Circadian chronotherapy for human cancers. *Lancet Oncol.* **2**, 307–315 (2001).
143. Hrushesky, W. J. Tumor chronobiology. *J. Control. Release* **74**, 27–30 (2001).
References 142 and 143 review the prospects for exploiting knowledge of circadian biology in cancer therapy.
144. Levi, F. *et al.* A multicenter evaluation of intensified, ambulatory, chronomodulated chemotherapy with oxaliplatin, 5-fluorouracil, and leucovorin as initial treatment of patients with metastatic colorectal carcinoma. International Organization for Cancer Chronotherapy. *Cancer* **85**, 2532–2540 (1999).
145. Filipski, E. *et al.* Host circadian clock as a control point in tumor progression. *J. Natl Cancer. Inst.* **94**, 690–697 (2002).
146. Fu, L. *et al.* The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response *in vivo*. *Cell* **111**, 41–50 (2002).
147. Fu, L. & Lee, C. C. The circadian clock: pacemaker and tumour suppressor. *Nature Rev. Cancer* **3**, 350–361 (2003).
An excellent review of molecular interactions between cancer and the circadian clockwork.
148. Hansen, J. Increased breast cancer risk among women who work predominantly at night. *Epidemiology* **12**, 74–77 (2001).
149. Schernhammer, E. S. *et al.* Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Natl Cancer Inst.* **93**, 1563–1568 (2001).
150. Davis, S., Mirick, D. K. & Stevens, R. G. Night shift work, light at night, and risk of breast cancer. *J. Natl Cancer Inst.* **93**, 1557–1562 (2001).
References 148–150 provide compelling epidemiological evidence of a link between shift work and cancer.
151. Hastings, M. The brain, circadian rhythms, and clock genes. *Br. Med. J.* **317**, 1704–1707 (1998).
152. Yamaguchi, S. *et al.* View of a mouse clock gene ticking. *Nature* **409**, 684 (2001).
An innovative *in vivo* study of circadian *Per::luc* activity in mouse SCN that has potential applications to other areas of neuroscience where real-time analysis of local brain activity might be used to probe the molecular and neural bases of behaviour.
153. Rocco, M. B., Nabel, E. G. & Selwyn, A. P. Circadian rhythms and coronary artery disease. *Am. J. Cardiol.* **59**, 13C–17C (1987).

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Alzheimer disease: http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?104300

At a glance

- Circadian rhythms are daily cycles of physiology and behaviour that are driven by an endogenous oscillator with a period of approximately (*circa*-) one day (*diem*). Exemplified in humans by the rhythm of sleep and wakefulness and their attendant neurophysiological and metabolic states, they are a pervasive feature of eukaryotes, enabling the organism to anticipate and thereby adapt to the solar cycle.
- In mammals, the principal oscillator are the suprachiasmatic nuclei (SCN) of the hypothalamus. The circadian timing mechanism is cell-autonomous and is expressed individually by SCN neurons. Synchrony across the SCN neuronal network is maintained by γ-aminobutyric acid (GABA) and peptide signalling. It is entrained to the light–dark cycle by glutamatergic retinal afferents, derived in part from a class of intrinsically photosensitive, melanopsin-positive retinal ganglion cells.
- The cellular oscillator consists of interlocked transcriptional and post-translational feedback loops. Heterodimeric complexes encoded by the *Clock* and *Bmal* genes drive expression of *Per* and *Cry* genes during circadian day, leading to accumulation of Per/Cry protein complexes that enter the nucleus and suppress transcription of their cognate genes, thereby establishing an oscillatory negative feedback loop. A feedforward loop, mediated by rhythmic expression of Rev-erbα, phases the expression of *Bmal* to circadian night, in antiphase to *Per* and *Cry*, thereby augmenting the core oscillation.
- This SCN cycle is synchronized to solar time by neurochemical cues that activate or suppress *Per* expression. Circadian organization within and beyond SCN neurons is mediated by rhythmic expression of clock-controlled genes that sit outside the feedback loop, but undergo periodic transcriptional activation and repression by Per, Cry and Rev-erbα.
- Circadian oscillators based on rhythmic *Per* gene expression are also present in non-neural, peripheral tissues and immortalized cell lines. They have tissue-specific variations in molecular composition and coordinate the local, tissue-specific programmes of gene expression that underpin circadian metabolic programmes.
- As with the SCN oscillator, where up- and down-regulation of *Per* resets circadian time, these peripheral oscillators can be reset or activated by various biochemical cues that acutely regulate *Per* expression. *In vivo*, this resonant network of peripheral oscillators is synchronized by behavioural, neural, endocrine and food-related cues that depend on the SCN. Manipulation of these cues *in vivo* can desynchronize peripheral oscillators from the SCN.
- Circumstances that disrupt the smooth temporal integration of metabolism within and between tissues impose a burden on health. Therapeutic managements should be designed to maintain circadian structure in the periphery. Circadian prevalence of chronic disease is a reflection of the activity of peripheral oscillators and their interactions. Targeted modification of these local endogenous clocks should provide avenues for selective and specific treatment. The contribution of circadian mechanisms to tumour progression highlights the value of incorporating and exploiting temporal specificity in therapeutic regimes.

Biographies

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