© 2014. Published by The Company of Biologists Ltd | Development (2014) 141, 3105-3111 doi:10.1242/dev.104851

REVIEW

# Circadian clock-mediated control of stem cell division and differentiation: beyond night and day

**Steven A. Brown***


## ABSTRACT

A biological 'circadian' clock conveys diurnal regulation upon nearly all aspects of behavior and physiology to optimize them within the framework of the solar day. From digestion to cardiac function and sleep, both cellular and systemic processes show circadian variations that coincide with diurnal need. However, recent research has shown that this same timekeeping mechanism might have been co-opted to optimize other aspects of development and physiology that have no obvious link to the 24 h day. For example, clocks have been suggested to underlie heterogeneity in stem cell populations, to optimize cycles of cell division during wound healing, and to alter immune progenitor differentiation and migration. Here, I review these circadian mechanisms and propose that they could serve as metronomes for a surprising variety of physiologically and medically important functions that far exceed the daily timekeeping roles for which they probably evolved.


**KEY WORDS: Mitochondrial activity, Circadian physiology, Elongation**

## Introduction

Exhaustive research over the past few decades has begun to elucidate the full range of human physiology that is regulated in synchrony with the solar day. With regard to neuronal function, this includes not only the control of sleep and wakefulness, but also modulation of mood, cognition, sensory acuity, breathing rate and body temperature (Schmidt et al., 2007). Nearly all aspects of digestion and detoxication – from gastric emptying time to fat processing and xenobiotic degradation by the liver – are under circadian control (Dallmann et al., 2014). Many aspects of the circulatory and immune systems, including heartbeat and blood pressure, vascular leakage and even plasma composition, are also regulated daily (Dallmann et al., 2012; Scheiermann et al., 2013).

Underlying this panorama of circadian physiology are circadian clocks that regulate cellular and molecular processes at all levels: in each tissue examined, the expression of one in ten genes is regulated in a circadian fashion, either through circadian initiation of transcription or through circadian control of post-transcriptional processes, such as elongation and message stability (Lim and Allada, 2013). Mitochondrial activity is also regulated by the circadian clock (Peek et al., 2013), along with a variety of intracellular signaling cascades (Robles et al., 2014). Supporting this widespread control, the circadian clock mechanism itself is cell-autonomous; most cells of the human body possess the same molecular clockwork. These clocks are then synchronized to one another via redundant systemic cues to ensure optimum correspondence with the environment (Brown and Azzi, 2013). For the most part, these cues originate from the suprachiasmatic nuclei (SCN) of the hypothalamus: the 'master clock' tissue in mammals. The SCN directs the timing of body clocks under most circumstances via autonomous nervous control of hormones such as glucocorticoids, or through direct innervation of other brain regions to send indirect signals via body temperature and rhythmic food intake (Dibner et al., 2010). Because the SCN is itself synchronized to light via the retinohypothalamic tract, the result is a flexible system of clocks, each with an intrinsic period of about one day, that is constantly readjusted to the timing of environmental light. At the same time, because of this redundant and partly autonomous hierarchical organization, considerable flexibility is possible. For example, repeated feeding signals at uncharacteristic times of the day in rodents result in the phase-shifting of clocks in peripheral tissues, such as the intestine, liver and heart, as well as in food anticipatory behavior, even as the SCN continues to track the daily light cycle (Patton and Mistlberger, 2013).

For most aspects of circadian-controlled physiology, one can easily imagine why such regulation exists: in a world divided into day and night, the rhythmic control of digestive function and detoxication can be synchronized with rhythmic food intake that is directed by rhythmic sleep and wakefulness. Likewise, diurnal cardiac function parallels the expected changes in energy needs on a systemic level, and circadian regulation of mitochondrial energy production could reflect the same optimization on a cellular level. In large part, teleological arguments for the evolutionary necessity of circadian clocks propose that the processes that clocks control run most optimally when correctly coordinated with the environment.

However, increasing evidence suggests that the circadian clock controls a much greater proportion of rhythmic physiology than can be easily explained in this fashion. Nowhere is this conundrum more apparent than in the case of stem cells, development and tissue regeneration. In plants, circadian control of development is well established (Nagel and Kay, 2012; Thines and Harmon, 2011), but recent studies suggest that circadian clocks could play a more important role in animals than previously suspected, even in developmental and regenerative processes that are considerably faster or slower than a biological day. In this Review, I first provide an overview of the molecular mechanisms involved in circadian clocks and then discuss how such mechanisms can influence stem cell biology and hence tissue development, homeostasis and regeneration.

## An overview of the mechanistic basis of molecular clocks

The molecular basis of known circadian clocks involves feedback loops of transcription and translation (Fig. 1). Therein, dedicated clock proteins [e.g. the heterodimeric transcription factors CLOCK and BMAL1 (ARNTL – Mouse Genome Informatics) and their

Institute of Pharmacology and Toxicology, University of Zürich, 190 Winterthurerstrasse, Zürich 8057, Switzerland.

*Author for correspondence (steven.brown@pharma.uzh.ch)

Development (2014) 141, 3105-3111 doi:10.1242/dev.104851

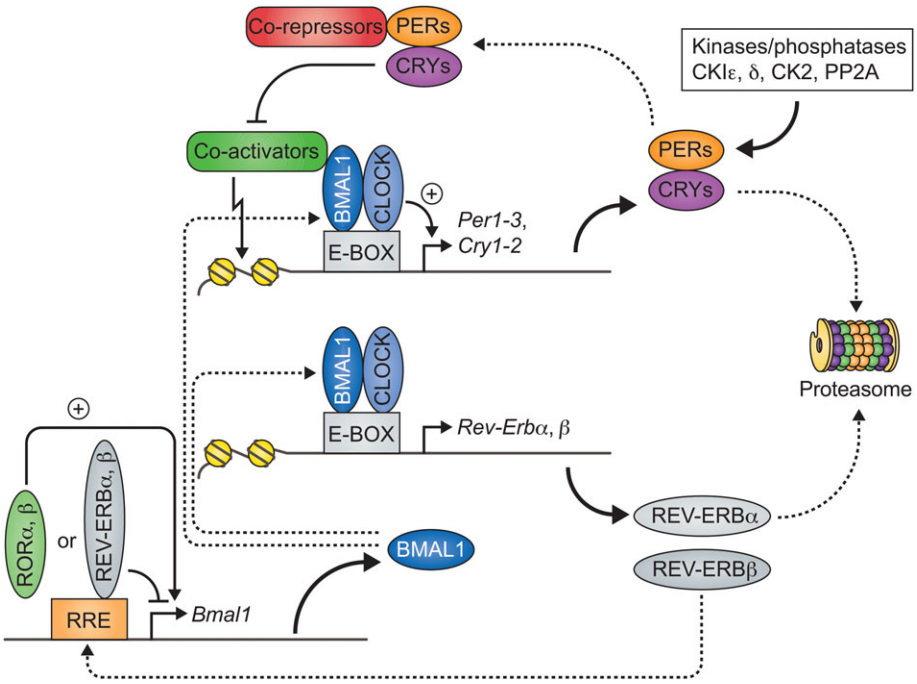


**Fig. 1 . The 'core' circadian clock.** The molecular basis of known circadian clocks involves feedback loops of transcription and translation. Dedicated clock proteins (e.g. the heterodimeric transcription factors CLOCK and BMAL1, and their homologs) activate the transcription of other clock factors (e.g. period loci *Per1-3* and cryptochrome loci *Cry1-2*), the protein products of which repress their own transcription by blocking CLOCK:BMAL1-mediated activation. Other interlocked feedback loops provide essential robustness within this simple mechanism, e.g. CLOCK:BMAL1-mediated transcription of the *Rev-Erbα* nuclear receptor, which itself represses *Bmal1* expression and competes with positively acting ROR factors. Within this simple mechanism, a host of other proteins, such as kinases and phosphatases, chromatin modifiers, transcriptional co-repressors and co-activators, and RNA-binding factors, are necessary for the precise regulation of clock RNA and protein levels. CK2, casein kinase 2; CRYs, cryptochrome proteins; PERs, period proteins; PP2A, protein phosphatase 2; RRE, REV-ERBα response element.

homologs] activate the transcription of other clock genes (e.g. period genes *Per1-Per3* and the cryptochromes *Cry1* and *Cry2*), the protein products of which repress their own transcription by blocking CLOCK:BMAL1-mediated activation (Brown et al., 2012). Other interlocked feedback loops provide essential robustness within this simple mechanism. For example, CLOCK:BMAL1 promotes transcription of the *Rev-Erbα* (*Nr1d1* – Mouse Genome Informatics) nuclear receptor, which itself represses *Bmal1* expression (Preitner et al., 2002), and oscillatory cAMP-dependent signaling within the SCN also reinforces circadian transcriptional oscillations (O'Neill et al., 2008). A host of other proteins – kinases and phosphatases (Reischl and Kramer, 2011), chromatin modifiers (Sahar and Sassone-Corsi, 2013) and RNA-binding factors (Kowalska et al., 2012) – contribute necessary roles to the precise regulation of clock RNA and protein levels (Fig. 2). In turn, the cellular regulation of clock-controlled processes can be achieved by the same cis-acting elements that direct clock gene expression, by circadian cascades of downstream transcription factors or by systemic regulation via hormones, metabolic products and body temperature (Brown and Azzi, 2013). Recently, transcription-independent oscillation of protein oxidation has also been reported, but its mechanism remains as yet unknown (O'Neill and Reddy, 2011).

## Circadian control of cell division

One example of a basic cell biological process that is modulated by the clock is the cell division cycle in mammals, which can run at widely varying speeds but nevertheless shows stage-dependent circadian gating. The cell cycle itself can be loosely divided into stages labeled G1, S, G2 and M, in which S indicates DNA synthesis/replication and M indicates mitosis. Both stages are flanked by intermediary periods (G1 and G2, respectively), terminating in kinase-controlled 'checkpoints'. On a molecular level, circadian control of the cell cycle has been documented at multiple levels and in multiple different scenarios (Fig. 3). For example, an initial report of cell division in the regenerating mouse liver documented circadian transcription of the *Wee1* checkpoint gene, suggesting control at the G2/M checkpoint. Consistent with this idea, CDC2, the target of the WEE1 kinase, shows circadian phosphorylation in the liver (Matsuo et al., 2003). A G1/S checkpoint regulator in hepatocytes, the *p21-waf1* (*Cdkn1a* – Mouse Genome Informatics) gene, is also clock-regulated via REV-ERBα response elements (RREs) within its promoter (Grechez-Cassiau et al., 2008). Further studies in cell culture have illuminated an even more complicated picture. Cultured mouse fibroblasts show a complex clock gating pattern that suggests multiple control points (Feillet et al., 2014; Nagoshi et al., 2004). Other subsequent studies have highlighted potential control via the CHK1/2 (CHEK1/2 – Mouse Genome Informatics) proteins binding to the clock-associated TIM protein (Unsal-Kacmaz et al., 2005; Yang et al., 2010) or via transcriptional control of the *p16-Ink4A* (*Cdkn2a* – Mouse Genome Informatics) locus by the clock protein NONO, which acts as a partner for PER proteins. Such regulations imply circadian G2/M checkpoint control and, consistent with the importance of these regulations, elimination of NONO has been shown to be sufficient to eliminate circadian cell cycle gating in fibroblasts (Kowalska et al., 2013). Recent cell-based studies have also provided strong evidence in reverse, for modulation of the circadian cycle by cell division (Bieler et al., 2014). Therefore, the ultimate picture that emerges is one of complex multilayer control, the cell- and tissue specificity of which still remains to be established.

The observations that the circadian clock regulates cell division in cultured mammalian cells (Nagoshi et al., 2004) and in adult animal tissues (Matsuo et al., 2003) can be understood in terms of diurnal optimization. As the oxidation reactions inherent to metabolic processes result in the production of mutation-causing free radicals, circadian control of the cell cycle to segregate DNA replication away from periods of maximum respiration would optimize the time available for the DNA repair process, without relying upon independent checkpoint mechanisms to pause the cell cycle during DNA repair (Chen and McKnight, 2007). The observation that multiple types of DNA damage repair are themselves coordinately regulated by the circadian clock supports this

Development (2014) 141, 3105-3111 doi:10.1242/dev.104851

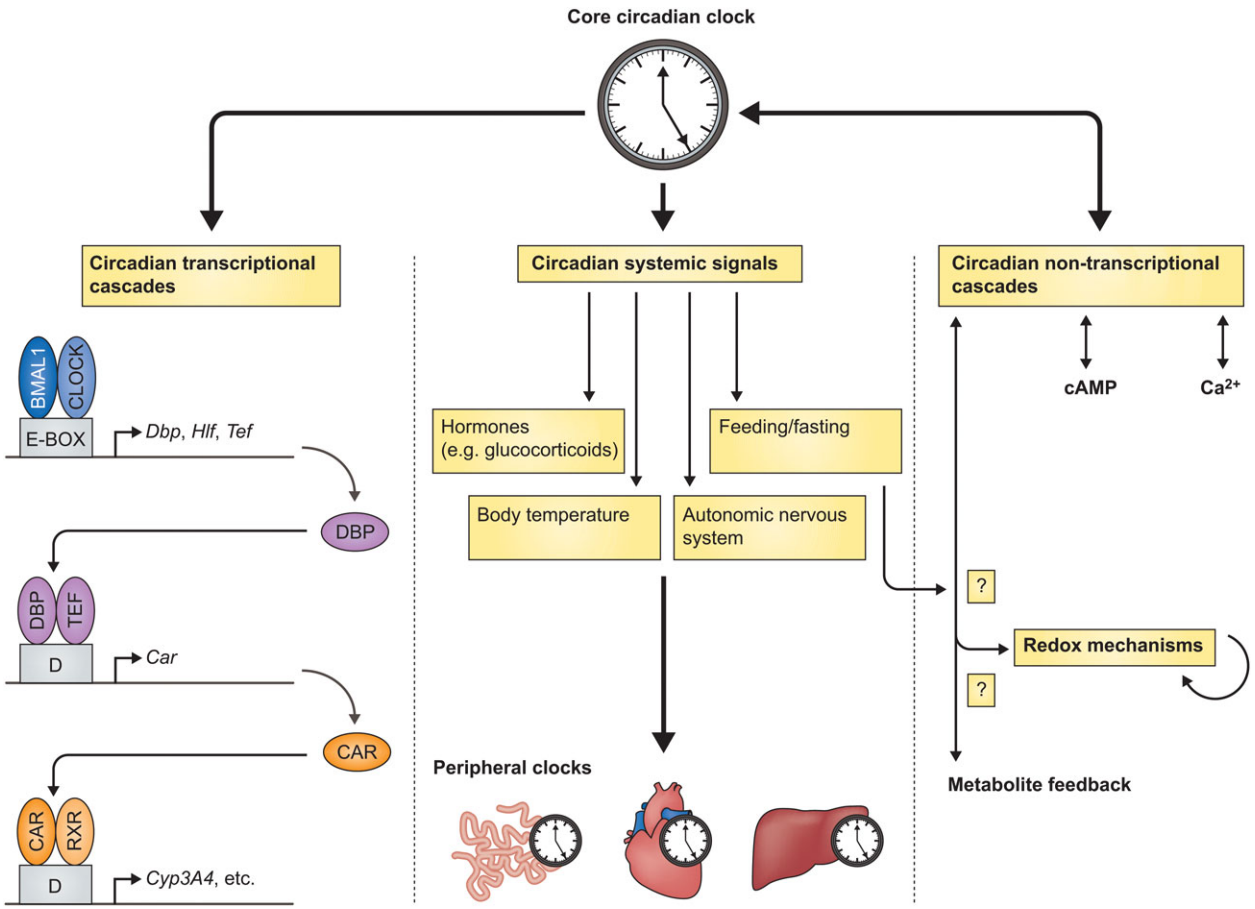


**Fig. 2. Signaling to and from the core clock.** The cellular regulation of clock-controlled processes can be achieved by the same cis-acting elements that direct clock gene expression. Regulated genes can in turn themselves encode further transcription factors, thereby creating circadian cascades of downstream transcription factors (left). For example, the transcription factors DBP, TEF and HLF are regulated by the core clock, and themselves regulate the constitutive androstane receptor [CAR (NR1I3 – Mouse Genome Informatics)], ultimately resulting in circadian control of cytochrome P450 isoforms controlling circadian xenobiotic metabolism. Circadian physiology in peripheral tissues can also be driven by systemic signals, such as hormones, metabolic products and body temperature (center). These signals also serve to synchronize circadian clocks in peripheral tissues with the central clock in the suprachiasmatic nuclei. Beyond transcriptional cascades and systemic signals, post-transcriptional cascades also serve to propagate circadian signals to physiology, and vice versa (right). These include regulatory molecules, such as cAMP, and metabolic and redox-based mechanisms.

hypothesis (Sancar et al., 2010). Similarly, clock-deficient mouse strains (notably *Per2*-deficient mice) have been documented to have increased spontaneous cancer rates in some, but not all studies (Antoch et al., 2013; Fu et al., 2002). This reflects possible increases in DNA damage and supports a role for clock genes as tumor suppressors (Cao et al., 2009; Yang et al., 2009). However, these observations are a simplification: other clock-deficient mice do not show increased cancer rates (Antoch et al., 2008; Gauger and Sancar, 2005), and cell lines from multiple clock-deficient strains possess normal DNA repair properties (Gaddameedhi et al., 2012). Therefore, other benefits of circadian regulation of the cell cycle might also exist, as we explore below.

## From cell cycle to tissues: circadian control of tissue homeostasis

The direct consequences of circadian control of the cell division throughout the body have been established in numerous recent studies. For example, elimination of the SCN 'master clock' results in tumor growth two to three times faster than in controls (Filipski et al., 2002). Consistent with the possible importance of this regulation, clock protein misexpression and/or a lack of circadian control has been documented in multiple tumor types (Hwang-Verslues et al., 2013; Luo et al., 2012; Zhao et al., 2013) and immortalized cell lines (Yeom et al., 2010). In normal physiology, circadian cell division has been documented in adult hippocampal neurogenesis (Bouchard-Cannon et al., 2013), in intestinal and skin epithelial cell division (Geyfman et al., 2012; Janich et al., 2013; Karpowicz et al., 2013), and in multiple immune cell populations (Fortier et al., 2011; Keller et al., 2009) – essentially anywhere that cell division occurs in adult animals.

Therefore, beyond a context in DNA repair and cancer prevention, circadian control could serve as a metronome to coordinate complex processes, whether or not they are related to the 24 h day. For example, in the case of dermal wound repair, disruption of circadian gating by eliminating PERIOD clock repressor proteins resulted in fibroblast and keratinocyte hyperproliferation, and in collagen undersecretion; eliminating the BMAL1 clock activator protein resulted in underproliferation of the same cells, whereas collagen was overproduced (Kowalska et al., 2013). One possible explanation for this phenomenon could be a reciprocal regulation of matrix production and cell division by fibroblasts, mediated via the circadian clock. Such an interpretation is supported by similar studies of regeneration in the *Drosophila* intestine after chemically induced damage. Here, not only is the division of intestinal stem

Development (2014) 141, 3105-3111 doi:10.1242/dev.104851

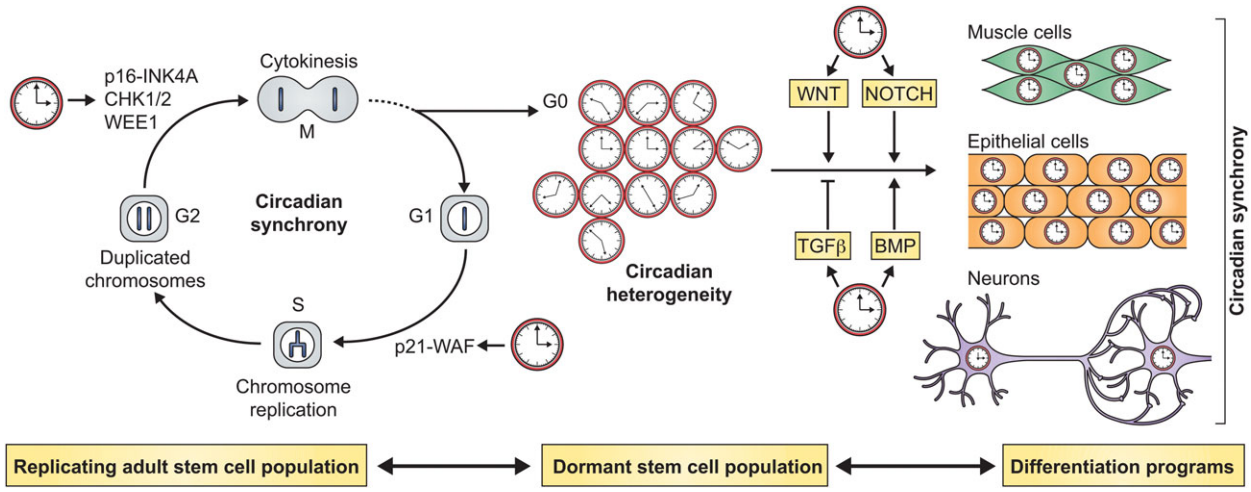


**Fig. 3. The daily life of a stem cell.** During proliferation, a stem cell follows the ordered stages of the cell cycle followed by any other cell type: S phase (DNA replication) and M phase (mitosis) are flanked by two rest phases, G1 and G2, for an overall sequence of G1-S-G2-M. Circadian control of this division process is probably exerted via the expression of 'checkpoint proteins' governing transition from G1 to S (e.g. p21WAF) and from G2 to M (e.g. WEE1, p16-INK4A, CHK1/2). After M phase and prior to G1, a stem cell may also exit the cell cycle to a dormant phase, G0. In this phase, circadian heterogeneity could play an important role in fate decisions. From G0, differentiation is possible in response to a variety of different signaling programs, notably the WNT, TGFβ, NOTCH, BMP and SHH pathways. Very generally, these pathways act antagonistically to each other to promote proliferation or differentiation to different lineages depending upon context. Some of these pathways, such as the WNT, TGFβ and NOTCH pathways, have been reported to be reciprocally regulated with clock genes in some instances, perhaps allowing regulation of the progression of differentiation by the circadian clock.

cells (ISCs) regulated in circadian fashion, but so too is the expression of hundreds of other genes in the healing intestine, regulating everything from the stress response to cell polarity (Karpowicz et al., 2013). In mammals, similar widespread circadian control of epithelial cell processes has also been documented and is necessary for gut homeostasis (Mukherji et al., 2013).

Analogous arguments can be drawn for most other instances of circadian cell division. The observed circadian regulation of adult hippocampal neurogenesis (Bouchard-Cannon et al., 2013) could complement circadian regulation of dendritic spine formation and stabilization (Liston et al., 2013), allowing for more efficient reintegration of new neurons into the adult brain. A circadian clock is also present in tooth ameloblasts, where it controls antiphase rhythms of enamel matrix endocytosis and secretion, as well as ameloblast maturation (Lacruz et al., 2012; Zheng et al., 2013). Circadian oscillations in the release of hematopoietic stem cells (HSCs), coupled with antiphase circadian expression of the chemokine *Cxcl12* and coordinated with GSK3β-dependent changes in HSC migratory properties (Lapid et al., 2013), could result in the coordinated release of HSCs and repopulation of the bone marrow stem cell niche (Méndez-Ferrer et al., 2009, 2008). Analogous rhythmic division and tissue colonization has also been documented for monocytes (Nguyen et al., 2013).

## From tissue homeostasis to stem cells: circadian control of cell fate

Because circadian clocks control the expression of cell cycle regulatory genes such as *p16-Ink4A* (Kowalska et al., 2013) and pathways, such as the NOTCH, WNT and HIPPO signaling pathways (Karpowicz et al., 2013), an immediate and obvious prediction of this widespread control is that not only cell division but also cell differentiation, e.g. that of stem cell populations, might be under circadian influence. As with other cell types, stem cells follow the ordered stages of the cell cycle, but they may also enter into a dormant G0 phase (Fig. 3), during which time circadian heterogeneity could play an important role in driving cell fate decisions, as I describe below.

Studies have shown that clock genes can indeed directly influence stem and progenitor cell fate. For example, the manipulation of *Per3* expression influences adipocyte fate, probably by regulating the expression of peroxisome proliferator-activated receptor γ (*Pparg* – Mouse Genome Informatics), which plays a key role in adipose tissue development (Costa et al., 2011). A similar role has been demonstrated for the circadian deadenylase nocturnin during adipogenesis (Kawai et al., 2010). Disruption of the clock gene *Bmal1* also led to increased adipogenesis and, correspondingly, the attenuation of *Bmal1* expression in pre-adipocytes *in vitro* led to downregulation of the WNT signaling pathway – probably through transcriptional control of multiple pathway members – and increased adipogenesis (Guo et al., 2012). The converse occurred upon *Bmal1* overexpression. An inversed role has been ascribed to *Bmal1* in the case of myogenesis: here, knockout of *Bmal1* led to reduced muscle mass and blunted expression of key myogenic regulators, presumably due to circadian control of WNT pathway activity (Chatterjee et al., 2013). *Bmal1* deficiency is also linked to reduced osteoblast proliferation in adult mice, although a circadian role here has not been proven (Chen et al., 2012). Furthermore, in the complex differentiation processes leading to the development of the immune system, $T_h17$ T helper cell development is regulated by the circadian factors REV-ERBα and RORγ (RORC – Mouse Genome Informatics) (Yu et al., 2013), and circadian cytokine release *in vivo* and *in vitro* is likely to influence other immune cell subpopulations (Fortier et al., 2011; Keller et al., 2009; Wang et al., 2011). Finally, in addition to regulating metabolism, multiple circadian clock genes directly regulate the size and differentiation of pancreatic islets (Marcheva et al., 2010).

Probably the most-studied case of circadian regulation of stem cells so far is that of the hair follicle. Hair tissues proceed through alternate stages of hair production (anagen) and inactivity (telogen), in which spatially distinct niches harbor populations of dormant stem cells, dividing stem cells or mixtures of the two at different times. Different roles for the circadian clock have been proposed in each of these phases and their transitions. During anagen, proliferating stem cells of the hair follicle show marked circadian

Development (2014) 141, 3105-3111 doi:10.1242/dev.104851

oscillations in cell division similar to those outlined above for other tissues. These oscillations in proliferation result in time-of-day-dependent hair growth, but circadian clock mutants surprisingly do not show altered hair dimensions overall. Instead, these animals lose a time-of-day-dependent genoprotective effect: whereas wild-type mice show time-of-day-dependent radiation toxicity, *Clock* mutants do not. Thus, a circadian clock has been suggested here to optimize DNA repair and replication cycles, primarily via CDC2/CYCLIN B-mediated synchronization of the G2/M checkpoint (Plikus et al., 2013).

During telogen and the transition to anagen, a very different picture has emerged. In the transition to anagen, a circadian pattern of cell division was again seen in dividing cells, but this time gating progression to anagen (Lin et al., 2009). In this study, the authors documented a circadian blockage of the G1 phase of the cell cycle, possibly due to clock-controlled elevation of p21. By contrast, the study of circadian function in dormant stem cells of the hair bulge (the permanent region of the hair follicle) during telogen resulted in a remarkable finding: although hair follicle 'stemness' genes – including those encoding members of the WNT, TGFβ, NOTCH and SHH pathways – in hair follicle stem cells were expressed in a circadian fashion dependent upon the expected clock genes, the phases of circadian oscillations in the hair follicle stem cell compartment were completely unsynchronized (Janich et al., 2011). In addition, genetic ablation of the circadian clock in these cells resulted in significant defects in the dynamics of hair follicle stem cell activation. With this finding, the authors suggested a possible second major role for circadian control of developmental processes: the generation of heterogeneity within the stem cell compartment (Janich et al., 2011). Given the importance of juxtacrine signaling during development, this heterogeneity might be important for optimizing cell fate decisions or could simply permit a broad population of cells expressing different receptors to respond to different cues at different times. In a subsequent study (Janich et al., 2013), the authors demonstrated the second possibility *in vitro* by showing that cultured and clock-synchronized keratinocyte stem cells responded better to certain differentiation cues at some times of day than at others. Consistent with their hypothesis, cells expressed different proliferation and differentiation pathway genes at different times of day. During times corresponding to early morning, differentiation pathways were high, whereas in the evening pathways corresponding to DNA replication and cell division predominated. Indeed, based on their gene expression data, the authors were able to distinguish 'functional intervals' during the stem cell differentiation process, in which activities of particular signaling pathways displayed maximum expression. In turn, clock genes themselves displayed ordered patterns of expression across these functional intervals, raising the possibility that circadian control might be necessary to achieve temporal orchestration of keratinocyte stem cell development within time windows even shorter than simply day and night (Janich et al., 2013). Further experiments using clock-deficient cells will no doubt shed further light upon this interesting hypothesis.

## From stem cells to development

Given the circadian coordination of transcriptomic programs present in adult stem cells such as the keratinocyte stem cells described above, it is logical to wonder which types of stem cells show circadian oscillations, and when in development they do so. *In vitro*, multiple laboratories have demonstrated that, whereas pluripotent embryonic stem (ES) cells show no visible rhythmicity, their differentiated counterparts – even multipotent neural precursor cells (NPCs) – show robust circadian oscillations of gene expression (Kowalska et al., 2010; Yagita et al., 2010). In elegant experiments, it was shown that these rhythms are lost upon de-differentiation of these cells back to ES cells, and regained upon re-differentiation to NPCs (Yagita et al., 2010). Thus, cell-intrinsic rhythms of circadian gene expression are probably present at the very earliest stages of development. It has even been suggested that circadian rhythms of energy consumption in ES cells might briefly precede the emergence of canonical circadian transcriptional oscillations (Paulose et al., 2012).

In spite of the evident potential for prenatal circadian oscillations that this research suggests, widespread and overt rhythms of behavior and physiology in mammals probably emerge much later in development, only after birth (Dolatshad et al., 2010). Circadian expression of certain clock genes in some tissues can be seen as early as embryonic day 20 (E20), but full adult-like oscillations develop as late as postnatal day 30 (P30), coincident with the emergence of circadian locomotor activity (Sladek et al., 2007). To date, no studies at cellular resolution have been performed *in vivo* in mammals to ascertain whether clocks in early developing tissues are absent or simply desynchronized, although *in utero* observations of rat embryos containing a circadian reporter also suggested that the first coherent circadian oscillations occur around or shortly before birth (Saxena et al., 2007). Nevertheless, it is likely that coherent circadian rhythms must exist in at least some tissues prior to birth, because phase at weaning is influenced directly by prenatal light timing, timed injection of a dopamine agonist or by exogenous melatonin (Davis, 1997; Davis and Gorski, 1985; Viswanathan et al., 1994).

A relatively late emergence of coherent circadian oscillations has also been documented in chicks, but evidence suggests that substantially earlier unsynchronized cell-autonomous oscillations exist (Gonçalves et al., 2012). In other vertebrates such as zebrafish, synchronized circadian oscillations of both the cell cycle (Dekens et al., 2003) and of cell cycle regulatory genes, including *p20* and *p21*, during embryonic development are well documented (Laranjeiro et al., 2013). The evolutionary or developmental benefit of this regulation remains mostly unknown, but a recent study showed that genetic or environmental disruption of these circadian oscillations resulted in defects in angiogenesis (Jensen et al., 2012).

Consistent with the late emergence of coherent circadian rhythmicity during mammalian development, the important roles shown by many groups for circadian rhythms in stem cells of adult mammals appear to be unsubstantiated during embryogenesis. In mouse models, disruption of any of the canonical circadian clock genes still results in the birth of a basically normal mouse. In contrast to the severe defects observed in circadian clock-deficient adult mice during epidermal wound healing, the skin in newborns is normal in morphology (Kowalska et al., 2013). Furthermore, despite the alterations in hair follicle regeneration observed in *Clock* mutants, initial hair growth in pups is unchanged (Plikus et al., 2013). Only in some instances has the importance of non-circadian expression of *Clock* genes during mammalian development been postulated, e.g. in the case of the developing pancreas through regulation of WNT, NOTCH and cell division rates (Li et al., 2007). Thus, one is forced to assume that many of the important regulatory processes described above are substituted for or are unnecessary within the context of embryonic development, perhaps due to the rapid pace of cell division that makes circadian regulation suboptimal. Such ideas, however, are pure speculation.

## Conclusions

It is clear that circadian clock-mediated regulation of both the division and differentiation of stem cells plays an important role in

adult tissue renewal. The control of these processes by the circadian clock might not only be essential for correct healing and regeneration, but might also be of benefit during ageing. As already noted above, mice deficient in different circadian clock genes suffer from pathologies ranging from diabetes to arthritis and cancer (Antoch et al., 2008; Bunger et al., 2005; Fu et al., 2002; Marcheva et al., 2010).

In this Review, I have postulated that, broadly speaking, circadian clocks could serve two equally important roles in stem cell development and differentiation. On the one hand, circadian gating of multiple aspects of complex tissue homeostasis and regeneration could permit the optimal coordination of mutually beneficial or antagonistic processes. On the other hand, dephased oscillators could provide a source of heterogeneity for stem cells, allowing them to respond optimally to a variety of signals (Fig. 3). If the latter were true, maintenance of such heterogeneity would represent an issue important in stem cell biology. Normal peripheral circadian oscillators are entrained to a particular phase by a wealth of direct and indirect timing cues from the environment and from physiology (Saini et al., 2011). Escape from such entrainment could be envisioned in a variety of ways. Stem cells might fail to respond to normal cues, or they might themselves represent a heterogeneous population that responds to different cues. Finally, the epidermal stem cells in which clock heterogeneity has been convincingly documented are themselves in a unique environment of extreme temperature variation, and temperature represents an important entrainment signal to peripheral oscillators (Brown et al., 2002; Morf and Schibler, 2013). Therefore, the epidermal stem cells might simply be confused by conflicting environmental signals. Further experiments will no doubt soon shed light on this interesting issue.

Altogether, a wealth of research suggests that the circadian clock indeed controls more than just daily timekeeping. Understanding such regulation could hold clues to novel treatments for disease. Moreover, it could also help explain the mechanisms by which chronic ‘mis-phasing’ of clocks in a modern society of extended artificial light and widespread shiftwork might contribute to the increased prevalence of cancer, cardiac and metabolic diseases observed in recent times.

#### Acknowledgements
I thank Dr Ludmila Gaspar for critical comments and figure editing in this manuscript.

#### Competing interests
The author declares no competing financial interests.

#### Funding
The above-cited work of this author has been made possible by grants from the Swiss National Science Foundation, the Swiss Cancer League, the Velux Foundation and the Zürich Clinical Priority project ‘Sleep and Health’.

#### References
**Antoch, M. P., Gorbacheva, V. Y., Vykhovanets, O., Toshkov, I. A., Kondratov, R. V., Kondratova, A. A., Lee, C. and Nikitin, A. Y.** (2008). Disruption of the circadian clock due to the Clock mutation has discrete effects on aging and carcinogenesis. *Cell Cycle* **7**, 1197-1204.

**Antoch, M. P., Toshkov, I., Kuropatwinski, K. K. and Jackson, M.** (2013). Deficiency in PER proteins has no effect on the rate of spontaneous and radiation-induced carcinogenesis. *Cell Cycle* **12**, 3673-3680.

**Bieler, J., Cannavo, R., Gustafson, K., Gobet, C., Gatfield, D. and Naef, F.** (2014). Robust synchronization of coupled circadian and cell cycle oscillators in single mammalian cells. *Mol. Syst. Biol.* **10**, 739.

**Bouchard-Cannon, P., Mendoza-Viveros, L., Yuen, A., Kaern, M. and Cheng, H. Y.** (2013). The Circadian molecular clock regulates adult hippocampal neurogenesis by controlling the timing of cell-cycle entry and exit. *Cell Rep.* **5**, 961-973.

**Brown, S. A. and Azzi, A.** (2013). Peripheral circadian oscillators in mammals. *Handb. Exp. Pharmacol.* **217**, 45-66.

**Brown, S. A., Zumbrunn, G., Fleury-Olela, F., Preitner, N. and Schibler, U.** (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr. Biol.* **12**, 1574-1583.

**Brown, S. A., Kowalska, E. and Dallmann, R.** (2012). (Re)inventing the circadian feedback loop. *Dev. Cell* **22**, 477-487.

**Bunger, M. K., Walisser, J. A., Sullivan, R., Manley, P. A., Moran, S. M., Kalscheur, V. L., Colman, R. J. and Bradfield, C. A.** (2005). Progressive arthropathy in mice with a targeted disruption of the Mop3/Bmal-1 locus. *Genesis* **41**, 122-132.

**Cao, Q., Gery, S., Dashti, A., Yin, D., Zhou, Y., Gu, J. and Koeffler, H. P.** (2009). A role for the clock gene per1 in prostate cancer. *Cancer Res.* **69**, 7619-7625.

**Chatterjee, S., Nam, D., Guo, B., Kim, J. M., Winnier, G. E., Lee, J., Berdeaux, R., Yechoor, V. K. and Ma, K.** (2013). Brain and muscle Arnt-like 1 is a key regulator of myogenesis. *J. Cell Sci.* **126**, 2213-2224.

**Chen, Z. and McKnight, S. L.** (2007). A conserved DNA damage response pathway responsible for coupling the cell division cycle to the circadian and metabolic cycles. *Cell Cycle* **6**, 2906-2912.

**Chen, Y., Xu, X., Tan, Z., Ye, C., Zhao, Q. and Chen, Y.** (2012). Age-related BMAL1 change affects mouse bone marrow stromal cell proliferation and osteo-differentiation potential. *Arch. Med. Sci.* **8**, 30-38.

**Costa, M. J., So, A. Y.-L., Kaasik, K., Krueger, K. C., Pillsbury, M. L., Fu, Y.-H., Ptacek, L. J., Yamamoto, K. R. and Feldman, B. J.** (2011). Circadian rhythm gene period 3 is an inhibitor of the adipocyte cell fate. *J. Biol. Chem.* **286**, 9063-9070.

**Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C. and Brown, S. A.** (2012). The human circadian metabolome. *Proc. Natl. Acad. Sci. USA* **109**, 2625-2629.

**Dallmann, R., Brown, S. A. and Gachon, F.** (2014). Chronopharmacology: new insights and therapeutic implications. *Annu. Rev. Pharmacol. Toxicol.* **54**, 339-361.

**Davis, F. C.** (1997). Melatonin: role in development. *J. Biol. Rhythms* **12**, 498-508.

**Davis, F. C. and Gorski, R. A.** (1985). Development of hamster circadian rhythms: prenatal entrainment of the pacemaker. *J. Biol. Rhythms* **1**, 77-89.

**Dekens, M. P. S., Santoriello, C., Vallone, D., Grassi, G., Whitmore, D. and Foulkes, N. S.** (2003). Light regulates the cell cycle in zebrafish. *Curr. Biol.* **13**, 2051-2057.

**Dibner, C., Schibler, U. and Albrecht, U.** (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* **72**, 517-549.

**Dolatshad, H., Cary, A. J. and Davis, F. C.** (2010). Differential expression of the circadian clock in maternal and embryonic tissues of mice. *PLoS ONE* **5**, e9855.

**Feillet, C., Krusche, P., Tamanini, F., Janssens, R. C., Downey, M. J., Martin, P., Teboul, M., Saito, S., Lévi, F. A., Bretschneider, T. et al.** (2014). Phase locking and multiple oscillating attractors for the coupled mammalian clock and cell cycle. *Proc. Natl. Acad. Sci. USA* **111**, 9828-9833.

**Filipski, E., King, V. M., Li, X., Granda, T. G., Mormont, M.-C., Liu, X., Claustrat, B., Hastings, M. H. and Lévi, F.** (2002). Host circadian clock as a control point in tumor progression. *J. Natl. Cancer Inst.* **94**, 690-697.

**Fortier, E. E., Rooney, J., Dardente, H., Hardy, M.-P., Labrecque, N. and Cermakian, N.** (2011). Circadian variation of the response of T cells to antigen. *J. Immunol.* **187**, 6291-6300.

**Fu, L., Pelicano, H., Liu, J., Huang, P. and Lee, C. C.** (2002). The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell* **111**, 41-50.

**Gaddameedhi, S., Reardon, J. T., Ye, R., Ozturk, N. and Sancar, A.** (2012). Effect of circadian clock mutations on DNA damage response in mammalian cells. *Cell Cycle* **11**, 3481-3491.

**Gauger, M. A. and Sancar, A.** (2005). Cryptochrome, circadian cycle, cell cycle checkpoints, and cancer. *Cancer Res.* **65**, 6828-6834.

**Geyfman, M., Kumar, V., Liu, Q., Ruiz, R., Gordon, W., Espitia, F., Cam, E., Millar, S. E., Smyth, P., Ihler, A. et al.** (2012). Brain and muscle Arnt-like protein-1 (BMAL1) controls circadian cell proliferation and susceptibility to UVB-induced DNA damage in the epidermis. *Proc. Natl. Acad. Sci. USA* **109**, 11758-11763.

**Gonçalves, L., Vinhas, M., Pereira, R., Pais De Azevedo, T., Bajanca, F. and Palmeirim, I.** (2012). Circadian clock genes Bmal1 and Clock during early chick development. *Dev. Dyn.* **241**, 1365-1373.

**Grechez-Cassiau, A., Rayet, B., Guillaumond, F., Teboul, M. and Delaunay, F.** (2008). The circadian clock component BMAL1 is a critical regulator of p21WAF1/CIP1 expression and hepatocyte proliferation. *J. Biol. Chem.* **283**, 4535-4542.

**Guo, B., Chatterjee, S., Li, L., Kim, J. M., Lee, J., Yechoor, V. K., Minze, L. J., Hsueh, W. and Ma, K.** (2012). The clock gene, brain and muscle Arnt-like 1, regulates adipogenesis via Wnt signaling pathway. *FASEB J.* **26**, 3453-3463.

**Hwang-Verslues, W. W., Chang, P.-H., Jeng, Y.-M., Kuo, W.-H., Chiang, P.-H., Chang, Y.-C., Hsieh, T.-H., Su, F.-Y., Lin, L.-C., Abbondante, S. et al.** (2013). Loss of corepressor PER2 under hypoxia up-regulates OCT1-mediated EMT gene expression and enhances tumor malignancy. *Proc. Natl. Acad. Sci. USA* **110**, 12331-12336.

**Janich, P., Pascual, G., Merlos-Suárez, A., Batlle, E., Ripperger, J., Albrecht, U., Cheng, H.-Y. M., Obrietan, K., Di Croce, L. and Benitah, S. A.** (2011). The circadian molecular clock creates epidermal stem cell heterogeneity. *Nature* **480**, 209-214.

**Janich, P., Toufighi, K., Solanas, G., Luis, N. M., Minkwitz, S., Serrano, L., Lehner, B. and Benitah, S. A.** (2013). Human epidermal stem cell function is regulated by circadian oscillations. *Cell Stem Cell* **13**, 745-753.
**Jensen, L. D., Cao, Z., Nakamura, M., Yang, Y., Bräutigam, L., Andersson, P., Zhang, Y., Wahlberg, E., Länne, T., Hosaka, K. et al.** (2012). Opposing effects of circadian clock genes bmal1 and period2 in regulation of VEGF-dependent angiogenesis in developing zebrafish. *Cell Rep.* **2**, 231-241.
**Karpowicz, P., Zhang, Y., Hogenesch, J. B., Emery, P. and Perrimon, N.** (2013). The circadian clock gates the intestinal stem cell regenerative state. *Cell Rep.* **3**, 996-1004.
**Kawai, M., Green, C. B., Lecka-Czernik, B., Douris, N., Gilbert, M. R., Kojima, S., Ackert-Bicknell, C., Garg, N., Horowitz, M. C., Adamo, M. L. et al.** (2010). A circadian-regulated gene, Nocturnin, promotes adipogenesis by stimulating PPAR-gamma nuclear translocation. *Proc. Natl. Acad. Sci. USA* **107**, 10508-10513.
**Keller, M., Mazuch, J., Abraham, U., Eom, G. D., Herzog, E. D., Volk, H.-D., Kramer, A. and Maier, B.** (2009). A circadian clock in macrophages controls inflammatory immune responses. *Proc. Natl. Acad. Sci. USA* **106**, 21407-21412.
**Kowalska, E., Moriggi, E., Bauer, C., Dibner, C. and Brown, S. A.** (2010). The circadian clock starts ticking at a developmentally early stage. *J. Biol. Rhythms* **25**, 442-449.
**Kowalska, E., Ripperger, J. A., Muheim, C., Maier, B., Kurihara, Y., Fox, A. H., Kramer, A. and Brown, S. A.** (2012). Distinct roles of DBHS family members in the circadian transcriptional feedback loop. *Mol. Cell. Biol.* **32**, 4585-4594.
**Kowalska, E., Ripperger, J. A., Hoegger, D. C., Bruegger, P., Buch, T., Birchler, T., Mueller, A., Albrecht, U., Contaldo, C. and Brown, S. A.** (2013). NONO couples the circadian clock to the cell cycle. *Proc. Natl. Acad. Sci. USA* **110**, 1592-1599.
**Lacruz, R. S., Hacia, J. G., Bromage, T. G., Boyde, A., Lei, Y., Xu, Y., Miller, J. D., Paine, M. L. and Snead, M. L.** (2012). The circadian clock modulates enamel development. *J. Biol. Rhythms* **27**, 237-245.
**Lapid, K., Itkin, T., D'Uva, G., Ovadya, Y., Ludin, A., Caglio, G., Kalinkovich, A., Golan, K., Porat, Z., Zollo, M. et al.** (2013). GSK3beta regulates physiological migration of stem/progenitor cells via cytoskeletal rearrangement. *J. Clin. Invest.* **123**, 1705-1717.
**Laranjeiro, R., Tamai, T. K., Peyric, E., Krusche, P., Ott, S. and Whitmore, D.** (2013). Cyclin-dependent kinase inhibitor p20 controls circadian cell-cycle timing. *Proc. Natl. Acad. Sci. USA* **110**, 6835-6840.
**Li, Z., Ruan, L., Lin, S. and Gittes, G. K.** (2007). Clock controls timing of mouse pancreatic differentiation through regulation of Wnt- and Notch-based and cell division components. *Biochem. Biophys. Res. Commun.* **359**, 491-496.
**Lim, C. and Allada, R.** (2013). Emerging roles for post-transcriptional regulation in circadian clocks. *Nat. Neurosci.* **16**, 1544-1550.
**Lin, K. K., Kumar, V., Geyfman, M., Chudova, D., Ihler, A. T., Smyth, P., Paus, R., Takahashi, J. S. and Andersen, B.** (2009). Circadian clock genes contribute to the regulation of hair follicle cycling. *PLoS Genet.* **5**, e1000573.
**Liston, C., Cichon, J. M., Jeanneteau, F., Jia, Z., Chao, M. V. and Gan, W.-B.** (2013). Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nat. Neurosci.* **16**, 698-705.
**Luo, Y., Wang, F., Chen, L.-A., Chen, X.-W., Chen, Z.-J., Liu, P.-F., Li, F.-F., Li, C.-Y. and Liang, W.** (2012). Deregulated expression of cry1 and cry2 in human gliomas. *Asian Pac. J. Cancer Prev.* **13**, 5725-5728.
**Marcheva, B., Ramsey, K. M., Buhr, E. D., Kobayashi, Y., Su, H., Ko, C. H., Ivanova, G., Omura, C., Mo, S., Vitaterna, M. H. et al.** (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* **466**, 627-631.
**Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F. and Okamura, H.** (2003). Control mechanism of the circadian clock for timing of cell division in vivo. *Science* **302**, 255-259.
**Méndez-Ferrer, S., Lucas, D., Battista, M. and Frenette, P. S.** (2008). Haematopoietic stem cell release is regulated by circadian oscillations. *Nature* **452**, 442-447.
**Méndez-Ferrer, S., Chow, A., Merad, M. and Frenette, P. S.** (2009). Circadian rhythms influence hematopoietic stem cells. *Curr. Opin. Hematol.* **16**, 235-242.
**Morf, J. and Schibler, U.** (2013). Body temperature cycles: gatekeepers of circadian clocks. *Cell Cycle* **12**, 539-540.
**Mukherji, A., Kobiita, A., Ye, T. and Chambon, P.** (2013). Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell* **153**, 812-827.
**Nagel, D. H. and Kay, S. A.** (2012). Complexity in the wiring and regulation of plant circadian networks. *Curr. Biol.* **22**, R648-R657.
**Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naef, F. and Schibler, U.** (2004). Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell* **119**, 693-705.
**Nguyen, K. D., Fentress, S. J., Qiu, Y., Yun, K., Cox, J. S. and Chawla, A.** (2013). Circadian gene Bmal1 regulates diurnal oscillations of Ly6C$^{hi}$ inflammatory monocytes. *Science* **341**, 1483-1488.
**O'Neill, J. S. and Reddy, A. B.** (2011). Circadian clocks in human red blood cells. *Nature* **469**, 498-503.
**O'Neill, J. S., Maywood, E. S., Chesham, J. E., Takahashi, J. S. and Hastings, M. H.** (2008). cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science* **320**, 949-953.
**Patton, D. F. and Mistlberger, R. E.** (2013). Circadian adaptations to meal timing: neuroendocrine mechanisms. *Front. Neurosci.* **7**, 185.
**Paulose, J. K., Rucker, E. B., III and Cassone, V. M.** (2012). Toward the beginning of time: circadian rhythms in metabolism precede rhythms in clock gene expression in mouse embryonic stem cells. *PLoS ONE* **7**, e49555.
**Peek, C. B., Affinati, A. H., Ramsey, K. M., Kuo, H.-Y., Yu, W., Sena, L. A., Ilkayeva, O., Marcheva, B., Kobayashi, Y., Omura, C. et al.** (2013). Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. *Science* **342**, 1243417.
**Plikus, M. V., Vollmers, C., de la Cruz, D., Chaix, A., Ramos, R., Panda, S. and Chuong, C.-M.** (2013). Local circadian clock gates cell cycle progression of transient amplifying cells during regenerative hair cycling. *Proc. Natl. Acad. Sci. USA* **110**, E2106-E2115.
**Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U. and Schibler, U.** (2002). The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* **110**, 251-260.
**Reischl, S. and Kramer, A.** (2011). Kinases and phosphatases in the mammalian circadian clock. *FEBS Lett.* **585**, 1393-1399.
**Robles, M. S., Cox, J. and Mann, M.** (2014). In-vivo quantitative proteomics reveals a key contribution of post-transcriptional mechanisms to the circadian regulation of liver metabolism. *PLoS Genet.* **10**, e1004047.
**Sahar, S. and Sassone-Corsi, P.** (2013). The epigenetic language of circadian clocks. *Handb. Exp. Pharmacol.* **217**, 29-44.
**Saini, C., Suter, D. M., Liani, A., Gos, P. and Schibler, U.** (2011). The mammalian circadian timing system: synchronization of peripheral clocks. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 39-47.
**Sancar, A., Lindsey-Boltz, L. A., Kang, T.-H., Reardon, J. T., Lee, J. H. and Ozturk, N.** (2010). Circadian clock control of the cellular response to DNA damage. *FEBS Lett.* **584**, 2618-2625.
**Saxena, M. T., Aton, S. J., Hildebolt, C., Prior, J. L., Abraham, U., Piwnica-Worms, D. and Herzog, E. D.** (2007). Bioluminescence imaging of period1 gene expression in utero. *Mol. Imaging* **6**, 68-72.
**Scheiermann, C., Kunisaki, Y. and Frenette, P. S.** (2013). Circadian control of the immune system. *Nat. Rev. Immunol.* **13**, 190-198.
**Schmidt, C., Collette, F., Cajochen, C. and Peigneux, P.** (2007). A time to think: circadian rhythms in human cognition. *Cogn. Neuropsychol.* **24**, 755-789.
**Sladek, M., Jindrakova, Z., Bendova, Z. and Sumova, A.** (2007). Postnatal ontogenesis of the circadian clock within the rat liver. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R1224-R1229.
**Thines, B. and Harmon, F. G.** (2011). Four easy pieces: mechanisms underlying circadian regulation of growth and development. *Curr. Opin. Plant Biol.* **14**, 31-37.
**Unsal-Kacmaz, K., Mullen, T. E., Kaufmann, W. K. and Sancar, A.** (2005). Coupling of human circadian and cell cycles by the timeless protein. *Mol. Cell. Biol.* **25**, 3109-3116.
**Viswanathan, N., Weaver, D. R., Reppert, S. M. and Davis, F. C.** (1994). Entrainment of the fetal hamster circadian pacemaker by prenatal injections of the dopamine agonist SKF 38393. *J. Neurosci.* **14**, 5393-5398.
**Wang, X., Reece, S. P., Van Scott, M. R. and Brown, J. M.** (2011). A circadian clock in murine bone marrow-derived mast cells modulates IgE-dependent activation in vitro. *Brain Behav. Immun.* **25**, 127-134.
**Yagita, K., Horie, K., Koinuma, S., Nakamura, W., Yamanaka, I., Urasaki, A., Shigeyoshi, Y., Kawakami, K., Shimada, S., Takeda, J. et al.** (2010). Development of the circadian oscillator during differentiation of mouse embryonic stem cells in vitro. *Proc. Natl. Acad. Sci. USA* **107**, 3846-3851.
**Yang, X., Wood, P. A., Ansell, C. and Hrushesky, W. J. M.** (2009). Circadian time-dependent tumor suppressor function of period genes. *Integr. Cancer Ther.* **8**, 309-316.
**Yang, X., Wood, P. A. and Hrushesky, W. J. M.** (2010). Mammalian TIMELESS is required for ATM-dependent CHK2 activation and G2/M checkpoint control. *J. Biol. Chem.* **285**, 3030-3034.
**Yeom, M., Pendergast, J. S., Ohmiya, Y. and Yamazaki, S.** (2010). Circadian-independent cell mitosis in immortalized fibroblasts. *Proc. Natl. Acad. Sci. USA* **107**, 9665-9670.
**Yu, X., Rollins, D., Ruhn, K. A., Stubblefield, J. J., Green, C. B., Kashiwada, M., Rothman, P. B., Takahashi, J. S. and Hooper, L. V.** (2013). TH17 cell differentiation is regulated by the circadian clock. *Science* **342**, 727-730.
**Zhao, N., Yang, K., Yang, G., Chen, D., Tang, H., Zhao, D. and Zhao, C.** (2013). Aberrant expression of clock gene period1 and its correlations with the growth, proliferation and metastasis of buccal squamous cell carcinoma. *PLoS ONE* **8**, e55894.
**Zheng, L., Seon, Y. J., Mourão, M. A., Schnell, S., Kim, D., Harada, H., Papagerakis, S. and Papagerakis, P.** (2013). Circadian rhythms regulate amelogenesis. *Bone* **55**, 158-165.