

In: *Solar Radiation and Human Health*  
Espen Bjertness, editor.  
Oslo: The Norwegian Academy of Science and Letters, 2008.

## **Cellular clocks and light effects on man**

**Ole Didrik Laerum, Oleg Tsinkalovsky, Tien-Sheng Huang**

*The Gade Institute, University of Bergen, Bergen, Norway*

Correspondence: The Gade Institute, Section of Pathology, University of Bergen, Haukeland  
University Hospital, N-5021 Bergen, Norway  
E-mail: ole.laerum@gades.uib.no Telephone +47 55972572 Fax: 47 55973158

### **Abstract**

In principle, there are two types of time regulation in humans as well as in other species: *Cyclic time* when the same phenomena occur with regular intervals, and *linear time*, when alterations occur along a straight line. Examples of the former are circadian and seasonal variations, while the latter time functions include periods of gestation, infancy, growth and adulthood, followed by senescence and death. For the former type of variations, a series of clock genes are operative, giving alternating stimulatory and inhibitory signals to cells.

The interplay between external light through a master clock in the brain and cyclic changes in the body due to multiple peripheral cellular clocks in the organs has recently been elucidated. As example of a peripheral clock we have recently shown that the expression of clock genes in both mouse and human hematopoietic stem cells undergoes significant circadian variations. Both species and cell type characteristic variations are present, with evidence of local as well as systemic regulation. Different patterns between nocturnal mice and diurnal humans are conformal with light induced systemic regulation. Clock functions in cells and their importance as transcription factors are now being explored in cell culture. Human mesenchymal stem cells lose their circadian variations of clock gene expression after some days in culture, but these can be reinduced with external cues as serum shock or cyclic AMP analogs and followed for several cycles. Therefore, these clocks seem to be dependent on continuous exposure to external cues.

## Introduction

Practically all functions in the body vary with time, with periodicities ranging from seconds to day and night, month and season. For a number of years it has been known that circadian rhythms are governed by cellular clocks through special clock genes. They are coding for proteins that mediate positive (*Clock*, *Bmal1*) and negative (*Per1-3*, *Cry1 and 2*, *Rev-erb  $\alpha$* ) regulatory signals in cells. It has been estimated that so-called clock controlled genes influence the transcription of about 10 percent of the genome in mammalian cells. Molecular clocks are active in most tissues and contribute to adjustment to the outer environment. At the same time it seems that such clock functions can both be systemic and local. Since time regulation is universal, both for coordinating cellular processes locally and for coordination of cells within a tissue as well homeostatic control in the whole body, the need for universal clock functions is obvious. Until recently, however, research on biological rhythms has mainly been descriptive, aiming at elucidating how and when different cell functions varied in time. Only during the last decade, a deeper understanding of cellular time regulation has been achieved. This adds to general knowledge about homeostatic mechanisms and how environmental light induces time variations through the eye and the nucleus suprachiasmaticus (For general review, see 1).

In this chapter, some recently elucidated aspects of local time regulation in human cells will be presented and with particular emphasis on haematopoietic tissues and stem cells. The reason for pursuing this type of research is twofold. Firstly, since stem cells are of critical importance for regeneration and for maintaining equilibrium in any tissue, their variations will be quantitatively more important than those of functional end cells. Second, the bone marrow is a rapidly dividing tissue with a high cell turnover, which is known to be subject to strong circadian variations. At the same time this organ is not exposed to environmental light. Therefore, only secondary regulatory mechanisms related to time and light biology will be applying.

### Some basic mechanisms

Basically, the mammalian cellular clock, including the human one, consists of a complex feedback mechanism, consisting of positive and negative regulators. This is achieved through an autoregulatory transcriptional / translational program occurring in a cyclic manner. The genes *Per1-3* and *Cry1 and 2* code for inhibitory proteins, while *Clock* and *Bmal 1* code for proteins that form a heterodimer enhancing transcription. The pattern is further modified by *Rev-erb  $\alpha$* , acting inhibitory on this sequence. Thereby an efficient switch mechanism for inhibitory and stimulatory signals is given, not only as feedback to the clock

genes, but also to other genes downstream of their action (1-3). This mechanism was first elucidated in the master clock in the brain, the nucleus suprachiasmaticus, which coordinates circadian timing in mammals (3). This pattern developed relatively early in evolution and is similar in a wide variety of organisms from plants to humans (4).

In the beginning, the main focus was on the results from studies of the central nervous system and the master clock. However, it was soon realized, that circadian oscillators were also operating in peripheral tissues (see e.g. 5,6). Surprisingly, the circadian oscillation of global gene expression was found to be highly variable between different tissues, e.g. between the liver and the heart (7). In addition, the different clock genes seem to be alternating differently in various peripheral tissues, and also with differences between species (1; see also later). The production of mutant mice for different clock genes has revealed that the circadian pattern can become obliterated, and also that there is a certain redundancy in the clock work (see e.g. 8). Thus, it was recently reported that the circadian variations are not obliterated in mice with a clock 0 mutation (9). Circadian expression of clock genes in human peripheral tissues has been observed both in the oral mucosa and the skin (10), as well as in hematopoietic stem and precursor cells (11).

Cellular clocks seem to be of particular importance for regulation of cell cycling and timing of cell division *in vivo*, linking their actions to circadian variations of cell division which have earlier been observed in most tissues (see e.g. 12).

### **Human light perception and the master clock**

For many years, only the visual functions of the eyes through rods and cones were known and characterized. However, there was increasing evidence for the existence of other types of photoreceptors in the retina, since light perception through the eyes also strongly influenced the circadian system. Later it has been shown that circadian, neuroendocrine and neurobehavioural responses in rodent, primates and humans are mediated through the eyes in the spectrum 446 to 484 nm, which is predominantly the blue part of the spectrum. This newly discovered photosensory system is composed of a small population of widely dispersed retinal ganglion cells that are intrinsically responsive to light. They have connections to the suprachiasmatic nucleus and other non-visual centers in the brain. The light sensitive retinal ganglion cells contain melanopsin, a vitamin A photopigment that mediates the whole phototransduction cascade in the cells. In contrast, the conventional photoreceptors rods and cones were not required for circadian photoreception (13-15). There is so far no indication that cells in peripheral tissues of man respond directly to light, neither *in vivo* nor *in vitro*. On the other hand, it is known that several external factors may act more or less

indirectly as circadian cues, such as the timing of meals, certain chemicals and changes in the environmental temperature (1).

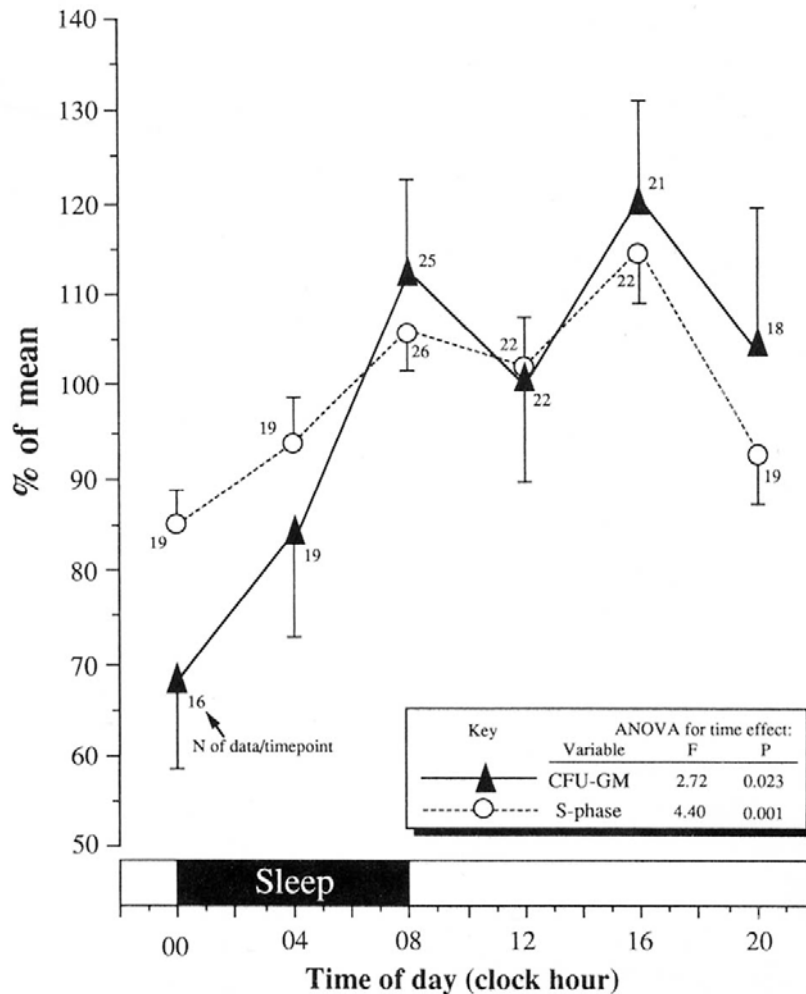
The identification of both the regulatory circuit of cyclic timing in man and other mammals and the differences between the master clock and the peripheral clocks have given a deeper understanding of the physiological regulation of multiple functions in the body (16). In addition, this has led to a molecular understanding of human circadian rhythm disorders, which may be due to inheritable conditions affecting different parts of the central regulation of biological rhythms (17). These include conditions as seasonal affective disorders, non 24-h sleep-wake syndrome, delayed sleep phase syndrome, advanced sleep phase syndrome as well as morningness-eveningness preference within the normal range (17).

### **Hematopoiesis and stem cells**

Rapidly proliferating tissues with a high cell turnover usually exhibit strong cyclic variations in their proliferative activity. Thus, cell cycling is coupled to both the circadian and seasonal timing of activity in tissues (see 1,12). For example, it has long been known that renewal of cells in actively proliferating tissues as the mucosal lining of the gastrointestinal tract, skin and hemopoiesis, occurs in rhythms (see 18). In hematopoiesis the cyclic variations both apply to the different types of peripheral blood cells (see e.g. 19), as well as in the bone marrow itself (20). These rhythms have been attributed to similar cyclic variations in various humoral regulators, and particular the cortisol rhythm. Thus, the circadian variations in serum cortisol and in circulating granulocytes are closely coordinated. In addition, both functions are markers for circadian variations of bone marrow proliferation (21). There are also circadian variations in the expression of various surface molecules leukocytes. For example, in rat blood lymphocytes several surface molecules change with day and night, including CD45 and CD5, indicating that the behaviour of the peripheral circulating cells may be varying according to an internal peripheral clock, as well (22).

Multipotent stem cells are of critical importance in maintenance of equilibrium in the hematopoietic system. In mammals one single stem cell can have a progeny of one million more or less differentiated daughter cells within a week. Thus, minor variations in stem cell renewal and differentiation may play an important quantitative role. It has earlier been shown that in human bone marrow, there is a close correlation between circadian variations of DNA synthesis in the total bone marrow population and clonability of progenitor cells in vitro (23). Recently, we have shown that the various clock genes are expressed in hematopoietic stem cells (11). In mouse and human bone marrow

the expression level is cyclic with strong circadian variations (24,25). This occurs as a sequential process with alternation between the different types of clock genes.



**Figure 1.** Circadian co-variation of DNA synthesis and clonogenicity by granulocyte-macrophage-forming progenitor cells (CFU-GM) in bone marrow in 16 clinically healthy men. (From Smaaland et al; 23).

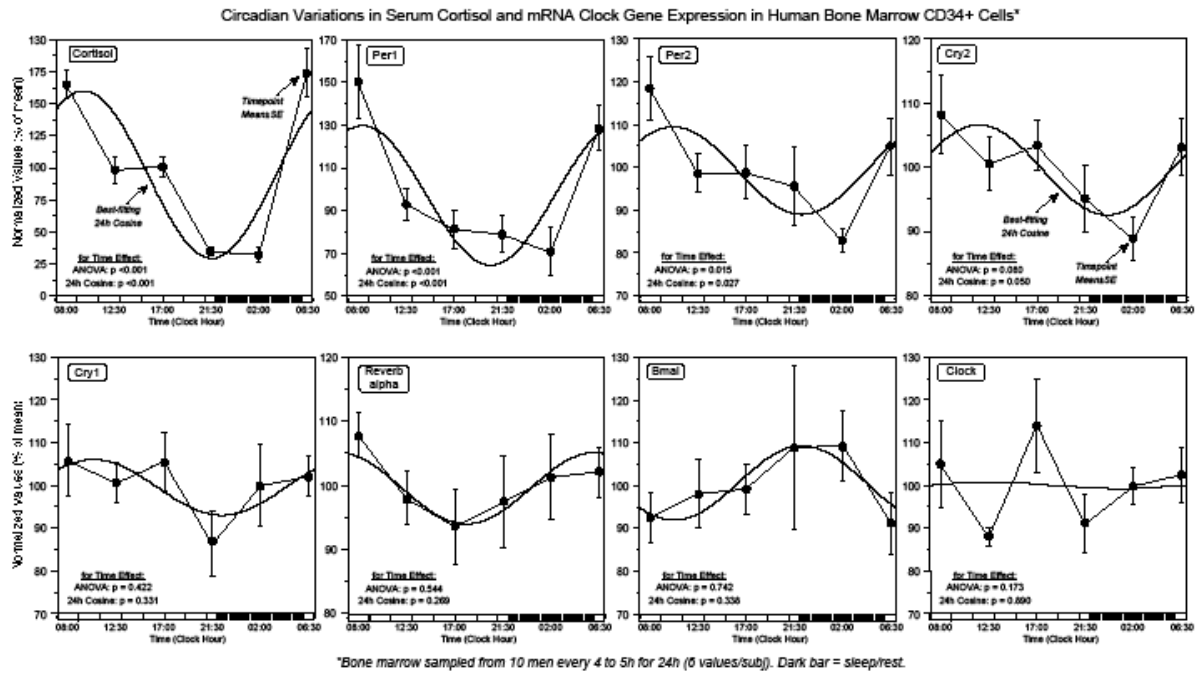
While significant variations of Per-1, Per-2 and Cry-2 were seen which closely followed the cortisol rhythm in peripheral blood, Cry-1 and Bmal-1 did not vary significantly. Contrary to precursor cells, most of the clock genes were not oscillating in a fully organized circadian manner in primitive mouse hematopoietic stem cells. This indicates that the circadian variations of murine clock gene expression in hematopoiesis are developmentally regulated, since significant variations were found both in whole bone marrow and in the liver, both representing mainly differentiating cells (24). This further emphasizes that peripheral clocks may be differently organized according to the cell type. On the

other hand, the close coordination between systemic cortisol levels and stem cell clock genes expression in humans indicates a significant systemic regulation mediated through the retina and the central master clock.

Specialised accessory mesenchymal cells are important for maintenance of haematopoiesis locally in the bone marrow, both through cellular and humoral factors in the microenvironment. Such accessory cells as well as adipose cells are also derived from more primitive stem cells, which can be obtained by aspiration from the bone marrow in man. They can be cultured for prolonged periods as primary cells, but once established *in vitro*, the cells do not exhibit circadian clock gene variations. However, these can be reinduced by various stimuli, such as serum shock and various cyclic AMP analogs (Huang, T-s et al in preparation). Upon such stimulation, alternations between stimulatory and inhibitory clock genes can be followed over several circadian cycles. Similar to clock functions in several other peripheral tissues, mesenchymal stem cells seem to be dependent on central or external cues for maintenance. The finding further points in direction of the master clock as regulator of peripheral clock in human haematopoiesis. The recent observation that bone marrow engraftability by transplantation to irradiated recipient mice was subject to significant circadian variations further supports this concept (26). If applicable to man, this may also be used for optimisation of bone marrow transplantation.

### **Human circadian and seasonal variations and disease**

The understanding of cellular clocks in the human CNS as well as in peripheral tissues may have clinical implications, for example by timing of medical treatment to periods where optimum effect and less side effects may be achieved (see e.g. 27,28). It is known that the occurrence of many different diseases in man may show circadian as well as seasonal variations. This includes infections, vascular diseases, the occurrence of genetic and malignant diseases, as well as the death rate in general (see e.g. 1). Such patterns may be due to the lighting conditions, where for example the occurrence of seasonal affective disorders show some variations with season as well as with latitude (28). Interestingly, chronic disturbance of the circadian rhythms may be associated with tumour progression in mice (29, 30). It has also been found that malignant lymphomas lack circadian variations in DNA synthesis (31). In addition, it has been reported that the expression of Per 1-3 genes is deregulated in breast cancers (32). Thus, the circadian clock pacemaker may act as a tumour suppressor, which upon perturbation facilitates malignant progression (33). Such findings provide further evidence for the fundamental importance of time keeping functions in normal human peripheral tissues.



**Figure 2.** Circadian variations of mRNA clock gene expression in human bone marrow stem cells (CD34+ Cells) as compared to the serum cortisol level. (From Tsinkalovsky et al; 25).

## Concluding remarks

New light has in the recent years been shed on fundamental mechanisms underlying photoperiodism in mammals, including man. In particular, this applies to the circadian system, where a large body of evidence is available on genetic regulation of central as well as peripheral clocks, including their coordination. To some extent data on cyclic time keeping have already had clinical consequences. Foremost, such knowledge is important for a deeper understanding of homeostatic mechanisms. As has already been indicated elsewhere (34), we may be moving towards a molecular biological calendar in humans, leading to improved conditions for maintenance of health and prevention of different diseases. While old farm life had a balance between cycling and linear time, and people were more exposed to natural changes in lighting conditions, our present electronic society implies exposure to a multitude of different artificial cues. A deeper understanding of light and time regulation in man might give the opportunity to reduce noxious influences perturbing the normal time keeping.

## References

1. Koukkari WL, Sothorn RB. *Introducing Biological Rhythms 2006*. Springer Science /Business Media, Inc. New York, 2006: 1-655.
2. Lowrey PL, Takahashi JS. Genetics of the mammalian circadian system: Photic entrainment, circadian pacemaker mechanisms, and post-translational regulation. *Annu Rev Genet* 2000; **34**: 533-562.
3. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature* 2002; **418**: 935-941.
4. Roenneberg T, Mrosovsky M. "What watch?... such much!" Complexity and evolution of circadian clocks. *Cell Tissue Res* 2002; **309**: 3-9.
5. Balsalobre A. Clock genes in mammalian peripheral tissues. *Cell Tissue Res* 2002; **309**: 193-199.
6. Fukuhara C, Tosini G. Peripheral circadian oscillators and their rhythmic regulation. *Front Biosci* 2003; **8**: d642-651.
7. Storch K-F, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. Extensive and divergent circadian gene expression in liver and heart. *Nature* 2002; **417**: 78-83.
8. Oster H, van der Horst TJ, Albrecht U. Daily variation of clock output gene activation in behaviorally arrhythmic mPer/mCry triple mutant mice. *Chronobiol Int* 2003; **20** (4): 683-695.
9. Collins B, Blau J. Keeping time without a clock. *Neuron* 2006; **50**: 348-350.
10. Bjarnason GA, Jordan RCK, Wood PA, Lincoln DW, Sothorn RB, Hrushesky WJM, Ben-David Y. Circadian expression of clock genes in human oral mucosa and skin. *Am J Pathol* 2001; **158** (5): 1793-1801.
11. Tsinkalovsky O, Rosenlund B, Laerum OD, Eiken HG. Clock gene expression in purified mouse hematopoietic stem cells. *Exp Hematol* 2005; **33**: 100-107.
12. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. *Science* 2003; **1126**: 1-10.
13. Beaulé C, Robinson B, Lamont EW, Amir S. Melanopsin in the circadian timing system. *J Mol Neurosci* 2003; **21** (1): 73-89.
14. Brainard GC, Hanifin JP. Photons, clocks and consciousness. *J Biol Rhythms* 2005; **20**(4): 314-325.
15. Hanifin JP, Brainard GC. Photoreception for circadian, neuroendocrine, and neurobehavioral regulation. *J Physiol Anthropol* 2007; **26** (2): 87-94.
16. Refinetti R. *Circadian physiology*. 2<sup>nd</sup>. Ed. CRC Press. Boca Raton, FL, 2005.
17. Cermakain N, Boivin DB. A molecular perspective of human circadian rhythm disorders. *Brain Res Rev* 2003; **42**: 204-220.
18. Laerum OD. Hematopoiesis occurs in rhythms. *Exp Hematol* 1995; **23**: 1145-1147.

19. Smaaland R, Sothorn RB, Laerum OD, Abrahamsen JF. Rhythms in human bone marrow and blood cells. *Chronobiol Int* 2002; **19**: 101-127.
20. Haus E, Smolensky, M. Biologic rhythms in the immune system. *Chronobiol Int* 1999; 16(5): 581-622.
21. Abrahamsen JF, Smaaland R, Sandberg S, Aadvaag A, Lote K. Circadian variation in serum cortisol and circulating neutrophils are markers for circadian variation of bone marrow proliferation in cancer patients. *Eur J Haematol* 1993; **50**: 206-212.
22. Pelegri C, Vilaplana J, Castellote C, Rabanal M, Franch A, Castell M. Circadian rhythms in surface molecules of rat blood lymphocytes. *Am J Physiol Cell Physiol* 2003; **284**: C67-C76.
23. Smaaland R, Laerum OD, Sothorn RB, Sletvold O, Bjerknes R, Lote K. Colony-forming unit-granulocyte-macrophage and DNA synthesis of human bone marrow are circadian stage-dependent and show covariation. *Blood* 1992; **79** (9): 2281-2287.
24. Tsinkalovsky O, Filipski E, Rosenlund B, Sothorn RB, Eiken HG, Wu MW, Claustrat B, Bayer J, Lévi F, Laerum OD. *Exp Hematol* 2006; **34**: 1248-1260.
25. Tsinkalovsky O, Smaaland R, Rosenlund B, Sothorn RB, Hirt A, Steine S, Badiie A, Abrahamsen JF, Eiken HG, Laerum OD. Circadian variations in clock gene expression of human bone marrow CD34+ cells. *J Biol Rhythms* 2007;22:140-150.
26. D'Hondt L, McAuliffe C, Daman J, Reilly J, Carlson J, Dooner M, Colvin G, Lambert J-F, Hsieh C-C, Habibian H, Stencel K, Quesenberry PJ. Circadian variations of bone marrow engraftability. *J Cell Physiol* 2004; **200**: 63-70
27. Bjarnason GA, Jordan R. Circadian variation of cell proliferation and cell cycle protein expression in man: clinical implications. *Prog Cell Cycle Res* 2000; **4**: 193-206.
28. Mersch PP, Middendorp HM, Bouhuys AL, Beersma DG, van den Hoofdakker RH. Seasonal affective disorder and latitude; a review of the literature. *J Affect Disord* 1999; **53** (1): 35-48.
29. Filipski E, King VM, Li XM, Granda RG, Mormont M-C, Liu XH, Claustrat B, Hastings MH. Host circadian clock as a control point in tumor progression. *J Nat Cancer Inst* 2002; **94**: 690-697.
30. Filipski E, Delaunay F, King VM, Wu M-W, Claustrat B, Gréchez-Cassiau A, Guettier C, Hastings MH, Francis L. Effects of chronic jet lag on tumor progression in mice. *Cancer Res* 2004; **64**: 7879-7884.
31. Smaaland R, Lote K, Sothorn RB, Laerum OD. DNA synthesis and ploidy in non-Hodgkin's lymphomas demonstrate inpatient variation depending on circadian stage of cell sampling. *Cancer Res* 1993; **53**: 3129-3138.
32. Chen S-T, Choo K-B, Hoou M-F, Yeh K-T, Kuo S-J, Chang J-G. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. *Carcinogenesis* 2005; **26** (7): 1241-1246.

33. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. *Nature Rev* 2003; **3**: 350-361.
34. Hastings MH, Follett BK. Toward a molecular biological calendar? *J Biol Rhythms* 2001; **16** (4): 424-430.