CIRCADIAN AND SEASONAL RHYTHMS

Integration of mammalian circadian clock signals: from molecule to behavior

H Okamura

Division of Molecular Brain Science, Department of Brain Sciences, Kobe University Graduate School of Medicine, Chuo-ku, Kobe 650-0017, Japan
(Requests for offprints should be addressed to H Okamura; Email: okamurah@kobe-u.ac.jp)

Abstract

The core oscillatory mechanism consisting of gene transcription and translation is a unique feature and the astonishing discovery in circadian biology is that the rhythm of gene transcription reflects the behavioral rhythm almost perfectly. This means that the clock gene oscillation generated by the core loop in each suprachiasmatic nucleus neuron is coupled and amplified, and harmonized strongly so that oscillating activities are spread

into the whole brain and to all those peripheral organs which contain peripheral clocks. Additionally, circadian changes are induced in behavior and hormone secretion. Investigations of biological clocks open the fascinating perspective to analyze the integrational mechanism of 'time', providing a bridge between single genes and the living organism as a whole.

Journal of Endocrinology (2003) **177**, 3–6

Introduction

Most organisms living on earth have an internal clock and the circadian rhythm represents a basic feature of life. The discovery of clock genes and a general principle of their oscillation have stimulated research on biological clocks and this research has provided a major impact to the field of life sciences. In many organisms, the circadian core oscillator is thought to be composed of an autoregulatory transcription–(post)translation–based feedback loop involving a set of clock genes. The feature of the circadian system is that the prevalence of the oscillation at the level of genes is reflected at cell, tissue and system levels. In this brief review I summarize the issue of how the time signal is born and how it is integrated into the organismic level.

Core feedback loop of clock genes

Generation and control of circadian rhythms have attracted the attention of many researchers and this has led to the discovery of clock genes in *Drosophila*, *Neurospora* and mammals (see Dunlap 1999, Young & Kay 2001). These molecular studies suggest that mammals and *Drosophila* utilize similar components to generate circadian (~24 h) rhythms, and that the negative autoregulatory loop present in *Drosophila* (Hardin *et al.* 1990) is also conserved in mammals.

The molecular feedback loops generating circadian oscillation in mammals can be summarized as follows. Two main oscillators, *mPer1* and *mPer2*, are speculated to exist in the cell body, because (i) targeted disruption of either *mPer1* or *mPer2* but not *mPer3* severely disrupts locomotor activity rhythms, and mice deficient in both *mPer1* and *mPer2* do not express circadian rhythms (Bae *et al.* 2001, Zheng *et al.* 2001), and (ii) introduction of mammalian *mPer1* or *mPer2* genes into the arrhythmic *per⁰¹* mutant of *Drosophila*, which is otherwise arrhythmic due to a lack of endogenous PER protein, is capable of restoring rhythmicity (Shigeyoshi *et al.* 2002). The heterodimers formed by the bHLH-PAS proteins (CLOCK and BMAL1) bind to the E-box of *mPer1* and *mPer2* promoters (Lee *et al.* 2001) and initiate the transcription of these *mPer* genes (Gekakis *et al.* 1998). Activated transcription results in the formation of *mPer1* and *mPer2* mRNAs, which are translated in the cytoplasm to mPER1 and mPER2 proteins. These proteins translocate into the nucleus, and form stable negative complexes that comprise mCRY1, mCRY2, mPER1 and mPER2, and these suppress the transcription of the *mPer1* and *mPer2* genes by binding to the positive factors (CLOCK/BMAL1). Since *mCry1/mCry2* double knock-out mice (Okamura *et al.* 1999, Van der Horst *et al.* 1999) and *Bmal1* (*Mop3*) knock-out mice (Bunger *et al.* 2000) show the immediate loss of a behavioral rhythm in constant darkness, *mCry1/mCry2* and *Bmal1* clearly play a key role in making up the core loop.

Figure 1 Hierarchical architecture of circadian rhythm from gene, to cell, to nerve nuclei, to brain, and to system. 'GENE' depicts rhythmic transcription of *mPer1* and *mPer2*. 'CELL' represents neuronal electrical activities of single SCN neuron. 'SCN' indicates the sum of the local neuronal and glial circuits. 'BRAIN' symbolizes functions produced by neuronal circuits in the brain such as sleep and recognition. 'SYSTEM' symbolizes behavior, peripheral neuronal activities and hormonal secretion. 'P' and 'N' at gene level represent positive and negative elements respectively. Positive factors stimulate the transcription of clock genes, and their translational products negatively regulate the transcription of their own gene. At the SCN, cell clocks interact with each other, and harmonize to make a strong rhythm in the SCN as a whole. At the system level, many of the peripheral organs have their own 'peripheral clock'. The master clock in the SCN receives light information via the retina, and the presumed peripheral clocks in the digestive system, such as that in the liver, receive feeding information.

Complementary molecular loops

The core feedback loop is very stable and accurately maintains a 24 h rhythm. The stability and high amplitude will be ascertained at multiple stages including intracellular, intercellular and systemic levels. There are several complementary molecular loops assisting the core feedback loop at the gene-transcription level. One is a loop consisting of a positive factor, BMAL1, which is in antiphase to the daily *mPer1/2* rhythm (Preitner *et al.* 2002, Ueda *et al.* 2002). Another type of regulation is the antagonistic regulation of PAR proteins (HLF, TEF and DBP) and E4 BP4 (Yamaguchi *et al.* 2000b, Mitsui *et al.* 2001). In the *mPer1* gene there is a specific sequence,

ATTACGTAAC, to which both PAR proteins and E4 BP4 can bind (Yamaguchi *et al.* 2000a). In fact, DBP or E4 BP4 directly bind to the upstream region of the second transcription initiation site (1B site) of the *mPer1* gene, and DBP increases and E4 BP4 decreases the BMAL1/CLOCK-induced transcription of *mPer1* (Yamaguchi *et al.* 2000b, Mitsui *et al.* 2001). In the case of the *dbp* gene, BMAL1/CLOCK binds to the E-box of the second intron, and increases the transcription (Ripperger *et al.* 2000, Yamaguchi *et al.* 2000a). Therefore, PAR proteins, such as DBP, expressed highly at the beginning of the daytime assist the enhancement, but E4 BP4 expressed abundantly at early night assists the suppression of *mPer1* transcription.

Spatial and temporal regulation of clock proteins

If the concentration of negative factors determines the time for the shut off of the transcription, the question remains as to what mechanisms determine the concentration of clock proteins. As in *Drosophila* (Kloss *et al.* 1998), phosphorylation of mPER1 and mPER2 by casein kinase I ϵ plays a crucial step in determining the accumulation of negatively active clock proteins (Lowrey *et al.* 2000, Vielhaber *et al.* 2000). Furthermore, there is growing evidence that clock proteins are regulated dynamically in both spatial (nuclear and cytoplasmic) and temporal (production and degradation) dimensions (Yagita *et al.* 2000, 2002, Akashi *et al.* 2002). The main clock oscillatory protein mPER2 usually shuttles between the cytoplasm and the nucleus and is easily degraded by ubiquitination and the proteasome pathway (Yagita *et al.* 2002). It is also evident that the ubiquitination of mPER proteins is inhibited in the presence of mCRY proteins and the mPER proteins appear to be more fragile if they do not dimerize with mCRY proteins. Moreover, we have recently demonstrated that the mCRY protein, which is the strongest suppressor of *mPer1* transcription, can be ubiquitinated when mPER proteins are absent (Yagita *et al.* 2002). Thus the stabilized negative loop proteins suppress *mPer1* and *mPer2* transcription. The decrease of mPER in the nucleus due to proteasome-dependent degradation causes destabilization of mCRY, and the decrease in mCRY will lead to the re-starting of *mPer1* and *mPer2* gene transcription.

Output of the core oscillatory feedback loop to clock-controlled genes

Since hundreds of genes are controlled by the circadian clock (Akhtar *et al.* 2002, Panda *et al.* 2002), it is very important to reveal the core clock output mechanism. Two routes have been proposed. The first involves direct control by the central loop of the mammalian clock by CLOCK:BMAL1 heterodimers through an E-box in the promoter region such as vasopressin (Jin *et al.* 1999). The second route is an indirect pathway, consisting of antagonistic regulation of PAR proteins and E4 BP4 (Yamaguchi *et al.* 2000b, Mitsui *et al.* 2001), which is also used as the accessory feedback loop of clock genes. Albumin, cholesterol 7 α -hydroxylase and cytochrome P450 (*Cyp2A5*), and possibly aromatic L-amino acid decarboxylase, are regulated by this mechanism (Lavery *et al.* 1999, Ishida *et al.* 2002), in which the positive PAR proteins and the negative E4 BP4 switch back and forth between the on-off conditions of the target genes.

Hierarchical architecture of clock gene oscillation

In mammals, circadian oscillations occur within the suprachiasmatic nucleus (SCN) of the basal hypothalamus.

Since *mPer1* and *mPer2* genes are expressed in most neurons in the SCN (Takumi *et al.* 1998), thousands of clock cells in the SCN might generate the rhythm. These cell clocks show the harmonized expression pattern in the SCN. Inside the SCN, there is a topographical gradient in the circadian expression of clock genes (Yan & Okamura 2002). Expression of genes in the SCN has been visualized by a real-time monitoring system of slice cultures from transgenic mice carrying *mPer1* promoter-driven luciferase reporter (Yamaguchi *et al.* 2000a, Asai *et al.* 2001). Circadian fluctuations of bioluminescence have also been detected in living animals through insertion of an optical fiber just above the SCN of *mPer1-luc* transgenic mice (Yamaguchi *et al.* 2001). This shows that a harmonized ticking of clock genes will also occur in the SCN of living mammals.

The molecular clocks previously thought to exist only in the SCN are now also being found in cells of many peripheral organs. In the liver the peripheral clock is entrained by a restriction of feeding (Damiola *et al.* 2000), and this entrainment is independent of the SCN (Hara *et al.* 2001). In fibroblast cell lines, external stimuli such as high concentrations of serum and endothelin can induce the circadian expression of the clock genes for several cycles (Balsalobre *et al.* 1998, Yagita *et al.* 2001). The similar transcription/translation mechanisms of the core feedback loop is used in this system as in the SCN (Yagita *et al.* 2001), although these peripheral clock rhythms damped after a few cycles (Yamazaki *et al.* 2000). Mammalian clock system displays a complex hierarchical structure headed by the eternally oscillating SCN at the top.

The unique feature of circadian biology is that the gene transcription reflects the behavioral rhythm in an almost perfect state. This means that the clock gene oscillation generated by the core loop in each SCN neuron is coupled and amplified, and spread into the whole brain and to all those peripheral organs containing local clocks, and finally behavioral and hormonal rhythms are induced. 'Time' will be a bridge between single genes and the living organism as a whole.

References

- Akashi M, Tsuchiya Y, Yoshino T & Nishida E 2002 Control of intracellular dynamics of mammalian period proteins by casein kinase I ϵ (CKI ϵ) and CKI δ in cultured cells. *Molecular and Cellular Biology* **22** 1693–1703.
- Akhtar A, Reddy AB, Maywood ES, Clayton JD, King VM, Smith AG, Gant TW, Hastings MH & Kyriacou CP 2002 Cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Current Biology* **12** 540–550.
- Asai M, Yamaguchi S, Isejima H, Jounouchi M, Moriya T, Shibata S, Kobayashi M & Okamura H 2001 Visualization of *mPer1* transcription *in vitro* by luciferase mediated bioluminescence: NMDA induces a rapid phase-shift of *mPer1* gene in cultured SCN. *Current Biology* **11** 1524–1527.

- Bae K, Jin XW, Maywood ES, Hastings MH, Reppert SM & Weaver DR 2001 Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. *Neuron* **30** 525–536.
- Balsalobre A, Damiola F & Schibler U 1998 A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* **93** 929–937.
- Bunger MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS & Bradfield CA 2000 Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell* **103** 1009–1017.
- Damiola F, Minh N, Prenitner N, Kormmann B, Fleury-Olela F & Schibler U 2000 Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes and Development* **14** 2950–2961.
- Dunlap JC 1999 Molecular bases for circadian clocks. *Cell* **96** 271–290.
- Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS & Weitz CJ 1998 Role of the CLOCK protein in the mammalian circadian mechanism. *Science* **280** 1564–1569.
- Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M & Shibata S 2001 Restricted feeding entrains circadian clock in the mouse liver without participation of suprachiasmatic nucleus. *Genes to Cells* **6** 269–278.
- Hardin PE, Hall JC & Rosbach M 1990 Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. *Nature* **343** 536–540.
- Ishida Y, Yokoyama C, Inatomi T, Yagita K, Dong X, Yan L, Yamaguchi S, Nagatsu I, Komori T, Kitahama K *et al.* 2002 Circadian rhythm of aromatic L-amino acid decarboxylase in the rat suprachiasmatic nucleus: gene expression and decarboxylating activity in clock oscillating cells. *Genes to Cells* **7** 447–459.
- Jin X, Shearman LP, Weaver DR, Zylka MJ, de Vries GJ & Reppert SM 1999 A molecular mechanism regulating rhythmic output from the suprachiasmatic nucleus. *Cell* **96** 57–68.
- Kloss B, Price JL, Saez L, Blau J, Rothenfluh A, Wesley CS & Young MW 1998 The *Drosophila* clock gene *double-time* encodes a protein closely related to human casein kinase 1 ϵ . *Cell* **94** 97–107.
- Lavery DJ, Lopez-Molina L, Margueron R, Fleury-Olela F, Conquet F, Schibler U & Bonfils C 1999 Circadian expression of the steroid 15 α -hydroxylase (Cryp2a4) and coumarin 7-hydroxylase (Cryp2a5) genes in mouse liver is regulated by the PAR leucine zipper transcription factor DBP. *Molecular and Cellular Biology* **19** 6488–6499.
- Lee C, Etchegaray JP, Cagampang FRA, Loudon ASI & Reppert SM 2001 Posttranslational mechanisms regulate the mammalian circadian clock. *Cell* **107** 855–867.
- Lowrey PL, Shimomura K, Antoch MP, Yamazaki S, Zemenides PD, Ralph MR, Menaker M & Takahashi JS 2000 Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* **288** 483–492.
- Mitsui S, Yamaguchi S, Matsuo T, Ishida Y & Okamura H 2001 Antagonistic role of E4 BP4 and PAR proteins in the circadian oscillatory mechanism. *Genes and Development* **15** 995–1006.
- Okamura H, Miyake S, Sumi Y, Yamaguchi S, Yasui A, Muijtjens M, Hoeijmakers JHJ & van der Horst GTJ 1999 Photic induction of mPer1 and mPer2 in Cry-deficient mice lacking a biological clock. *Science* **286** 2531–2534.
- Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS & Hogenesch JB 2002 Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* **109** 307–320.
- Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U & Schibler U 2002 The orphan nuclear receptor REV-ERBa controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* **110** 251–260.
- Ripperger JA, Shearman LP, Reppert SM & Schibler U 2000 CLOCK, an essential pacemaker component, controls expression of the circadian transcription factor DBP. *Genes and Development* **14** 679–689.
- Shigeyoshi Y, Meyer-Bernstein E, Yagita K, Fu W, Chen Y, Takumi T, Schotland P, Sehgal A & Okamura H 2002 Restoration of circadian behavioral rhythms in a period null *Drosophila* mutant (*per⁰¹*) by mammalian period homologues mPer1 and mPer2. *Genes to Cells* **7** 163–171.
- Takumi T, Matsubara C, Shigeyoshi Y, Taguchi K, Yagita K, Maebayashi Y, Sakakida Y, Okumura K, Takashima N & Okamura H 1998 A new mammalian period gene predominantly expressed in the suprachiasmatic nucleus. *Genes to Cells* **3** 167–176.
- Ueda RH, Chen W, Adachi A, Wakamatsu H, Hayashi S, Takatsuji T, Nagano M, Nakahara K, Suzuki Y, Sugano S *et al.* 2002 A transcription factor response element for gene expression during circadian night. *Nature* **418** 534–539.
- Van der Horst GT, Muijtjens M, Kobayashi K, Takano R, Kanno S, Takao M, de Wit J, Verkerk A, Eker AP, van Leenen D *et al.* 1999 Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* **398** 627–630.
- Vielhaber E, Eide E, Rivers A, Gao ZH & Virshup DM 2000 Nuclear entry of the circadian regulator mPER1 is controlled by mammalian casein kinase 1 ϵ . *Molecular and Cellular Biology* **20** 4888–4899.
- Yagita K, Yamaguchi S, Tamanini F, van der Horst GTJ, Hoeijmakers JHJ, Yasui A, Loros JJ, Dunlap JC & Okamura H 2000 Dimerization and nuclear entry of mPER proteins in mammalian cells. *Genes and Development* **14** 1353–1363.
- Yagita K, Tamanini F, van der Horst G & Okamura H 2001 Molecular mechanisms of the biological clock in cultured fibroblasts. *Science* **292** 278–281.
- Yagita K, Tamanini F, Yasuda M, Hoeijmakers JHJ, van der Horst GTJ & Okamura H 2002 Nucleocytoplasmic shuttling and mCRY dependent inhibition of ubiquitination of the mPER2 clock protein. *EMBO Journal* **21** 1301–1314.
- Yamaguchi S, Mitsui S, Miyake S, Yan L, Onishi H, Yagita K, Suzuki M, Shibata S, Kobayashi M & Okamura H 2000a The 5' upstream region of mPer1 gene contains two promoters and is responsible for circadian oscillation. *Current Biology* **10** 873–876.
- Yamaguchi S, Mitsui S, Yan L, Yagita K, Miyake S & Okamura H 2000b Role of DBP in the circadian oscillatory mechanism. *Molecular and Cellular Biology* **20** 4773–4781.
- Yamaguchi S, Kobayashi M, Mitsui S, Ishida Y, van der Horst GTJ, Suzuki M, Shibata S & Okamura H 2001 Gene expression – view of a mouse clock gene ticking. *Nature* **409** 684.
- Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M & Tei H 2000 Resetting central and peripheral circadian oscillators in transgenic rats. *Science* **288** 682–685.
- Yan L & Okamura H 2002 Gradients in the circadian expression of Per1 and Per2 genes in the suprachiasmatic nucleus. *European Journal of Neuroscience* **15** 1153–1162.
- Young MW & Kay SA 2001 Time zones: a comparative genetics of circadian clocks. *Nature Reviews Genetics* **2** 702–715.
- Zheng B, Albrecht U, Kaasik K, Sage M, Lu W, Vaishnav S, Li Q, Sun ZS, Eichele G, Bradley A *et al.* 2001 Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. *Cell* **105** 683–694.

Received 9 July 2002

Accepted 1 October 2002