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## Healthy clocks, healthy body, healthy mind

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### Abstract

Circadian rhythms permeate mammalian biology. They are manifested in the temporal organisation of behavioural, physiological, cellular and neuronal processes. Whereas it has been shown recently that these ~24-hour cycles are intrinsic to the cell and persist *in vitro*, internal synchrony in mammals is largely governed by the hypothalamic suprachiasmatic nuclei that facilitate anticipation of, and adaptation to, the solar cycle. Our timekeeping mechanism is deeply embedded in cell function and is modelled as a network of transcriptional and/or post-translational feedback loops. Concurrent with this, we are beginning to understand how this ancient timekeeper interacts with myriad cell systems, including signal transduction cascades and the cell cycle, and thus impacts on disease. An exemplary area where this knowledge is rapidly expanding and contributing to novel therapies is cancer, where the *Period* genes have been identified as tumour suppressors. In more complex disorders, where aetiology remains controversial, interactions with the clockwork are only now starting to be appreciated.

### Introduction

Circadian (*circa*-, ‘approximately’; *-diem*, ‘day’) rhythms are a fundamental property of living cells. When held in temporal isolation, organisms from unicells to humans exhibit behavioural and physiological rhythms that persist with a period of approximately 24 h [1]. These rhythms are driven by biological clocks that have two essential features. First, their free-running period of ~24 h is temperature-compensated: clocks do not run slower at lower temperatures or speed up when it is hot – a remarkable and necessary feat of biochemical engineering. Second, they can synchronise to temporally relevant stimuli such as light, temperature or feeding schedules, and thus their definition of internal time becomes predictive of external (solar) time [2]. Entrained in this way, clocks confer selective advantages to organisms by facilitating anticipation of, and thereby adaptation to, the alternating day–night cycle as well as temporally segregating mutually antagonistic processes that might otherwise result in a futile cycle – for example, glycolysis (day) and gluconeogenesis (night) in hepatocytes [3]. The competitive value of circadian clocks has been demonstrated in prokaryotes and higher plants [4,5], and disturbance of circadian timing in humans, as seen in rotational shift workers for example, carries significant long-term health costs [6].

Rhythmic regulation of behaviour and physiology results from the circadian modulation of diverse processes and pathways, and therefore interactions between the clock and health are necessarily pleiotropic in nature. Two clear trends can be identified however. Namely, that organisms whose internal clocks are synchronised with the external environment are healthier (more adept at dealing with environmental challenge) [7], and that genetic or acute lesions affecting timekeeping reduce temporal homeostasis with concomitant health consequences, albeit often indirectly [8]. For example, in the context of cancer, it has been shown that the circadian cycle gates cell division [9], and thus loss of cellular rhythmicity might be expected to correlate with increased cellular transformation. Indeed, a number of canonical clock genes

have been identified independently as tumour suppressors; for example, *Bmal1* [10]. When otherwise healthy humans or rodents are repeatedly desynchronised from the external environment, however, an increased cancer risk and reduced longevity is also observed [11,12].

## Healthy clockwork = healthy body and mind

The human body is a cyclical machine, and circadian variation in physical and cognitive performance is readily observable at both the individual and population levels [13,14] (Box 1, panels A,B). These behavioural outputs stem from circadian regulation of neuronal, physiological and endocrine function; examples include rhythms in core body temperature, heart rate, and in cortisol and melatonin secretion [15,16]. Indeed, the majority of body and cell functions, where studied, appear to have some circadian component. For example, elements of both the adaptive and innate immune system are subject to circadian regulation [17], as is the severity of many disease states including myocardial infarction [18] and depression [19]. Indeed, more than 20% of gene expression in a given tissue has been estimated to be under circadian regulation at either the transcriptional or protein level, with further circadian regulation being evident through post-translational protein modification [20]. There is no doubt, therefore, that our bodies are temporally orchestrated by the clockwork, but what are the consequences when our internal clocks are disrupted, or become misaligned with the external environment, as occurs in jet-lag and in shift work?

There is mounting evidence to suggest that long-term disruption of rhythmic behaviour correlates with disease states, leading to profound implications for healthcare in the future [21]. Interestingly, diseases such as ischaemic stroke, that share risk factors with cardiovascular disease, have similarly been found to occur more frequently in female long-term shift workers [22]. Moreover, in this cohort there appears to be a clear link between breast cancer risk and long-term shift working [23,24], and this is being taken seriously by a number of governments in view of increasing litigation in this area [25]. A number of additional studies have found that chronic shift work is significantly associated with an increased risk of colorectal, endometrial and prostate cancers [26]. Similarly, recent work highlights that cardiovascular and metabolic dysfunction (glucose intolerance) occur in situations analogous to rotational shift work [27], meaning that how we work could have consequences for the development of such conditions. Obesity, diabetes and related metabolic syndromes are on the increase globally, and novel ways to combat them are needed. In this context, the observation that 'statins' are most efficacious when administered during subjective night has been known anecdotally for years [28], but when coupled with a recent report that mice fed at the 'wrong time' of day (i.e. when they are supposed to be sleeping) gain weight more rapidly than littermates fed at the 'right time' [29], one may infer that attempts to cure or prevent diseased states in humans will be hindered unless the circadian context of treatment is considered. There thus exists a clear need to understand the molecular mechanisms that sustain our clockwork and to elucidate its interactions with other biological systems.

## From rhythmic behaviour to molecular events

Our view of circadian rhythms has changed immensely over the last decade or so. For many years the consensus view was that mammalian timekeeping function was highly centralised within a so-called master clock – the suprachiasmatic nuclei (SCN) that integrate relevant environmental cues (photic and non-photic) and signal timing information to peripheral tissues through via neuronal efferents and diffusible factors [30–32] (Box 1, panel C). This bilateral structure comprises approximately 10,000 neurons and resides in the basal hypothalamus, above the optic nerve crossing (chiasm), and is ideally situated to be entrained by ambient lighting cues relayed from a sub-population of intrinsically photosensitive retinal ganglion cells

[33]. The SCN was shown to be indispensable for coherent behavioural rhythmicity in mammals in the 1970s. When it is ablated in mice, or damaged in humans, behavioural cycles including sleep and wakefulness become arrhythmic or disorganised [31,34]. Indeed, intrinsic rhythmic activity in the SCN is so robust that rhythms have been shown to persist for months in organotypic slices *in vitro* [35]. Moreover, mutations affecting the ability of the SCN to function as an assemblage (e.g. in VPAC2 receptor knockout mice), and mutations that either 'accelerate' the clockwork (e.g. homozygous *Tau* mutant mice or hamsters exhibit a significantly shorter free-running periodicity of ~21 hours) or slow it down (e.g. *Fbxl3* mutant mice, with an endogenous periodicity of ~28 hours), all directly impact on rhythmic behaviour, further illustrating the SCN's pivotal orchestrating role *in vivo* [36–41].

Significantly, however, in the last 5 years it has been shown beyond doubt that whereas the SCN clearly plays a key role in synchronising rhythms across the body's various tissues, cells within those tissues themselves exhibit self-sustained rhythms in gene expression that also persist in culture [35,42] (Box 1, panel D). Indeed, in mice with a conditionally inactive clock in the liver, the number of transcripts that continued to oscillate and were therefore driven directly by humoral factors was only ~10% compared to wild-type controls [43,44], implying that the majority of oscillating transcripts are reliant upon cell-intrinsic mechanisms. Moreover, in addition to serum factors and pharmacological cues, very mild 24-hour temperature cycles (that mimic circadian variation in body temperature) are sufficient to entrain the phase of fibroblasts *in vitro*, implying that peripheral tissues are also competent to be stably entrained to timing cues in the absence of SCN signalling [9,45–49]. Finally, when entrained via feeding or pharmacological cues, it has been shown that the SCN, and even several identified 'clock genes', are dispensable for rhythmic behaviour [50,51]. Thus, the generic mechanism that sustains intracellular rhythms in all tissues has become a major focus for circadian research (Box 1 overviews the current molecular model and its relation to higher-order biological structures and behaviour).

The early successes of molecular approaches to identifying clock components revealed a number of transcription factors (Per1–3, Cry1,2, Bmal1, Clock etc.) that appeared to act in a day-long auto-regulatory negative feedback loop, regulating the rhythmic expression of many clock-controlled genes in a tissue-specific manner [42,52]. More recently, however, several rhythmic outputs from this core loop have been shown, in turn, to also feedback into it – rendering the principle of a core mechanism increasingly semantic. Strikingly, these additional loops include not only additional transcription factors (e.g. Dec1,2, Rev-erba) [53] but also several ubiquitous pathways that are heavily implicated in other cellular processes. For example, AMPK is involved in cellular energy homeostasis but was recently shown to also display rhythmic activity and localisation, and regulate the stability of Cry1 [54]. Similarly, cAMP signalling is an essential signal transduction pathway, but is also described as a core clock component, governing the period, amplitude and phase of rhythms in gene expression [55]. In addition, the NAD/NADH redox balance, so crucial to cellular metabolism, has been shown to have reciprocal regulation with the core clock mechanism (see below) [56]. Whether the participation of these essential cellular systems reflects an inbuilt distributed functional redundancy or a deeper biological truth remains to be seen. Certainly, our picture of what constitutes the minimal cellular timing architecture has become somewhat cluttered of late, and our drive to separate cause from effect dictates that a quantitative assessment of the relevant functional contribution of each putative component to timekeeping is overdue, especially because transfer of this understanding to clinical relevance is the foremost objective.

## The clock and cancer: a tale of two cycles

Possible interactions between the cell cycle and the clockwork have been known for some time [57–61], in the sense that the clock gates cell division to specific circadian phases. From an

evolutionary perspective this is intuitive because DNA synthesis and replication performed at night are not exposed to harmful UV radiation that might otherwise have deleterious effects on replicative fidelity. In mammals, there is a vast body of evidence, spanning more than two decades, that has defined circadian variation in mitotic indices in a multitude of tissues including oral mucosa, skin, intestinal epithelium, and bone marrow [62]. Only recently, however, have the mechanisms underlying this link been revealed and, lately, translated to the clinical oncology arena [62–65].

Beyond the observation that circadian disruption shortens survival and accelerates malignant growth, insights broadly split into two categories: clock genes as tumour suppressors or oncogenes and therefore putative prognostic indicators, and chronotherapeutic applications resulting from circadian regulation of cell proliferation and detoxification pathways. In the case of the former, defects in core clock components (such as *Period 1* and *2*; putative tumour suppressors) have been shown to be involved in the response to radiation-induced DNA damage, and hence the propensity for tumour formation *in vivo* [66,67]. In addition, the core clock components Clock:Bmal1, and the close homologue Npas2, have been shown to protect against chemical and radiation-induced damage [68–70]. Indeed, Npas2 is being investigated as a prognostic biomarker for breast cancer [71]. By contrast, loss of the putative oncogene *Cryptochrome* significantly reduces cancer risk in *p53* mutant mice [72] and *Cryptochrome2* has further been implicated in the development of non-Hodgkin's Lymphoma [73]. Moreover, a variety of classical cell cycle and/or proliferation genes (such as *c-Myc* and *Wee1*) have been shown to be under direct clock control, and their expression (driven by Clock:Bmal1 complexes via E-box elements in their regulatory regions) effectively gates the division of non-transformed cells to specific circadian phases [62,64,66]. Other well-recognised cell-cycle regulators (e.g. *Cyclin D1* and *Mdm-2* and *Gadd45a*) are likely to be controlled indirectly [66] but, strikingly, the interaction is bidirectional in that DNA damage can reset the phase of the clockwork (Figure 1A) [74,75], presumably because repair to genetic material has to take priority over a system that contributes to cellular homeostasis in the longer term.

In tumours themselves, consistent changes in the expression of clock genes (e.g. *Per1–3*) have been demonstrated, as well as changes in the methylation state of their regulatory regions in various tissues including breast, liver and endometrial cancers [76–78]. The extent to which cancer cells are able to dispense with normal circadian gating of cell division is however unclear [79], and it could well be that tumours instead become insensitive to peripheral cues and, in effect, free run [80,81]. Demonstrably though, when the daily timing of animals is upset using conditions mimicking jet-lag or shift work, implanted malignant tumours grow more rapidly than in unperturbed controls [81], correlating well with reports of shortened survival in cancer patients with abnormal rhythms [82–84] and supported by epidemiological meta-analyses of tumour induction [85]. Indeed, several clock gene polymorphisms are being actively investigated as cancer risk factors [73,86]. Thus there is compelling evidence that 'clock mechanisms' are also inexorably tied up in cell proliferation and its control at the DNA (epigenetic), RNA and protein levels.

How is this new knowledge being applied clinically? Changes in clock gene expression might find use as biomarkers for cancers [73], but the most exciting avenue has been the development of 'chronotherapy' for cancer – using medications at times when they will be most effective on cancer cells, while simultaneously minimising side effects [65,87]. Circadian dosing time influences the extent of toxicity of more than thirty anticancer drugs, and it has been shown in animal models that survival rate varies by at least 50% depending on when a 'lethal dose' of drug is given [15]. This might well be because cellular repair mechanisms, such as nucleotide excision, are subject to circadian regulation [88], as are many key genes associated with xenobiotic metabolism and transport [89]. Even more strikingly though, the administration of a drug at a circadian time when it is best tolerated usually achieves the best anti-tumour activity

[90]. This knowledge is being applied increasingly in clinical trials. For example, Giacchetti and colleagues recently conducted a Phase III trial comparing 'chronomodulated' administration of fluorouracil (5-FU), leucovorin, and oxaliplatin against the standard regime in patients with newly diagnosed metastatic colorectal cancer (Figure 1B,C) [91]. There was a significant survival advantage of the chronomodulated regime, but this was interestingly only confined to men, whereas women fared better on conventional delivery, highlighting the need for further such studies. Moreover, rodent studies using the drug seliciclib, a cyclin-dependent kinase inhibitor, showed that drug treatment during subjective day reduced tumour growth by more than 50% compared with subjective night [92], and that this increased reduction apparently resulted from restoration of normally phased clock gene expression patterns in the tumours [93].

## The ageing clock: neurodegeneration and rhythmic behaviour

At the behavioural level, it is well established that aged organisms behave less rhythmically [12]. Indeed, loss of regular sleeping patterns in humans is the one of the prime motivations for institutionalisation of the elderly [94]. Furthermore, this loss of daily rhythms in sleep-wake activity has been speculated to contribute to the onset of neurodegenerative disorders and could be considered a pre-symptomatic correlate [94–96]. Conversely, behaviourally arrhythmic animals exhibit accelerated ageing – for example the *Bmal1*<sup>-/-</sup> mouse exhibits loss of behavioural and molecular circadian rhythms because it lacks Bmal1-driven rhythmic transcription of downstream clock genes from their cognate E-box promoter sequences [97]. These mice also exhibit reduced body weight and show mortality at around six months in the absence of major pathological changes in their major organ systems [98]. Thus, whereas a bidirectional interaction between the circadian clock and the ageing process has long been suspected, until recently there was little molecular evidence to substantiate it. Many theories of cellular senescence, however, posit impaired redox regulation at their core [99], and so the observation that Npas2, a functional homologue of Clock, could also be affected by the energy/redox status of the cell, and thus potentially influence circadian transcription, was a surprise to the circadian field [100,101]. More specifically, it is the redox balance of nicotinamide adenine dinucleotide (NAD) cofactors that influence this: the reduced forms NAD(H) and NADP(H) strongly enhance DNA binding of the Clock:Bmal1 and Npas2:Bmal1 heterodimers, whereas the oxidized forms inhibit binding [101]. Additional redox-sensing ligands (e.g. heme) have subsequently been identified in the regulation of *Per2* and *Rev-Erba* activity [102,103]; other recent work in this area has produced further insights into this core biochemical machinery and has highlighted a role for the deacetylase SIRT1 (homologous to Sir2 in yeast) in this process [104–107]. SIRT1 has broad biological functions in growth regulation, stress response, tumorigenesis, endocrine signalling, and in extending lifespan [108]. In the present context SIRT1 appears to counteract the transcription-activating function of Clock protein that was recently shown to exhibit histone acetyl-transferase activity, and thus controls chromatin remodelling around target genes. SIRT1 therefore facilitates repressive chromatin structures in anti-phase to Clock:Bmal1 because, crucially, SIRT1 activity is NAD-dependent and therefore sensitive to the redox balance of the cell, that is itself subject to circadian regulation through modulation of NAD synthesis pathways [104]. It remains to be seen how the clockwork and cell ageing interact at the molecular level to produce the whole-animal effects observed during senescence.

An additional correlate of ageing in humans is the increased incidence of neurodegenerative disorders. Whereas it has been known for many years that poor sleep health is associated with a range of neuronal diseases, the links between circadian dysfunction and Alzheimer's or Huntington's disease, for example, has only recently begun to be studied in detail. Disturbed sleep cycles are the principal cause of institutionalisation in dementia, and therefore represent a major clinical problem [94]. Certainly, disturbances in the activity–rest cycles of patients

with certain dementias (most notably Alzheimer-type and fronto-temporal dementia) have suggested that circadian rhythm disturbance might contribute to the disease process, or be a reflection of it [94,95,109] (Figure 2A). Recently, there has been much interest in the disturbance of circadian rhythms in Huntington's disease. Disintegration of sleep–wake cycles and circadian gene expression across the brain occurs in the R6/2 mouse model of Huntington's disease, and this disturbance becomes more profound as the animal's brain degenerates (Figure 2B) (Ref. [110]). More impressively, cognitive decline and dysfunctional circadian gene expression can be reversed by imposing a daily cycle of sleep in R6/2 mice with the benzodiazepine alprazolam, and this leads to a significant improvement in survival of the diseased mice [111,112]. This latest evidence suggests therefore that circadian and sleep disruption contribute to the neuronal damage that occurs in Huntington's disease, and that targeting the clockwork could be a novel way to combat this archetypal genetic disorder, with obvious implications for other related neurodegenerative conditions.

## Concluding Remarks

Our emerging view of circadian clocks at the cellular and molecular levels revolutionises the way we view the physiological processes that occur in our bodies. Whether it is the regulation of daily cell metabolism, the cell division cycle or the modulation of mood and neurological function, the circadian clockwork is hard-wired into these processes. A key example of this is provided by the Period proteins that are involved in tumour suppression. Understanding the programmes that mould tissue-specific gene and protein expression is beginning to lead to insights into how we can best use this knowledge to direct existing therapies and interventions. This is being applied currently in oncology, but endocrinologists in the future are likely to use drugs modulating clock outputs to treat obesity and its sequelae, including diabetes and metabolic syndrome. Nuclear hormone receptors are emerging targets for drug therapy and could link disease states in multiple tissues to tractable therapeutics in the future. Current barriers to translating basic findings to the clinic can only be addressed with further research into the complex interactions between the distributed network of clocks around the body and how they are synchronised (Box 2). Moreover, new discoveries will propel the development of completely novel therapeutic regimens.

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## References

1. Dunlap J.C. Molecular bases for circadian clocks. *Cell* 1999;96:271–290. [PubMed: 9988221]
2. Takahashi J.S. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat. Rev. Genet.* 2008;9:764–775. [PubMed: 18802415]
3. Kohsaka A. Bass J. A sense of time: how molecular clocks organize metabolism. *Trends Endocrinol. Metab.* 2007;18:4–11. [PubMed: 17140805]
4. Woelfle M.A. The adaptive value of circadian clocks; an experimental assessment in cyanobacteria. *Curr. Biol.* 2004;14:1481–1486. [PubMed: 15324665]
5. Dodd A.N. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* 2005;309:630–633. [PubMed: 16040710]
6. Barger L.K. Neurobehavioral, health, and safety consequences associated with shift work in safety-sensitive professions. *Curr. Neurol. Neurosci. Rep.* 2009;9:155–164. [PubMed: 19268039]
7. Preuss F. Adverse effects of chronic circadian desynchronization in animals in a 'challenging' environment. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2008;295:R2034–2040. [PubMed: 18843092]

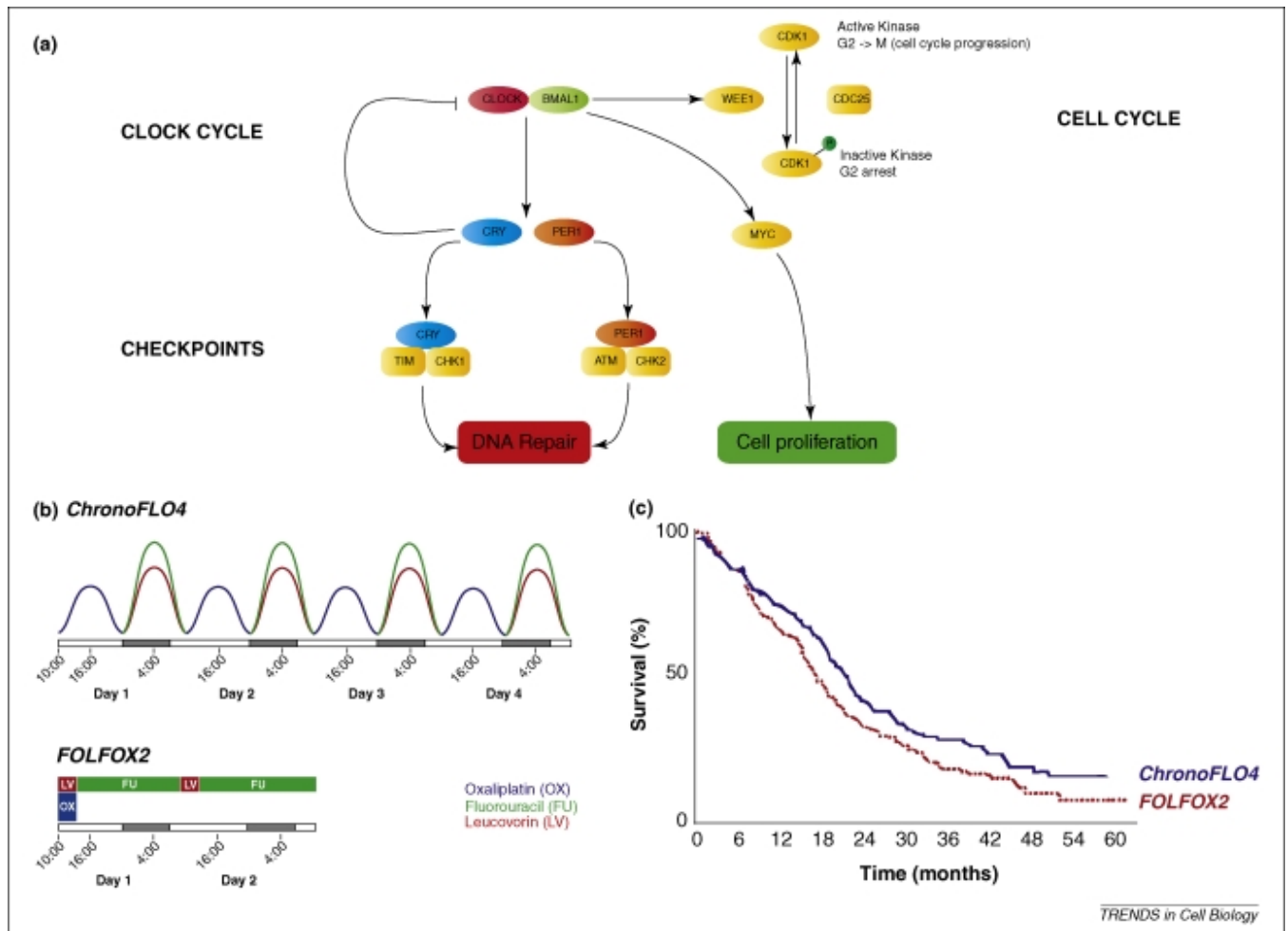
8. Kondratov R.V. Antoch M.P. The clock proteins, aging, and tumorigenesis. *Cold Spring Harb. Symp. Quant. Biol.* 2007;72:477–482. [PubMed: 18419307]
9. Nagoshi E. Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell* 2004;119:693–705. [PubMed: 15550250]
10. Mullenders J. A large scale shRNA barcode screen identifies the circadian clock component ARNTL as putative regulator of the p53 tumor suppressor pathway. *PLoS One* 2009;4:e4798. [PubMed: 19277210]
11. Filipinski E. Disruption of circadian coordination and malignant growth. *Cancer Causes Control* 2006;17:509–514. [PubMed: 16596304]
12. Gibson E.M. Aging in the circadian system: considerations for health, disease prevention and longevity. *Exp. Gerontol.* 2009;44:51–56.
13. Reilly T. Jet lag and air travel: implications for performance. *Clin. Sports Med.* 2005;24:367–380. xii. [PubMed: 15892930]
14. Schmidt C. A time to think: circadian rhythms in human cognition. *Cogn. Neuropsychol.* 2007;24:755–789. [PubMed: 18066734]
15. Levi F. Schibler U. Circadian rhythms: mechanisms and therapeutic implications. *Annu. Rev. Pharmacol. Toxicol.* 2007;47:593–628. [PubMed: 17209800]
16. Hu K. The endogenous circadian pacemaker imparts a scale-invariant pattern of heart rate fluctuations across time scales spanning minutes to 24hours. *J. Biol. Rhythms* 2008;23:265–273. [PubMed: 18487418]
17. Habbal O.A. Al-Jabri A.A. Circadian rhythm and the immune response: a review. *Int. Rev. Immunol.* 2009;28:93–108. [PubMed: 19241255]
18. Portaluppi F. Lemmer B. Chronobiology and chronotherapy of ischemic heart disease. *Adv. Drug. Deliv. Rev.* 2007;59:952–965. [PubMed: 17675179]
19. Wirz-Justice A. Diurnal variation of depressive symptoms. *Dialogues Clin. Neurosci.* 2008;10:337–343. [PubMed: 18979947]
20. Reddy A.B. Circadian orchestration of the hepatic proteome. *Curr. Biol.* 2006;16:1107–1115. [PubMed: 16753565]
21. Costa G. The impact of shift and night work on health. *Applied Ergonomics* 1996;27:9–16. [PubMed: 15676307]
22. Brown D.L. Rotating night shift work and the risk of ischemic stroke. *Am. J. Epidemiol.* 2009;169:1370–1377. [PubMed: 19357324]
23. Schernhammer E.S. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Natl. Cancer. Inst.* 2001;93:1563–1568. [PubMed: 11604480]
24. Schernhammer E.S. Night work and risk of breast cancer. *Epidemiology* 2006;17:108–111. [PubMed: 16357603]
25. Wise J. Danish night shift workers with breast cancer awarded compensation. *Br. Med. J.* 2009;338:b1152. [PubMed: 19297443]
26. Reiter R.J. Light at night, chronodisruption, melatonin suppression, and cancer risk: a review. *Crit. Rev. Oncog.* 2007;13:303–328. [PubMed: 18540832]
27. Scheer F.A. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci. U. S. A* 2009;106:4453–4458. [PubMed: 19255424]
28. Muck W. Pharmacokinetics of cerivastatin when administered under fasted and fed conditions in the morning or evening. *Int. J. Clin. Pharmacol. Ther.* 2000;38:298–303. [PubMed: 10890578]
29. Arble D.M. Circadian timing of food intake contributes to weight gain. *Obesity (Silver Spring)* 2009;17:2100–2102. [PubMed: 19730426]
30. Hastings M.H. Analysis of circadian mechanisms in the suprachiasmatic nucleus by transgenesis and biolistic transfection. *Methods Enzymol.* 2005;393:579–592. [PubMed: 15817313]
31. Weaver D.R. The suprachiasmatic nucleus: a 25-year retrospective. *J. Biol. Rhythms* 1998;13:100–112. [PubMed: 9554572]
32. Hastings M.H. Two decades of circadian time. *J. Neuroendocrinol.* 2008;20:812–819. [PubMed: 18601704]

33. Guler A.D. Multiple photoreceptors contribute to nonimage-forming visual functions predominantly through melanopsin-containing retinal ganglion cells. *Cold Spring Harb. Symp. Quant. Biol.* 2007;72:509–515. [PubMed: 18522518]
34. Akhtar R.A. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr. Biol.* 2002;12:540–550. [PubMed: 11937022]
35. Yoo S.H. PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc. Natl. Acad. Sci. U. S. A.* 2004;101:5339–5346. [PubMed: 14963227]
36. Harmar A.J. The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell* 2002;109:497–508. [PubMed: 12086606]
37. Busino L. SCFFbx13 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. *Science* 2007;316:900–904. [PubMed: 17463251]
38. Godinho S.I. The after-hours mutant reveals a role for Fbx13 in determining mammalian circadian period. *Science* 2007;316:897–900. [PubMed: 17463252]
39. Siepka S.M. Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. *Cell* 2007;129:1011–1023. [PubMed: 17462724]
40. Lowrey P.L. Positional syntenic cloning and functional characterisation of the mammalian circadian mutation tau. *Science* 2000;288:483–491. [PubMed: 10775102]
41. Maywood E.S. Genetic and molecular analysis of the central and peripheral circadian clockwork of mice. *Cold Spring Harb. Symp. Quant. Biol.* 2007;72:85–94. [PubMed: 18419265]
42. Reppert S.M. Weaver D.R. Coordination of circadian timing in mammals. *Nature* 2002;418:935–941. [PubMed: 12198538]
43. Kornmann B. System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. *PLoS Biol* 2007;5:e34. [PubMed: 17298173]
44. Reddy A.B. Maywood E.S. Circadian rhythms: perturbations in the liver clock. *Curr. Biol.* 2007;17:R292–294. [PubMed: 17437708]
45. Reddy A.B. Glucocorticoid signaling synchronizes the liver circadian transcriptome. *Hepatology* 2007;45:1478–1488. [PubMed: 17538967]
46. Balsalobre A. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* 2000;289:2344–2347. [PubMed: 11009419]
47. Balsalobre A. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* 1998;93:929–937. [PubMed: 9635423]
48. Balsalobre A. Multiple signaling pathways elicit circadian gene expression in cultured rat-1 fibroblasts. *Curr. Biol.* 2000;10:1291–1294. [PubMed: 11069111]
49. Brown S.A. Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr. Biol.* 2002;12:1574–1583. [PubMed: 12372249]
50. Mohawk J.A. The methamphetamine-sensitive circadian oscillator does not employ canonical clock genes. *Proc. Natl. Acad. Sci. U. S. A.* 2009;106:3519–3524. [PubMed: 19204282]
51. Storch K.F. Weitz C.J. Daily rhythms of food-anticipatory behavioral activity do not require the known circadian clock. *Proc. Natl. Acad. Sci. U. S. A.* 2009;106:6808–6813. [PubMed: 19366674]
52. Bozek K. Regulation of clock-controlled genes in mammals. *PLoS One* 2009;4:e4882. [PubMed: 19287494]
53. Ukai-Tadenuma M. Proof-by-synthesis of the transcriptional logic of mammalian circadian clocks. *Nat. Cell. Biol.* 2008;10:1154–1163. [PubMed: 18806789]
54. Lamia K.A. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* 2009;326:437–440. [PubMed: 19833968]
55. O'Neill J.S. cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science* 2008;320:949–953. [PubMed: 18487196]
56. Eckel-Mahan K. Sassone-Corsi P. Metabolism control by the circadian clock and vice versa. *Nat. Struct. Mol. Biol.* 2009;16:462–467. [PubMed: 19421159]
57. Clausen O.P. Circadian rhythms in mouse epidermal basal cell proliferation. Variations in compartment size, flux and phase duration. *Cell Tissue Kinet.* 1979;12:319–337. [PubMed: 476779]

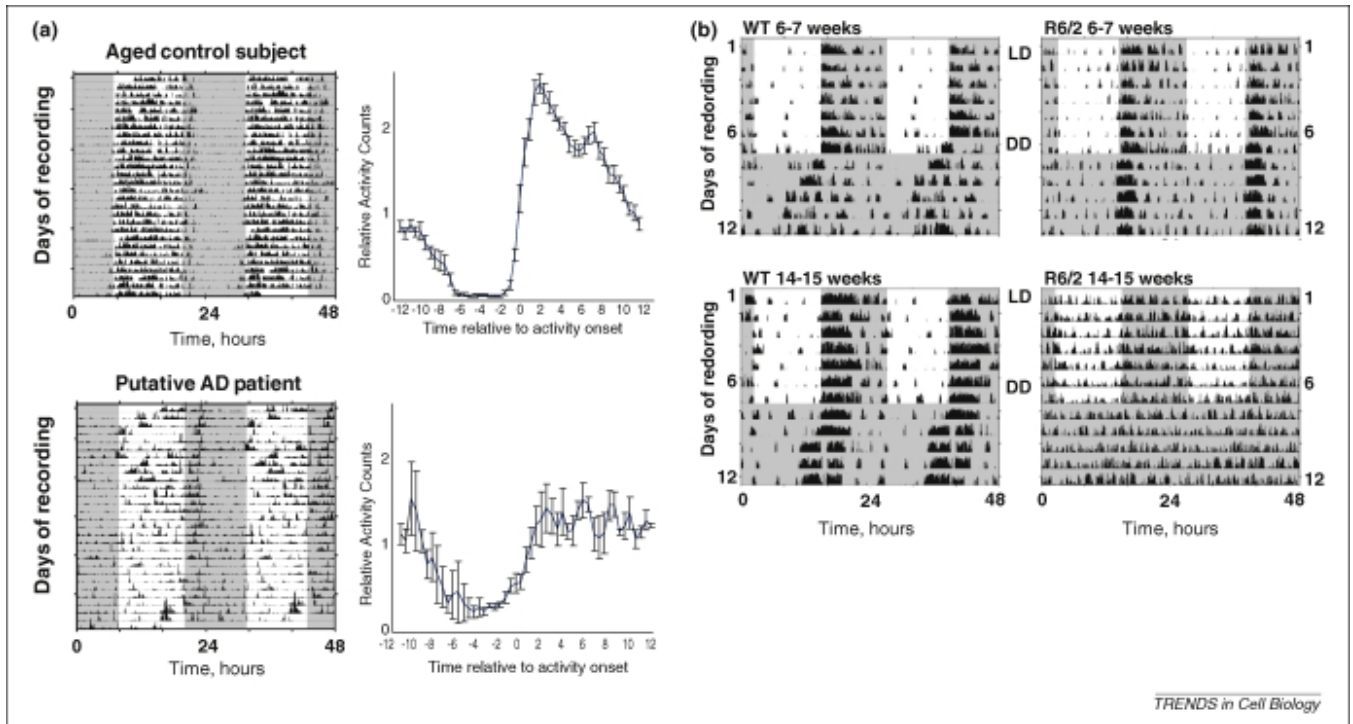
58. Rensing L. Goedeke K. Circadian rhythm and cell cycle: possible entraining mechanisms. *Chronobiologia* 1976;3:853–865. [PubMed: 179764]
59. Scheving L.E. Pauly J.E. Cellular mechanism involving biorhythms with emphasis on those rhythms associated with the S and M stages of the cell cycle. *Int. J. Chronobiol.* 1973;1:269–286. [PubMed: 4776538]
60. Gushchin V.A. [Relation of the duration of phases S and M of the cell cycle, the labelling index and the mitotic index in order to sustain circadian rhythm]. *Tsitologiya* 1971;13:1035–1038. [PubMed: 5117432]
61. Hunt T. Sassone-Corsi P. Riding tandem: circadian clocks and the cell cycle. *Cell* 2007;129:461–464. [PubMed: 17482541]
62. Reddy A.B. Circadian clocks: neural and peripheral pacemakers that impact upon the cell division cycle. *Mutat. Res.* 2005;574:76–91. [PubMed: 15914209]
63. Borgs L. Cell 'circadian' cycle: new role for mammalian core clock genes. *Cell Cycle* 2009;8:832–837. [PubMed: 19221497]
64. Matsuo T. Control mechanism of the circadian clock for timing of cell division in vivo. *Science* 2003;302:255–259. [PubMed: 12934012]
65. Levi F. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. *Philos. Trans. R. Soc. Lond. A Math. Phys. Sci.* 2008;366:3575–3598.
66. Fu L. The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 2002;111:41–50. [PubMed: 12372299]
67. Lee C.C. Tumor suppression by the mammalian *Period* genes. *Cancer Causes Control* 2006;17:525–530. [PubMed: 16596306]
68. Antoch M.P. Disruption of the circadian clock due to the *Clock* mutation has discrete effects on aging and carcinogenesis. *Cell Cycle* 2008;7:1197–1204. [PubMed: 18418054]
69. Gorbacheva V.Y. Circadian sensitivity to the chemotherapeutic agent cyclophosphamide depends on the functional status of the *CLOCK/BMAL1* transactivation complex. *Proc. Natl. Acad. Sci. U. S. A.* 2005;102:3407–3412. [PubMed: 15689397]
70. Hoffman A.E. The circadian gene *NPAS2*, a putative tumor suppressor, is involved in DNA damage response. *Mol. Cancer Res.* 2008;6:1461–1468. [PubMed: 18819933]
71. Yi, C. *et al.* (2009) The circadian gene *NPAS2* is a novel prognostic biomarker for breast cancer. *Breast Cancer Res. Treat.* (in press)
72. Ozturk N. Loss of cryptochrome reduces cancer risk in p53 mutant mice. *Proc. Natl. Acad. Sci. U. S. A.* 2009;106:2841–2846. [PubMed: 19188586]
73. Hoffman A.E. Clock–cancer connection in non-Hodgkin's lymphoma: a genetic association study and pathway analysis of the circadian gene cryptochrome 2. *Cancer Res.* 2009;69:3605–3613. [PubMed: 19318546]
74. Gamsby J.J. A phylogenetically conserved DNA damage response resets the circadian clock. *J. Biol. Rhythms* 2009;24:193–202. [PubMed: 19465696]
75. Oklejewicz M. Phase resetting of the mammalian circadian clock by DNA damage. *Curr. Biol.* 2008;18:286–291. [PubMed: 18291650]
76. Kuo S.J. Disturbance of circadian gene expression in breast cancer. *Virchows Arch.* 2009;454:467–474. [PubMed: 19296127]
77. Lin Y.M. Disturbance of circadian gene expression in hepatocellular carcinoma. *Mol. Carcinog.* 2008;47:925–933. [PubMed: 18444243]
78. Chen S.T. Deregulated expression of the *PER1*, *PER2* and *PER3* genes in breast cancers. *Carcinogenesis* 2005;26:1241–1246. [PubMed: 15790588]
79. Singletary J. Imaging multidimensional therapeutically relevant circadian relationships. *Int. J. Biomed. Imaging* 2009;2009:231539. [PubMed: 19688113]
80. Nakagawa H. Modulation of circadian rhythm of DNA synthesis in tumor cells by inhibiting platelet-derived growth factor signaling. *J. Pharmacol. Sci.* 2008;107:401–407. [PubMed: 18678981]
81. Filipski E. Disruption of circadian coordination accelerates malignant growth in mice. *Pathol. Biol. (Paris)* 2003;51:216–219. [PubMed: 12852994]

82. Sephton S.E. Diurnal cortisol rhythm as a predictor of breast cancer survival. *J. Natl. Cancer. Inst.* 2000;92:994–1000. [PubMed: 10861311]
83. Mormont M.C. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clin. Cancer Res.* 2000;6:3038–3045. [PubMed: 10955782]
84. Innominato P.F. Circadian rhythm in rest and activity: a biological correlate of quality of life and a predictor of survival in patients with metastatic colorectal cancer. *Cancer Res.* 2009;69:4700–4707. [PubMed: 19470769]
85. Megdal S.P. Night work and breast cancer risk: a systematic review and meta-analysis. *Eur. J. Cancer* 2005;41:2023–2032. [PubMed: 16084719]
86. Marino J.L. Shift work, hCLOCK T3111C polymorphism, and endometriosis risk. *Epidemiology* 2008;19:477–484. [PubMed: 18379422]
87. Mormont M.C. Levi F. Cancer chronotherapy: principles, applications, and perspectives. *Cancer* 2003;97:155–169. [PubMed: 12491517]
88. Kang T.H. Circadian oscillation of nucleotide excision repair in mammalian brain. *Proc. Natl. Acad. Sci. U. S. A.* 2009;106:2864–2867. [PubMed: 19164551]
89. Lim F.L. Emerging evidence for the interrelationship of xenobiotic exposure and circadian rhythms: a review. *Xenobiotica* 2006;36:1140–1151. [PubMed: 17118921]
90. Levi F. Chronotherapeutics: the relevance of timing in cancer therapy. *Cancer Causes Control* 2006;17:611–621. [PubMed: 16596317]
91. Giacchetti S. Phase III trial comparing 4-day chronomodulated therapy versus 2-day conventional delivery of fluorouracil, leucovorin, and oxaliplatin as first-line chemotherapy of metastatic colorectal cancer: the European Organisation for Research and Treatment of Cancer Chronotherapy Group. *J. Clin. Oncol.* 2006;24:3562–3569. [PubMed: 16877722]
92. Iurisci I. Improved tumor control through circadian clock induction by Seliciclib, a cyclin-dependent kinase inhibitor. *Cancer Res.* 2006;66:10720–10728. [PubMed: 17108108]
93. Iurisci I. Liver circadian clock, a pharmacologic target of cyclin-dependent kinase inhibitor seliciclib. *Chronobiol. Int.* 2009;26:1169–1188. [PubMed: 19731111]
94. Hatfield C.F. Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia. *Brain* 2004;127:1061–1074. [PubMed: 14998915]
95. Hu K. Reduction of scale invariance of activity fluctuations with aging and Alzheimer's disease: Involvement of the circadian pacemaker. *Proc. Natl. Acad. Sci. U. S. A.* 2009;106:2490–2494. [PubMed: 19202078]
96. van Someren E.J.W. Circadian rest-activity rhythm disturbances in Alzheimer's disease. *Biol. Psychiatry* 1995;38:1–12. [PubMed: 7548467]
97. Kondratov R.V. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes Dev.* 2006;20:1868–1873. [PubMed: 16847346]
98. Sun Y. The mortality of MOP3 deficient mice with a systemic functional failure. *J. Biomed. Sci.* 2006;13:845–851. [PubMed: 16944268]
99. Shimokawa I. Longevity genes: insights from calorie restriction and genetic longevity models. *Mol. Cells* 2008;26:427–435. [PubMed: 18799928]
100. Rutter J. Metabolism and the control of circadian rhythms. *Annu. Rev. Biochem.* 2002;71:307–331. [PubMed: 12045099]
101. Rutter J. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 2001;293:510–514. [PubMed: 11441146]
102. Yang J. A novel heme-regulatory motif mediates heme-dependent degradation of the circadian factor period 2. *Mol. Cell. Biol.* 2008;28:4697–4711. [PubMed: 18505821]
103. Yin L. Rev-erb $\alpha$ , a heme sensor that coordinates metabolic and circadian pathways. *Science* 2007;318:1786–1789. [PubMed: 18006707]
104. Ramsey K.M. Circadian clock feedback cycle through NAMPT-mediated NAD<sup>+</sup> biosynthesis. *Science* 2009;324:651–654. [PubMed: 19299583]
105. Nakahata Y. Circadian control of the NAD<sup>+</sup> salvage pathway by CLOCK-SIRT1. *Science* 2009;324:654–657. [PubMed: 19286518]

106. Nakahata Y. The NAD<sup>+</sup>-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 2008;134:329–340. [PubMed: 18662547]
107. Asher G. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 2008;134:317–328. [PubMed: 18662546]
108. Kim E.J. Um S.J. SIRT1: roles in aging and cancer. *BMB Rep.* 2008;41:751–756. [PubMed: 19017485]
109. Anderson K.N. Disrupted sleep and circadian patterns in frontotemporal dementia. *Eur. J. Neurol.* 2009;16:317–323. [PubMed: 19170747]
110. Morton A.J. Disintegration of the sleep–wake cycle and circadian timing in Huntington's disease. *J. Neurosci.* 2005;25:157–163. [PubMed: 15634777]
111. Pallier P.N. Pharmacological imposition of sleep slows cognitive decline and reverses dysregulation of circadian gene expression in a transgenic mouse model of Huntington's disease. *J. Neurosci.* 2007;27:7869–7878. [PubMed: 17634381]
112. Pallier P.N. Morton A.J. Management of sleep/wake cycles improves cognitive function in a transgenic mouse model of Huntington's disease. *Brain Res.* 2009;1279:90–98. [PubMed: 19450569]
113. Yu B.H. Cho D.Y. Jeong D.U. The effects of circadian rhythm in subjective alertness on the occurrence of traffic accidents. *Sleep Med. Psychophysiol.* 1994;1:68–75.
114. Hastings M.H. A clockwork web: circadian timing in brain and periphery, in health and disease. *Nat. Rev. Neurosci.* 2003;4:649–661. [PubMed: 12894240]
115. Gallego M. Virshup D.M. Post-translational modifications regulate the ticking of the circadian clock. *Nat. Rev. Mol. Cell Biol.* 2007;8:139–148. [PubMed: 17245414]
116. Preitner N. The orphan nuclear receptor REV-ERB $\alpha$  controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 2002;110:251–260. [PubMed: 12150932]
117. Panda S. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 2002;109:307–320. [PubMed: 12015981]
118. Storch K.F. Extensive and divergent circadian gene expression in liver and heart. *Nature* 2002;417:78–83. [PubMed: 11967526]
119. Cheng H.Y. microRNA modulation of circadian-clock period and entrainment. *Neuron* 2007;54:813–829. [PubMed: 17553428]
120. Gatfield D. Integration of microRNA miR-122 in hepatic circadian gene expression. *Genes Dev.* 2009;23:1313–1326. [PubMed: 19487572]
121. Dibner C. Circadian gene expression is resilient to large fluctuations in overall transcription rates. *EMBO J.* 2009;28:123–134. [PubMed: 19078963]
122. Hastings M.H. Cellular circadian pacemaking and the role of cytosolic rhythms. *Curr. Biol.* 2008;18:R805–R815. [PubMed: 18786386]



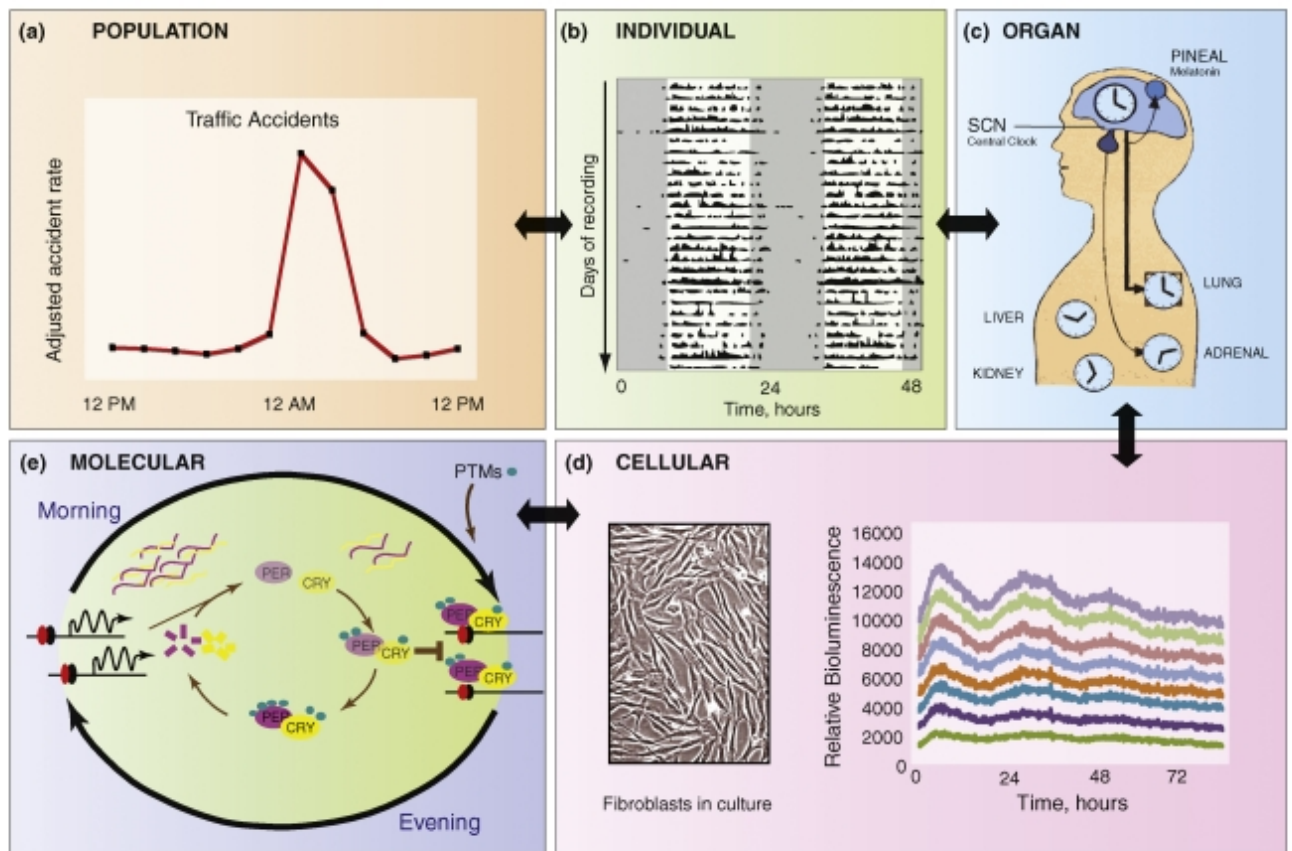
**Figure 1.** Clocks, cancer and the cell cycle. **(A)** The circadian system is linked to the cell-division cycle through circadian control of gene expression and post-translational mechanisms. Transcription of the myelocytomatosis (*Myc*) oncogene and of *Wee1* is circadian and this appears to be a direct target of the CLOCK:BMAL1 complex. The expression of *Wee1* is coregulated with that of period homologue genes (*Per*) and the entry of the cell cycle into M phase is suppressed during the daytime when the transcription of *Per* (and *Wee1*) is high. In addition, the PER1 protein interacts with the checkpoint proteins ataxia telangiectasia mutated (ATM) and checkpoint kinase 2 (CHK2), whereas related work has linked the timeless (TIM) and cryptochrome (CRY) proteins with CHK1. Activation of the DNA-damage pathway can also reset the phase of the circadian clock. CDC25, cell division cycle 25; CDK1, cyclin-dependant kinase 1 (adapted from Ref. [2]). **(B)** Treatment schedules combining oxaliplatin (Oxal), fluorouracil (FU), and leucovorin (LV) administered as a chronomodulated infusion over 4 days (chronofLO4) or as a conventional infusion over 2 days (FOLFOX2). The abscissa represents alternating spans of 8 hours of darkness, corresponding to the average rest span at night, and 16 hours of light, corresponding to the average duration of daytime wakefulness, over the course of chemotherapy delivery. **(C)** Overall survival curve for men, indicating a superior survival at 5 years in the chronomodulated (chronofLO4) chemotherapy group (adapted from Ref. [91]).



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**Figure 2.**

The clockwork and neurodegenerative disorders. (A) Representative actograms from healthy control (top) and moderately demented (bottom) patients. Data from 28 consecutive days are double-plotted on a 48-hour time base for clarity. Group daily activity profiles (plotted as means  $\pm$  SEM) and moderately demented subjects are shown to the right; adapted from Ref. [94]. (B) Progressive changes in the activity–rest cycles of control mice (left) and R6/2 mice (right) before they develop motor and/or cognitive symptoms (6–7 weeks) and after they exhibit overt signs of disease (14–15 weeks). LD, light–dark cycle; DD, constant dim red light.



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**Figure I.** Montage showing the hierarchical nature of circadian rhythms.