Mini Review

Genetic clock of biologic rhythms

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Abstract

Biologic rhythms are tightly regulated in all species, in order to adapt to circadian variations of light, temperature and other biological related living conditions. The intrinsic mechanisms of rhythm regulation from every cell to a whole organism is embedded into the genetic clockwork. The mammalian clock genes are reviewed with their interrelation. The per gene promoter is stimulated by clock/bmal1 heterodimer and trigger the per proteins synthesis. Initially considered as a simple negative feed-back, from the phosphorylated per/cry heterodimer towards clock/bmal1 transcription stimulus, the clockwork of the cell became complex. General characteristics of cell clock genes are integrated in a model. In addition, the involvement of clock genes in peripheral tissues is discussed.

Keywords: clock genes • circadian • rhythm • oscillator

Introduction

Biologic clocks reflect the four temporal cycles prevailing life on Earth: tides, day-night, month and year, regulate their temporal programs. Since life on Earth depends of Sunlight, all the organisms use Sunlight to survive and adjust the biological circadian rhythms.

The mechanisms inducing circadian rhythms are regulated at cellular and molecular level. Not only

environmental variations, but also endogenous changes in energy supplies related to time, influence the biologic rhythms. Like unicellular organisms, specific cells of complex organisms have the capacity of rhythm generation (pacemaker) and include the elements of an oscillatory system (entrance-generator-exit): receptors, the transmission pathway of intracellular signal, the mechanism generating the rhythm and expression of rhythmic activity (e.g. neuronal potentials) (Fig. 1).

The cellular clocks are entrained by a micro-medium temporally organized, using transmitters, hormones and other different chemical signals. Some cells (e.g. retinal and pineal cells) respond

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Fig. 1 An oscillator is regulated by input synchronizing signals (zeitgeber) and provides data for synchronizing other elements of the system (output). Such an oscillator is in the suprachiasmatic nucleus.

directly to exogenous synchronizing stimuli, named **zeitgeber**, like light. The rhythms can be generated by environmental changes or can be driven by an endogenous generator, hardly modified by external disturbances. Endogenous rhythms present the following features [1]:

1. the rhythm is realized through feedback;
2. the oscillatory period is around 24 h (circadian);
3. the amplitude is strong enough to entrain exogenous rhythms;
4. there is a temperature compensation;
5. the oscillatory pattern can be modified by external entraining.

The discovery of mutant genes and consequently of distortional circadian properties provided improvement in understanding the genetic adjustment of rhythms.

Clock genes identification

The unicellular algae *Chlamydomonas*, the fungus *Neurospora* and the fly *Drosophila*, usually utilized for genetic studies, have provided also material for clock genes identification. At the beginning, the period gene (*per*) at *Drosophila* [2], and the frequency gene (*frq*) at *Neurospora* [3] were cloned. Screening of stable mutations affecting the circadian rhythm for the genes *per* and *frq* followed the experiments for determination of mutant circadian genes. Not only eukaryotes, but also prokaryotes (eg. *Cyanobacteria*), coordinate their rhythms driven by an endogenous temporal program. *Cyanobacteria* offer many advantages for the study of clock genes: generation life time is short, with a huge number of individuals available for mutagen-

esis and screening. Recordings of circadian rhythm is improved with luciferase as reporter gene, when the bacterial luciferase was fused with a promoter controlled by the clock. The chimeric gene emits light, such structures being utilized in *Drosophila*. Automatization facilitates the screening of thousands of individuals, identifying mutants with low amplitude, altered periods or bimodal structure of rhythm. In *Drosophila*, six other clock genes were identified, named clock (*clk*) [4], cycle (*cyc*) [5], timeless (*tim*) [6], doubletime (*dbt*) [7], cryptochrome (*cry*) [8], and vril (*vri*) [9].

The first clock-related gene (*tau*) in mammals with a shorter period than normal, was discovered by chance, in a mutant hamster [10]. In the homozygous form, the periodicity is 20 hours and in the heterozygous form is 22, comparative with 24 in normal. This was proved to encode casein kinase 1 epsilon, (CKIε) involved in PER protein turnover. Systematic studies of mutagenesis which prolongs the period of circadian rhythm [11], have disclosed the mammalian clock genes in mice. The conservation of clock mechanisms from insects to mammals allowed the identification of mammalian orthologs to *Drosophila* clock genes, as follows: 3 mammalian Period genes, (*mPer1*, *mPer2*, *mPer3*), [12] *Clock* [13], *Brain and muscle RNA t-like protein* (*Bmal1*, ortholog to *cyc*) [14], two Cryptochrome genes (*mCry1* and *mCry2*) [15], *CKIε*, ortholog to *dbt* [16] and *Rev-erba* [17]. After clock gene has been cloned in mice, the challenge has started for finding the partners of CLOCK protein. Since CLOCK could be a regulator of transcription only as a heterodimer, a cofactor has been necessary, which was found to be *Bmal1* [18]. The heterodimer CLOCK/BMAL1 is the activator factor of *per* gene transcription.

In just few years, the missing elements of molecular clock have been discovered, a number of homologues have been described, lightening the fact that molecular mechanisms are preserved from insects to mammals.

Circadian expression of these genes was first investigated in the suprachiasmatic nucleus (SCN), by immunohistochemistry, *in situ* hybridization or receptor autoradiography, in wild type or transgenic animals. Circadian variation of bioluminescence in *mPer1* - luc transgenic mice were recorded *in vivo* from SCN via a fiber-optic system [19]. From a simple feed-back regulation, the cellular clock

gradually became highly regulated but also sophisticated in synchronism of phases for mRNA, protein products, or their complexes. Large scale screening of genes using DNA microarrays allowed observation of numerous genes with circadian oscillation.

Building-up of an elementary cellular clock

Demonstration of molecular generation of circadian rhythm was based on five criteria elaborated over 20 years ago and slightly changed since (quoted by [20]).

1. Mutations of a clock constituent affect the rhythmic properties and gene deletion abolishes normal rhythmicity.
2. Quantity (activity) of the constituent has to oscillate in a self sustained manner, with a proper periodicity.
3. Quantity or activity of a clock constituent are feed back regulated.
4. Oscillatory phase of a constituent has to be reset by shifting light/dark cycle and the stable rhythm will be reset by changing the activity of a constituent.
5. Preventing the oscillation of a constituent causes the lost of a stable rhythm.

These criteria are the features of a circadian oscillator, which is involved in feed back mechanisms: e.g., a molecule, periodically synthesized, synchronic with circadian signals, affects its own oscillation through feed-back and responds at synchronizing signals named “zeitgeber”. A mechanism that generates rhythm has to possess a feed back negative loop [21]. These criteria are accomplished for *per* and *cry* genes. The gene (*per* or *cry*) produces a protein that inhibits transcription of its own mRNA; the protein synthesis starts again when mRNA has been removed from cell and the self-inhibition has disappeared.

Molecular models of genetic clock have been established, the criteria for *per* and *cry* genes have been accomplished, but the intimate details of functioning of this system remained to be understood. Which processes are responsible for maintenance of this long rhythm of 24 hours? How much does *de novo* synthesis of mRNA matter and how much does mRNA and proteins turnover mean for the rhythm

generation? Which are the mechanisms of activation and inhibition? How is this loop affected by the “zeitgeber” signals?

The two genes participate in a negative feed back loop, the protein products inhibit their own transcription [22, 23]. Once synthesized, PER1 and 2 are phosphorylated by CKI ϵ and interact with corresponding cryptochromes CRY1/2. These have circadian expression in retina and suprachiasmatic nucleus. The expression of mPer 1 and mPer 2 is higher in the morning. Light induces resetting of rhythm generated by rapid induction of mPer1. The mPer2 rhythm is shifted with 4 hours comparative to mPer1. Degradation of protein products PER and CRY allows restart of the cycle. The promoter regions of *per* and *cry* genes are activated by heterodimers of Clock and Bmal1 proteins. The PER/CRY complexes obtained in the cytoplasm are transported back into the nucleus and inhibit expression of *Clock* and *Bmal1* genes. The two subtypes of protein complexes are required for a two different light - sensitive oscillators: a morning oscillator PER1/CRY1 and an evening oscillator PER2/CRY2 [24]. In addition, new regulators of the central double heterodimers feedback loop appeared. An orphan nuclear receptor, Rev-Erba, inhibits Bmal1 synthesis [25], while its synthesis is stimulated by Bmal1 itself. This allows the midnight peak of Bmal1 in mammalian SCN.

Subtle changes induce a shifting phase of the stable rhythm and also, the synchronizers (zeitgebers) can induce alterations of oscillatory phases of clock genes, while constitutional expression abolishes the rhythm.

Actual models of molecular circadian oscillator

The actual model of animal circadian oscillatory is based on two pairs of heterodimers (Fig. 2). CLOCK/BMAL1 represents the transcription activator of gene promoter for *per* and *cry*. The PER1 and PER2 proteins are phosphorylated in the cytoplasm by CKI ϵ , influencing their stability and CRY binding properties. PER and CRY form a heterodimer that is translocated into nucleus and inhibits transcription of own genes, interacting with CLOCK/BMAL1. Recently, it was shown how the

Fig. 2 Clock genes and their regulation in the suprachiasmatic nucleus. The signal is transmitted from glutamergic fibers via NMDR1 to the neurons from the core SCN. The light input stimulates transcription of *per* and *cry* genes by binding of Clock/Bmal1 heterodimers in the promoter region (E-box) of *per* and *cry* genes. Synthesised into the cytoplasm, *per* is then phosphorylated by CKI ϵ . In this state, it binds to Clock/Bmal1 complexes and inhibits the further activation of *per/cry*. The *Bmal1* gene transcription is rhythmic, under the influence of orphan nuclear receptor Rev-Erb α , while *Clock* transcription is constitutive constant.

rhythm coming from the suprachiasmatic nucleus in mammals is controlled by the afore mentioned loop of negative feed back [26]. It results that the function of clock genes is to control the transcription of other genes, generic denominated clock controlled genes (CCG). As example, the CLOCK/BMAL1 complex activates also the vasopressin gene, which is controlled similar to the clock constituents. An important feature of circadian regulation of vasopressin is that the promoter isn't integrated in negative feed back, so other effects of the main mechanism generate the rhythmicity. Considering the

vasopressin model, experiments can analyze the impact of circadian clock on different rhythms involved in phase shifting. Phase specificity can be achieved by other elements activated in different phases of the circadian rhythm. In mammals, distinct *per* genes (*mper1* and *mper2*), oscillating with 4 hours shift, could be involved. Another possibility is the existence of "second" oscillators, that can hinder the circadian pathway, determining a specific phase of the signal.

Although the molecular mechanisms of circadian loop in different species have the same elements,

there are some species to species differences. The peak of mRNA occurs at daytime in mammals and at night in *Drosophila*. In insects, the mRNA level of *cry* oscillates, but in mice it is constant. Similarly, CLOCK oscillates weekly in fly, but it is constant in mice. BMAL1 is rhythmic in the suprachiasmatic nucleus in rats, but it is constitutive at *Drosophila*. In conclusion, in mammals and insects, both activator and inhibitory heterodimers are rhythmic. The rhythmicity demands only one oscillating constituent. In mammals, BMAL1/CLOCK is rhythmic due to BMAL1 oscillation while in insects CLOCK is the rhythmic one. In mammals, PER/CRY is rhythmic, but only PER oscillates, while in insects both of them oscillate.

Synchronization: from Sun to cell clock

The generation of an endogenous circadian rhythm is one of the most important qualities of circadian systems, together with the synchronization of the 24 hours cycle to environment [27]. Recent experiments have studied the molecular mechanisms involved in synchronization to light.

In certain situations the difference between oscillator and entrance pathway of light signal is difficult to establish. The promoter of *per1* at mice has an oscillatory pattern with elements related to light, it is induced by light, like the immediate early genes *c-fos* and *jun*. The *per2* gene in mice is also induced by light, but with a slower kinetic [12]. The regulation of *per* promoter by light hasn't been revealed in *Drosophila*; in this case the induction by light is dependent on rapid degradation of TIM, that make PER unstable. In mammals, the rapid induction by light of *per1* gene is similar to induction of *frq* mRNA in *Neurospora* after light exposure [28].

The central oscillator is located in the SCN, which consists of two neuronal structures: the core SCN, immunoreactive for vasoactive intestinal polypeptide (VIP) and gastrin releasing peptide (GRP), and the shell, synthesizing AVP and somatostatin. Circadian time is driven by the daylight (via another photoreceptive pigment, melanopsin) through the retinal ganglion cells (RGCs) which projects glutamatergic and PACAP-immunoreactive fibers onto core SCN via the retinohypothala-

mic tract [29]. Synchronization photic signals from retina are entrained by light. Activation of glutamate receptors trigger Ca^{2+} influx and CREB / mitogen activating protein (MAP) kinase phosphorylation pathway, which starts *per* gene expression.

Clock genes in peripheral tissues

Many cellular functions are involved in circadian rhythmicity: transcription, translation, RNA degradation, energetic metabolism and elements which convert the synchronic "zeitgeber" signals to clock. Extensive studies focused on SCN clock genes expression, but it can be observed in most tissues and cells.

It has been demonstrated that in many peripheral tissues clock genes do exist and function in a manner dependent on the central hypothalamic clock. However, in special conditions, like restricted-feeding, they can disconnect from the central driven and become autonomous at least for a limited period of time [30]. The restriction of feeding induces the liver clock genes, independent of SCN [31]. PCR for clock transcripts revealed that all components of the clock are expressed in similar fashion in most of the cells outside the suprachiasmatic nucleus, including liver, muscle, kidney, heart and even mononuclear leukocytes [30, 32, 33]. Elegant experiments conducted in rats tissues, using the mouse *Per1:luc* transgene, proved spontaneous circadian rhythms of luciferase activity in multiple organs for 2-7 days [34]. In these tissues the genes oscillates in phase, but with a four hours delay compared with SCN [30]. Unlike the clock in the SCN, which has the intrinsic generating rhythmicity, peripheral oscillators lose their rhythm in an independent state, either because they lack the central synchronizing input, or they lose synchronicity between them. Several lines of evidence sustain the hypothesis that subordinated relationship between SCN and peripheral tissues is maintained mainly by humoral signals. Silver et al imagined an experiment with encapsulated SCN neurons in mice, which regain rhythm despite prevention of any synaptic contacts of the grafted neurons [35]. Furthermore, the functioning graft of SCN neurons in damaged nucleus resumed the rhythmic activity [36]. Many studies were designed to unravel these humoral timing ligands: glucocorticoids [37],

forskolin, calcimycin, retinoic acid, some growth factors, agonists of adenylate cyclase [38,39]. They use different intracellular pathways and is postulated that each of them could signal different external cues.

The external environment synchronizes the internal circadian activity mainly through photoperiod, but other mechanisms can reset the circadian gene expression in peripheral tissues by-passing the SCN. In mammals, the most studied is restricted feeding, which phase-shifts the peripheral clocks in different tissues (e.g. liver, lung), without synchronicity amongst them [31,40]. The intimate mechanism that directly resets the circadian gene expression in periphery during the restricted feeding is likely to be the metabolic activity, namely the modified ratios of NAD/NADH and NADP/NADPH, which act at the translational level [41].

Recent efforts were involved in determining the precise molecular mechanisms by which peripheral clocks control the circadian processes like digestion, sleep, diurnal variations of pulse and arterial blood pressure and possibly many others, yet unrecognized as circadian. The supposed image of the orchestrated activity of physiological processes is one of an conductor (SCN) which gives the tone (circadian rhythm coordinated with day-night period), but allows the soloists (peripheral clocks) to perform under precise circumstances their own music.

Liver

The local clock regulates the expression of many enzymes, involved in the metabolism of basic nutrients and in synthesis and detoxification of exogenous and endogenous products (steroid hormones, drugs, biliary acids). For example, the *Dbp* gene, that encodes a transcriptional factor in many tissues, including the liver, in a circadian manner, is controlled by CLOCK/BMAL 1 heterodimers through an E-box located on the *per* promoter [42]. In its turn, the DBP transcription factor controls the circadian expression of several enzymes in the liver [43]. A single clock-controlled gene, *Dbp*, drives the timing expression of Cyp 7a, implicated in bile acid production and Cyp 2a4, which catalyses the steroids metabolism. The circadian expression of the enzymes family glutathione S-transferase could explain the patterns of toxicity for many drugs, associated with a circadian rhythmicity.

Gastrointestinal tract

In relationship with circadian variation, the gut physiology is quite poorly studied and understood. However, recent experiments showed that nutrients ingested during the night are less rapidly metabolized and utilized, leading to increased availability for tissue deposition. Thus, postprandial levels of triacylglycerol, an important risk factor for cardiovascular disease, are higher after a meal taken at night, providing an additional explanation for the increased cardiovascular mortality in shift-working people.

Cancer

Knowing that cell proliferation occurs in a circadian manner opened new areas in cancer research, in order to improve the cytotoxic potency and, conversely, to diminish the untoward effects of chemotherapy. It has been demonstrated that pulsatile administration of cytotoxic drugs, chronomodulated, is more effective in metastatic colorectal carcinoma [44]. Even more, clock genes could have a pathogenic role in tumour initiation and progression. The proto-oncogene *c-myc* is a CCG which, in *mPer2* mutant mice, generates an attenuated expression of its controlled tumor suppressor gene, *Gadd45a*, one possible mechanism of increasing proliferation, as demonstrated by Fu and coworkers, [45]. The quantitative role of disturbed circadian rhythmicity on overall malignancy prevalence is under investigation, but at least in breast cancer it was certified by epidemiological studies that long-term shift work increases the risk by 10-60% [46].

Cardiovascular and cerebrovascular pathophysiology

It is well known that acute myocardial infarction and hemorrhagic stroke have a circadian pattern, with the peak occurring early in the morning. This suggests a direct pathogenic correlation with increasing blood pressure and cardiac output in this critical period. The local, cardiovascular clock genes, have been intensive studied. In normal rat heart a circadian rhythm of contractile function

exists in direct relationship with genes expression involved in carbohydrate and fatty acid metabolism and in mitochondrial function. Also, the vascular smooth muscle shows a circadian variation in contractility, partially dependent on circadian sympathetic tone, partially related to synchronous variation in renin-angiotensin system [47]. Vascular smooth muscle cells express circadian molecular oscillators *mper2*, *dbp* and *Bmal1*. Angiotensin II induces increase of amplitudes of circadian oscillation of *mPer2* followed by synchronous cycling of other elements, via AT1 receptors [48].

In addition, the secretory capacity of the vascular endothelium, in particular endothelins, potent vasoconstrictors, can induce the circadian oscillator through *per* and thus can coordinate the vasculature layers in an "optimal" vasoconstrictive response. In fibroblasts stimulated by high concentrations of endothelin, the clock genes are induced for several cell cycles [49].

Blood factors display concerted changes, the overall result being the increasing coagulation after waking: *Pai 1* is one of the vascular CCG, controlled by Clock/Bmal2. Its circadian peak in the rat heart occurs at the onset of nocturnal locomotor activity [50]; the platelets are more available to aggregate in the morning; on contrary, the potency of plasminogen activator (tPA) is lowest in the morning [51].

While the circadian rhythm of SCN is rapidly adjusted to day-light period in alterations of light/dark cycle, the peripheral tissues are more slowly connected to the new cycle, and each of them in a different period of time, so the peripheral synchronicity is lost and also the central drive. This dis-rhythmic activity, frequently encountered in modern society in shift-work, is likely to cause aberrant and discordant responses of variant tissues, leading to increased cardiovascular mortality [52]. From molecular genetics to functional genomics and proteomics, a whole range of data are still brought together to decipher the impact of time-set mechanisms upon basic mechanisms of life. The ion channels activity, transcription and translation, various phosphorylation processes are involved in this circadian clockweb [53].

Conclusions

Recent advances in molecular biology made possible the discovery of intimate mechanisms of circadian time-keeping for most cells. The main molecular clock consists of several clock-dedicated genes that impose a rhythm via a complex web of transcription factors, interrelated in order to keep the biologic time precisely regulated. Compensatory mechanisms for temperature adjustment, summer / winter settings of the biological clock, exist and allow subsequent changes in the whole organism. The main tick generator in the mammalian brain is the hypothalamic SCN, the coordinator of biological and behavioral rhythms. The central coordinator allows synchronization of all the other cell clocks, according to the specific requirements of every tissue. Disclosure of clock-controlled genes in various tissues using DNA microarray, and their specific interrelation in health and disease, is under investigation.

References

1. Roenneberg T., Merrow M., Circadian clocks: Omnes viae Romam ducunt, *Curr Biol*, **10**: R742-R745, 2000
2. Bargiello T.A., Jackson F.R., Young M.W., Restoration of circadian behavioural rhythms by gene transfer in *Drosophila*, *Nature*, **312**: 752-754, 1984
3. McClung C.R., Fox B.A., Dunlap J.C., The Neurospora clock gene frequency shares a sequence element with the *Drosophila* clock gene period, *Nature*, **339**: 558-562, 1989
4. Allada R., White N.E., So W.V., Hall J.C., Rosbash M., A mutant *Drosophila* homolog of mammalian Clock disrupts circadian rhythms and transcription of period and timeless, *Cell*, **93**: 791-804, 1998
5. Rutila J.E., Suri V., Le M., So W.V., Rosbash M., Hall J.C., CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of *Drosophila* period and timeless, *Cell*, **93**: 805-814, 1998
6. Myers M.P., Wager-Smith K., Wesley C.S., Young M.W., Sehgal A., Positional cloning and sequence analysis of the *Drosophila* clock gene, timeless, *Science*, **270**: 805-808, 1995
7. Kloss B., Price J.L., Saez L., Blau J., Rothenfluh A., Wesley C.S., Young M.W., The *Drosophila* clock gene double-time encodes a protein closely related to human casein kinase Iepsilon, *Cell*, **94**: 97-107, 1998
8. Emery P., So W.V., Kaneko M., Hall J.C., Rosbash M., CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity, *Cell*, **95**: 669-679, 1998
9. Blau J., Young M.W., Cycling vril expression is required for a functional *Drosophila* clock, *Cell*, **99**: 661-671, 1999

10. **Ralph M.R., Menaker M.**, A mutation of the circadian system in golden hamsters, *Science*, **241**: 1225-1227, 1988
11. **Vitaterna M.H., King D.P., Chang A.M., Kornhauser J.M., Lowrey P.L., McDonald J.D., Dove W.F., Pinto L.H., Turek F.W., Takahashi J.S.**, Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior, *Science*, **264**: 719-725, 1994
12. **Albrecht U., Sun Z.S., Eichele G., Lee C.C.**, A differential response of two putative mammalian circadian regulators, *mper1* and *mper2*, to light, *Cell*, **91**: 1055-1064, 1997
13. **Antoch M.P., Song E.J., Chang A.M., Vitaterna M.H., Zhao Y., Wilsbacher L.D., Sangoram A.M., King D.P., Pinto L.H., Takahashi J.S.**, Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue, *Cell*, **89**: 655-667, 1997
14. **Honma S., Ikeda M., Abe H., Tanahashi Y., Namihira M., Honma K., Nomura M.**, Circadian oscillation of *BMAL1*, a partner of a mammalian clock gene *Clock*, in rat suprachiasmatic nucleus, *Biochem Biophys. Res Commun.*, **250**: 83-87, 1998
15. **Kume K., Zylka M.J., Sriram S., Shearman L.P., Weaver D.R., Jin X., Maywood E.S., Hastings M.H., Reppert S.M.**, *mCRY1* and *mCRY2* are essential components of the negative limb of the circadian clock feedback loop, *Cell*, **98**: 193-205, 1999
16. **Vielhaber E., Eide E., Rivers A., Gao Z.H., Virshup D.M.**, Nuclear entry of the circadian regulator *mPER1* is controlled by mammalian casein kinase I epsilon, *Mol. Cell Biol.*, **20**: 4888-4899, 2000
17. **Balsalobre A., Marcacci L., Schibler U.**, Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts, *Curr. Biol.*, **10**: 1291-1294, 2000
18. **Gekakis N., Staknis D., Nguyen H.B., Davis F.C., Wilsbacher L.D., King D.P., Takahashi J.S., Weitz C.J.**, Role of the *CLOCK* protein in the mammalian circadian mechanism [see comments], *Science*, **280**: 1564-1569, 1998
19. **Yamaguchi S., Kobayashi M., Mitsui S., Ishida Y., van der Horst G.T., Suzuki M., Shibata S., Okamura H.**, View of a mouse clock gene ticking, *Nature*, **409**: 684, 2001
20. **Aronson B.D., Bell-Pedersen D., Block G.D., Bos N.P., Dunlap J.C., Eskin A., Garceau N.Y., Geusz M.E., Johnson K.A., Khalsa S.B.**, Circadian rhythms, *Brain Res. Rev.*, **18**: 315-333, 1993
21. **Roenneberg T., Mrosovsky M.**, Molecular circadian oscillators: an alternative hypothesis, *J. Biol. Rhythms*, **13**: 167-179, 1998
22. **Aronson B.D., Johnson K.A., Loros J.J., Dunlap J.C.**, Negative feedback defining a circadian clock: autoregulation of the clock gene frequency, *Science*, **263**: 1578-1584, 1994
23. **Hardin P.E., Hall J.C., Rosbash M.**, Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels, *Nature*, **343**: 536-540, 1990
24. **Daan S., Albrecht U., van der Horst G.T., Illnerova H., Roenneberg T., Wehr T.A., Schwartz W.J.**, Assembling a clock for all seasons: are there M and E oscillators in the genes?, *J. Biol. Rhythms*, **16**: 105-116, 2001
25. **Preitner N., Damiola F., Lopez-Molina L., Zakany J., Duboule D., Albrecht U., Schibler U.**, The orphan nuclear receptor *REV-ERB*alpha controls circadian transcription within the positive limb of the mammalian circadian oscillator, *Cell*, **110**: 251-260, 2002
26. **Jin X., Shearman L.P., Weaver D.R., Zylka M.J., de Vries G.J., Reppert S.M.**, A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock, *Cell*, **96**: 57-68, 1999
27. **Roenneberg T., Foster R.G.**, Twilight times: light and the circadian system, *Photochem. Photobiol.*, **66**: 549-561, 1997
28. **Crosthwaite S.K., Dunlap J.C., Loros J.J.**, *Neurospora* *wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity, *Science*, **276**: 763-769, 1997
29. **Hattar S., Liao H.W., Takao M., Berson D.M., Yau K.W.**, Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity, *Science*, **295**: 1065-1070, 2002
30. **Balsalobre A., Damiola F., Schibler U.**, A serum shock induces circadian gene expression in mammalian tissue culture cells, *Cell*, **93**: 929-937, 1998
31. **Damiola F., Le Minh N., Preitner N., Kornmann B., Fleury-Olela F., Schibler U.**, Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus, *Genes Dev.*, **14**: 2950-2961, 2000
32. **Zylka M.J., Shearman L.P., Weaver D.R., Reppert S.M.**, Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain, *Neuron*, **20**: 1103-1110, 1998
33. **Allada R., Emery P., Takahashi J.S., Rosbash M.**, Stopping time: the genetics of fly and mouse circadian clocks, *Annu. Rev. Neurosci.*, **24**: 1091-1119, 2001
34. **Yamazaki S., Numano R., Abe M., Hida A., Takahashi R., Ueda M., Block G.D., Sakaki Y., Menaker M., Tei H.**, Resetting central and peripheral circadian oscillators in transgenic rats, *Science*, **288**: 682-685, 2000
35. **Silver R., LeSauter J., Treco P.A., Lehman M.N.**, A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms, *Nature*, **382**: 810-813, 1996
36. **Allen G., Rappe J., Earnest D.J., Cassone V.M.**, Oscillating on borrowed time: diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts, *J. Neurosci.*, **21**: 7937-7943, 2001
37. **Balsalobre A., Brown S.A., Marcacci L., Tronche F., Kellendonk C., Reichardt H.M., Schutz G., Schibler U.**, Resetting of circadian time in peripheral tissues by glucocorticoid signaling, *Science*, **289**: 2344-2347, 2000
38. **Akashi M., Nishida E.**, Involvement of the MAP kinase cascade in resetting of the mammalian circadian clock, *Genes Dev.*, **14**: 645-649, 2000
39. **Yagita K., Okamura H.**, Forskolin induces circadian gene expression of *rPer1*, *rPer2* and *dbp* in mammalian rat-1 fibroblasts, *FEBS Lett.*, **465**: 79-82, 2000
40. **Stokkan K.A., Yamazaki S., Tei H., Sakaki Y., Menaker M.**, Entrainment of the circadian clock in the liver by feeding, *Science*, **291**: 490-493, 2001
41. **Rutter J., Reick M., Wu L.C., McKnight S.L.**, Regulation of clock and *NPAS2* DNA binding by the

- redox state of NAD cofactors, *Science*, **293**: 510-514, 2001
42. **Ripperger J.A., Shearman L.P., Reppert S.M., Schibler U.**, CLOCK, an essential pacemaker component, controls expression of the circadian transcription factor DBP, *Genes Dev.*, **14**: 679-689, 2000
 43. **Lavery D.J., Lopez-Molina L., Margueron R., Fleury-Olela F., Conquet F., Schibler U., Bonfils C.**, Circadian expression of the steroid 15 alpha-hydroxylase (Cyp2a4) and coumarin 7-hydroxylase (Cyp2a5) genes in mouse liver is regulated by the PAR leucine zipper transcription factor DBP, *Mol. Cell. Biol.*, **19**: 6488-6499, 1999
 44. **Levi F., Zidani R., Brienza S., Dogliotti L., Perpoint B., Rotarski M., Letourneau Y., Llory J.F., Chollet P., Le Rol A., Focan C.**, A multicenter evaluation of intensified, ambulatory, chronomodulated chemotherapy with oxaliplatin, 5-fluorouracil, and leucovorin as initial treatment of patients with metastatic colorectal carcinoma. International Organization for Cancer Chronotherapy, *Cancer*, **85**: 2532-2540, 1999
 45. **Fu L., Lee C.C.**, The circadian clock: pacemaker and tumour suppressor, *Nat. Rev. Cancer*, **3**: 350-361, 2003
 46. **Davis S., Mirick D.K., Stevens R.G.**, Night shift work, light at night, and risk of breast cancer, *J. Natl. Cancer Inst.*, **93**: 1557-1562, 2001
 47. **Naito Y., Tsujino T., Fujioka Y., Ohyanagi M., Iwasaki T.**, Augmented diurnal variations of the cardiac renin-angiotensin system in hypertensive rats, *Hypertension*, **40**: 827-833, 2002
 48. **Nonaka H., Emoto N., Ikeda K., Fukuya H., Rohman M.S., Raharjo S.B., Yagita K., Okamura H., Yokoyama M.**, Angiotensin II induces circadian gene expression of clock genes in cultured vascular smooth muscle cells, *Circulation*, **104**: 1746-1748, 2001
 49. **Yagita K., Tamanini F., Der Horst G.T., Okamura H.**, Molecular mechanisms of the biological clock in cultured fibroblasts, *Science*, **292**: 278-281, 2001
 50. **Maemura K., de la Monte S.M., Chin M.T., Layne M.D., Hsieh C.M., Yet S.F., Perrella M.A., Lee M.E.**, CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression, *J. Biol. Chem.*, **275**: 36847-36851, 2000
 51. **Braunwald E.**, Morning resistance to thrombolytic therapy, *Circulation*, **91**: 1604-1606, 1995
 52. **Knutsson A.**, Health disorders of shift workers, *Occup. Med. (Lond)*, **53**: 103-108, 2003
 53. **Haulica I.**, From molecular genetics to functional genomics and physiome, *J. Cell. Mol. Med.*, **6**: 648-652, 2002