Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 346 (2006) 169–174
BBRC
www.elsevier.com/locate/ybbrc

Circadian rhythm generation in a glioma cell line

Atsuko Fujioka [a], Naoyuki Takashima [a,b], Yasufumi Shigeyoshi [a,*]

[a] Department of Anatomy and Neurobiology, Kinki University School of Medicine, Osaka-Sayama, Osaka 589-8511, Japan
[b] Course of Medical Biosignaling, The Public Relations Committee, Graduate School of Medicine, Faculty of Medicine, Osaka University, 2-2 Yamadaoka Suita, Osaka 565-0871, Japan

Received 4 May 2006
Available online 24 May 2006

Abstract

In mammals, the principal circadian oscillator resides in the hypothalamic suprachiasmatic nucleus. However, the basic components and the ability to generate a circadian rhythm are also characteristic of most peripheral tissues and some cell lines. In our present study, we show that the rat C6 glioma cell line displays circadian oscillations of reporter luciferase bioluminescence driven by the mouse *Per2* promoter and of clock-related gene transcripts. *Per2::luc* expressing C6 cells display circadian rhythm in their bioluminescence levels for more than seven days. In addition, clock and clock-controlled genes show dynamic circadian oscillation in C6 cells after exposure to dexamethasone. It is also significant that *Per1* is not induced in C6 cells by a calcium ionophore, which is in stark contrast to Rat-1 cells. The C6 glioma cell line has therefore the potential to be a useful tool in future investigations of the underlying molecular machinery of the circadian clock.

Keywords: C6; Glioma; Circadian rhythm; *Per1*; *Per2*; *Per3*; *Bmal1*; *Rev-erbAα*; *Dbp*

Circadian rhythms are generated by endogenous timekeeping mechanisms that are conserved across evolution. In mammals, the master circadian oscillator resides in the hypothalamic suprachiasmatic nucleus (SCN) [1] in which individual neurons are equipped with autonomous oscillators that generate circadian firing rhythms [2]. Recent studies involving the genetic dissection of circadian rhythms in several organisms have further revealed the nature of these intracellular oscillators. The basic circadian clock mechanism comprises a feedback loop in which the expression of clock genes is suppressed periodically by their protein products [3,4]. The major negative elements in this process are the period genes (*Per1* and *Per2*) and cryptochrome genes (*Cry1* and *Cry2*), whereas positive elements include *Clock* and *Bmal1*. Each of these genes is abundantly expressed in the SCN and whereas *Per1*, *Per2*, *Cry1*, *Cry2*, and *Bmal1* mRNAs show dynamic endogenous oscillations [5–8], *clock* transcripts are constitutively expressed in the SCN [9].

The Rat-1 and NIH3T3 fibroblast cell lines, immortalized SCN and GnRH neurons, show a circadian rhythm in the expression of clock-controlled genes [10–16]. However, the oscillation that can be observed in populations of these cells is eventually dampened after several cycles. Recently, however, studies of these fibroblast cell lines have demonstrated that the lack of synchronization prevents a continuous circadian oscillation in the whole population of cells in culture, despite the maintenance of a dynamic oscillation in individual cells [17,18]. Cell lines that have the ability to generate a circadian rhythm may thus be a suitable model system for investigating the molecular mechanisms underlying circadian pathways. In our present study, we demonstrate that a rat glioma cell line, C6, generates a circadian oscillation of its clock genes which is more stable than Rat-1 cells. C6 cells also show specific responses to a variety of chemical compounds. This glioma cell line might therefore provide a valuable experimental tool for the study of intercellular synchronization

* Corresponding author. Fax: +81 72 368 1031.
E-mail address: shigey@med.kindai.ac.jp (Y. Shigeyoshi).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.05.094

mechanisms and tissue specific regulation of the endogenous circadian clock.

Materials and methods

Cell culture. Rat-1 fibroblasts, C6 glioma cells, Hek293 cells, and Cos7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS, ICN) at 37 °C in a 5% CO_2 atmosphere.

Construction of dLuc. dLuc was constructed following the method proposed by Ueda et al. [19]: Luciferase cDNA was amplified with pyrobest DNA polymerase (Takara) and primers that incorporated a *Nco*I recognition sequence at the 5′ end and a *Eco*RV sequence at the 3′ end. The stop codon of *Luciferase* (*Luc*) was deleted to generate a continuous open reading frame with PEST. The PEST sequence was amplified from pd1EGFP-N1 (Clontech) with pyrobest and primers that incorporated an *Eco*RV recognition sequence at the 5′ end and an *Xba*I sequence at the 3′ end. The amplified PCR products were ligated at the *Eco*RV site, and the fusion product was cloned into the *Nco*I and *Xba*I sites of the pGV-B2 basic vector (TOYO B-Net). The primers used were as follows: PEST-forward, tacaaggatatcagccatgg; PEST-reverse, tcgcggcctctagactacacattg; *Luc*-forward, tggtaaagccaccatggaag; *Luc*-reverse, cgactctagatatccacggcg.

Construction of mPer2::dLuc. The m*Per2* promoter was amplified with LA-*taq* DNA polymerase (Takara) and primers that incorporated a *Kpn*I recognition sequence at the 5′ end and a *Bgl*II sequence at the 3′ end. 3410 bp of the 5′ upstream sequence from the mouse *Per2* was thus obtained by PCR and then subcloned into the *Kpn*I/*Bgl*II sites of the *dLuc* vector to generate the m*Per2::dLuc* construct. The primers were as follows: m*Per2*-forward, atggtaccgtgtgcctttcaccttagctgt; m*Per2*-reverse, atagatctcgtctgtcccttgctcg.

Transfection and real-time monitoring of circadian bioluminescence. Rat-1 fibroblasts, C6 cells, Hek293 cells, and Cos7 cells were grown in DMEM supplemented with 10% FBS (Sigma). Cells were plated at a density of 1.0×10^6 cells per dish in 35-mm dishes 24 h before transfection. The cells were then transfected with 1 μg *mPer2::dLuc* plasmid using LipofectAMINE 2000 reagent (Invitrogen) according to the manufacturer's instructions. After 16 h, the medium was replaced with 1.5 ml DMEM supplemented with 10% FBS. Forty-eight hours after the transfection, the cells in each dish were treated with 100 nM dexamethasone (Dex), and following a further 2 h the medium was replaced with 2 ml DMEM with 25 mM Hepes (GIBCO), supplemented with 10% FBS and 0.1 μM luciferin (Nacalai Tesque). Bioluminescence was measured using photomultiplier tube (PMT) detector assemblies (Kronos, ATTO).

Treatment with various chemical agents. Cells (5×10^5) (Rat-1 or C6) were plated on 35 mm dishes and cultured in DMEM containing 10% FBS for several days. After the cells had reached confluence, they were incubated in serum-free DMEM for a further 24 h and then separately stimulated by supplementation with 100 nM Dex (ICN), 50% horse serum (HS) (ICN), 10 μM forskolin (FK) (WAKO), 100 nM phorbol 12-myristate 13-acetate (TPA) (WAKO) or 100 nM ionomycin (IM) (Calbiochem). After 2 h of treatment with these agents, the culture medium was replaced with serum-free DMEM.

Quantitative PCR. At the indicated time points after the treatment with each substance listed above, cultured cells were harvested in either 400 μl Trizol (Invitrogen) or Sepasol RNA1 Super (Nacalai Tesque) and total RNA was extracted. 0.5 μg of each total RNA preparation was then reverse-transcribed using ReverTra Ace (Toyobo) and 2.5 μM oligo(dT). TaqMan real time PCR (qPCR) was next carried out with an ABI PRISM 7700 Sequence Detector, in a total volume of 25 μl using Quantitect Probe PCR Kit (Qiagen) or in a total volume of 15 μl using Premix Ex Taq™ (Perfect Real Time) (Takara), according to the supplier's instructions. mRNA quantification was performed using two primers and a fluorescent probe as follows: *rPer1*: forward primer, GCTCTCAGAGTTT GTGCGATGA; reverse primer, AAAAGACACAAGCAGTCACAC AAATA; probe, TTGTTCATGCGCAAACCAAACGTACC; *rPer2*: forward primer, GCTCTCAGAGTTTGTGCGATGA; reverse primer, AAAAGACACAAGCAGTCACACAAATA; probe, TTGTTCATGCG CAAACCAAACGTACC; *rCry1*: forward primer, TTCGTCAGGAG GGCTGGAT; reverse primer, GCCGCGGGTCAGGAA; probe, CACC ATCTAGCCCGACATGCAGTTGC; *rBmal1*: forward primer, CTGAG CTGCCTCGTTGCA; reverse primer, CCCGTATTTCCCCGTTCACT; probe, TCGGGCGACTGCACTCACACATG; *Rev-ErbAα* (*Nr1d1*): forward primer, TGAAAAACGAGAACTGCTCCATT; reverse primer, CCAACGGAGAGACACTTCTTGAA; probe, TATCAATCGCAACC GCTGCCAGC; and *rDbp*: forward primer, TGCCCTGTCAAGCA TTCCA; reverse primer, AGGCTTCAATTCCTCCTCTGAGA; probe, CCATGAGACTTTTGACCCTCGGAGGC. For the normalization of template concentrations, primers and a probe for glycaldehyde-3-phosphate dehydrogenase (GAPDH (ABI)) were used. The resulting C_T values from the cDNA amplifications were thus normalized to the C_T values for GAPDH and then analyzed according to the $\Delta\Delta C_T$ method.

Results

C6 cells display more stable circadian gene expression of Per2 than Rat-1 cells

Following the treatment with Dex, C6 cells that had been transfected with the m*Per2::dluc* expression vector displayed a circadian rhythm of bioluminescence, with a period close to 24 h. This circadian oscillation was generally sustained for more than 7 days (Fig. 1A) but a continuous attenuation in amplitude was found to occur. In contrast to C6 cells, Rat-1 cells transfected with the same vector showed a circadian oscillation that dampened within three or four cycles (Fig. 1B). The period length in the Rat-1 cells was 21.7 ± 0.11 ($n = 6$), whereas C6 cells exhibited a circadian period length of 23.5 ± 0.06 ($n = 9$), which was significantly longer ($P < 0.0001$, Student's *t*-test). We studied the circadian rhythmicity in the *Per2::luc* in the other cell lines, Cos7 cells, and Hek293 cells. Those cells did not display any oscillation after the treatment (Fig. 1C and D). We found that C6 cells exposed to Dex displayed the most stable circadian oscillation with a little dampening of all experimented cell lines. The circadian rhythms in the C6 and Rat-1 cells could also be demonstrated by measurement of clock gene transcripts using quantitative PCR (Fig. 1E). Both of these immortalized cell lines showed a circadian rhythm in their *Per2* mRNA expression pattern, with a period length of approximately 24 h (Fig. 1E). In both cases also, the *Per2* transcript levels displayed a peak at around 4 h after treatment, which then rapidly decreased to basal levels at 16–20 h, followed by a second peak and trough at approximately 28 and 40 h, respectively (Fig. 1E). The ratio of the second peak to the second trough was much larger in C6 than in Rat-1 cells, which was consistent with our finding that the damping of circadian oscillation was much more rapid in Rat-1 cells.

Various clock genes display circadian oscillations in C6 cells following treatment with Dex

We next tested the temporary expression of the other clock and clock-controlled genes in Dex-administered C6 cells and compared these patterns with the expression profiles in Rat-1 cells (Fig. 2). *Per3*, *Cry1*, *Bmal1*, *Rev-erbAα*,

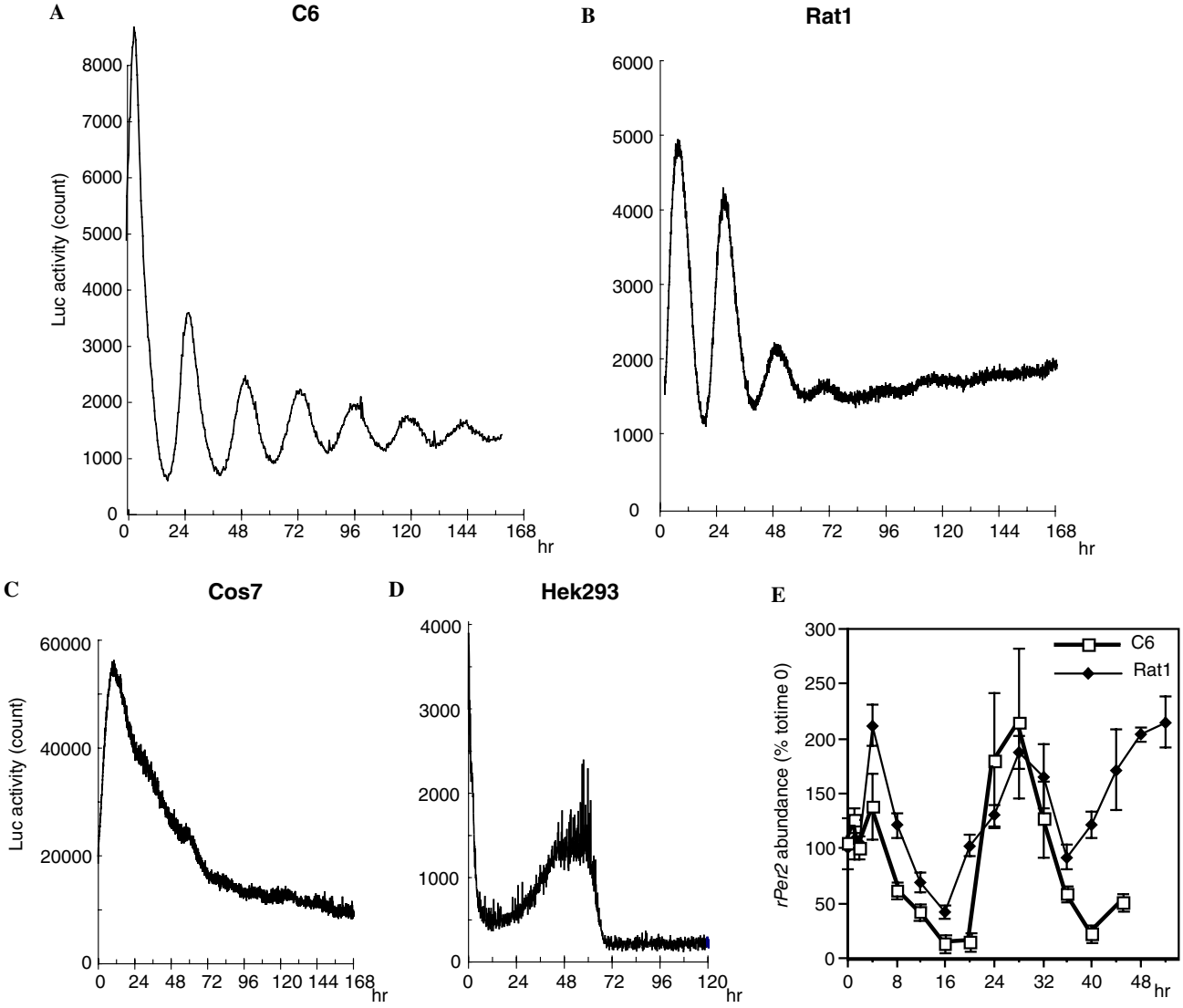

Fig. 1. Circadian oscillation and transcriptional activity of *Per2* mRNA in C6 and Rat-1 cells. (A–D) Representative graphs showing a time course of transcriptional activity for m*Per2::dLuc* in C6 (A), Rat-1 (B), Cos7 (C), and Hek293 (D) cells treated with 100 nM Dex. Each experiment was independently performed at least three times. (E) The temporary expression of r*Per2* mRNA in C6 and Rat-1 cells after 100 nM Dex treatment. The results are expressed as relative quantities of *Per2* (±SEM) and are representative of means of three independent experiments.

and *Dbp* displayed a circadian oscillation with a periodicity of about 24 h in both C6 and Rat-1 cells. In C6 cells, Dex treatment induced a rapid increase in *Per1* gene expression of more than 13-fold and this was followed by a sharp reduction. This increase was not evident for any of the other genes, which showed a rapid increase of no more than 2-fold. *Per2*, *Per3*, *Rev-erbAα*, and *Dbp* also demonstrated synchronous cycling (Figs. 1E and 2). A robust cycling of *Bmal1* mRNA was observed, with a mRNA oscillation that was antiphase to the *Per2*, *Per3*, and *Dbp* mRNA cycles. *Cry1* expression showed rhythmicity, peaking 4–8 h after the peak times of *Per2*/*Per3* mRNAs.

Although C6 cells and Rat-1 cells demonstrated similar profiles of clock and clock-controlled genes after Dex treatment (Fig. 2), some differences were observed in the expression profiles of clock and clock-controlled genes. In both C6 and Rat-1 cells, *Per1* was immediately upregulated after Dex treatment and reached a peak at 2 h (Fig. 2A). In C6 cells, however, *Per1* was continuously upregulated for 48 h or longer after exposure to Dex and remained at a level that was more than 6-fold higher than basal. In contrast, *Per1* expression in Rat-1 cells reached a trough below the basal level at 16 h and was then found to be upregulated to levels about 2-fold higher than basal. One other difference is that *Per3* was rapidly induced by Dex treatment in Rat-1 cells but not in C6 cells (Fig. 2B). In Rat-1 cells, *Per3* was immediately induced and peaked at 2 h after the Dex treatment. In contrast, no apparent induction was observed in C6 cells.

The immediate induction of Per1, Per2, and Per3 by various compounds that stimulate signal transduction pathways

Balsalobre et al. [20] previously demonstrated that multiple signaling pathways are involved in the immediate

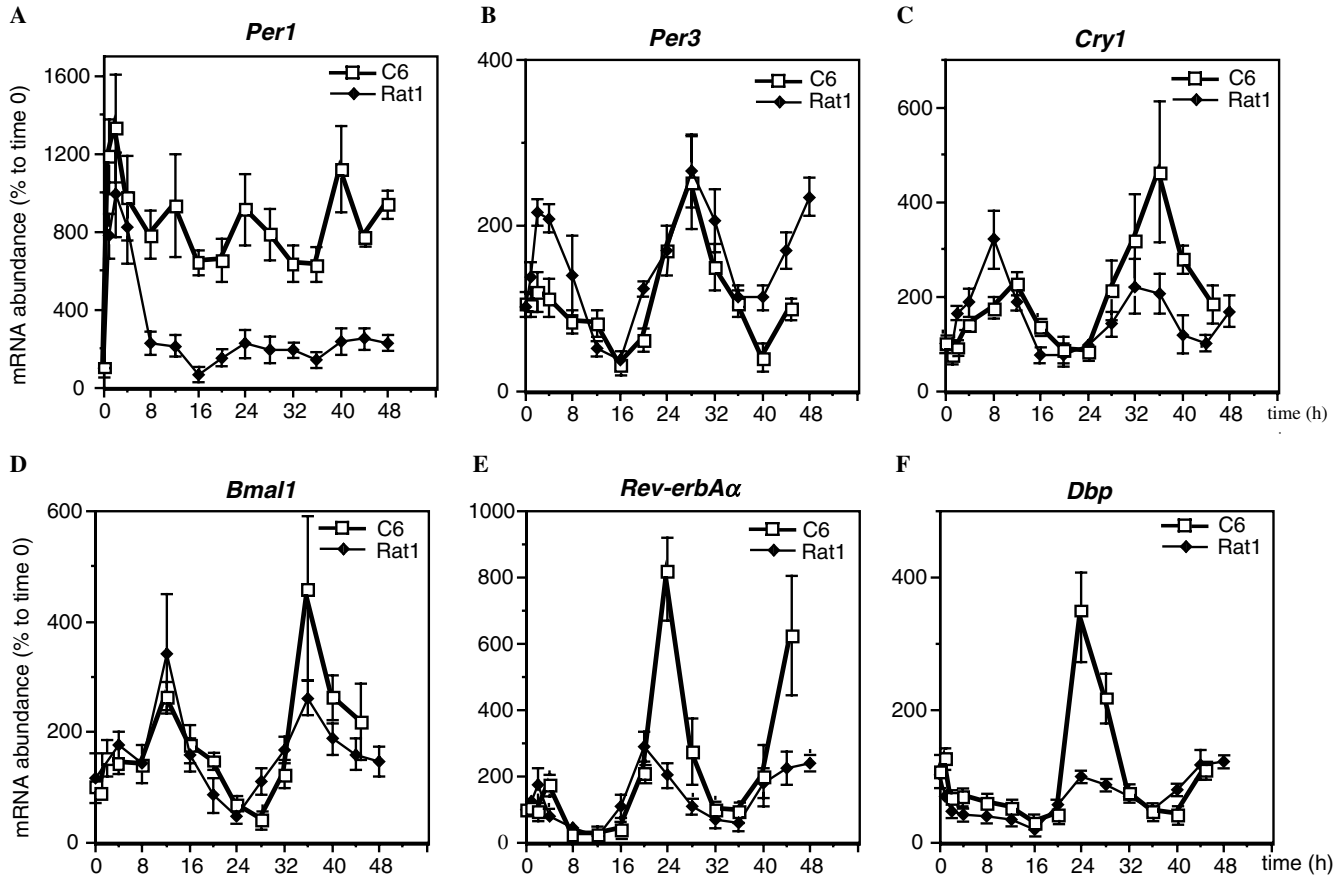

Fig. 2. Time course of clock gene mRNA expression in C6 and Rat-1 cells after 100 nM Dex treatment. The results are expressed as relative quantities of *Per1* (A), *Per3* (B), *Cry1* (C), *Bmal1* (D), *Rev-erbAα* (E), and *Dbp* (F). The values (±SEM) are means of at least three independent experiments.

induction of *Per1*. FK, an activator of adenylate cyclase, TPA, an activator of protein kinase C, ionomycin (IM), a calcium ionophore, and high concentrations of HS have been found to elicit the immediate induction of *Per1* in Rat-1 cells. We therefore assessed in our current experiments whether these same compounds would also similarly induce the immediate upregulation of *Per1*, *Per2*, and *Per3* in C6 cells (Fig. 3). In Rat-1 cells, serum stimulation and four compounds were found to induce *Per1* within 1 h, followed by a reduction below basal levels within 4 h. *Per2* was significantly upregulated only by serum stimulation in both C6 and Rat-1 cells (Fig. 3B). A differential response to treatment with the calcium ionophore was observed; exposure to IM immediately induced *Per1* and *Per3* in Rat-1 cells but not in C6 cells (Fig. 3A and C).

Discussion

In our present study, we demonstrate that the rat glioma cell line C6 shows a circadian oscillation in the expression of its clock genes that is maintained for at least seven days. In previous similar studies in fibroblasts, the damping of oscillation was found to be primarily due to desynchrony among cells, whereas individual cells were shown to generate stable circadian oscillation without damping [17,18]. Moreover, when such fibroblast populations were synchronized by extrinsic stimulation, Rat-1 cells were found to be desynchronized due to differences in the period of the circadian oscillation in each cell and the lack of synchronization overall. In our present experiments, we observe that the oscillation in C6 cells is sustained for a much longer period than in Rat-1 cells. This more stable oscillation in C6 cells may be attributable to a smaller variation in the circadian period and/or greater ability to maintain synchronization among the cell population. The ability of C6 cells to generate a circadian rhythm might also reflect the ability of the SCN to maintain the circadian activity rhythm. A previous study has demonstrated that cultured astrocytes also show circadian oscillation of clock gene activity [21]. Glial rhythms were significantly sustained when these cells were cocultured with explants of the adult SCN, but not with cortical explants. This finding suggests that diffusible signals released from the SCN in a circadian manner entrain the co-cultured astrocytes. In addition, 25% of the cells within the SCN are astrocytes [22] and it is thus probable that in addition to the neurons equipped with a cell-autonomous circadian clock, the astrocytes play significant roles in the maintenance of circadian oscillation in the SCN.

High amplitude oscillation in C6 cells was shown not only for *Per2* expression but also for *Per3*, *Cry1*, *Bmal1*, *Rev-erbAα*, and *Dbp*. Most of the temporal expression profiles and the phase relationship of the various mRNA

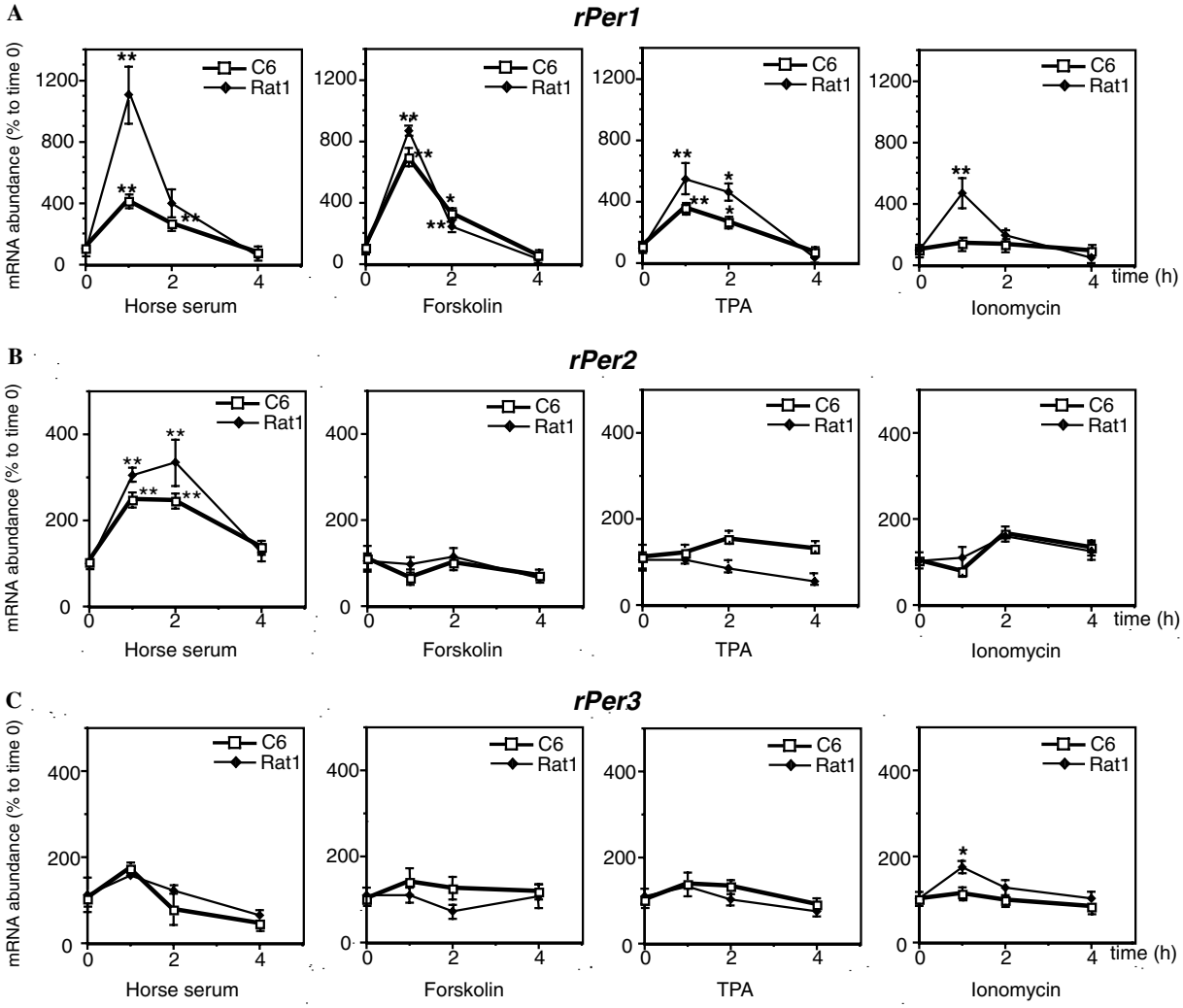

Fig. 3. The induction of r*Per1* (A), r*Per2* (B), and r*Per3* (C) mRNA in C6 and Rat-1 cells exposed to HS, FK, TPA or IM. The results are expressed as the relative quantities of each transcript. The values (±SEM) are means of three independent experiments. The amounts of mRNA at each time point were compared with the basal levels by ANOVA. $^{**}P < 0.01$, $^{*}P < 0.05$ (ANOVA).

rhythms of clock genes examined in the present study were consistent with those in the SCN and in Rat-1 cells. This suggests that a similar molecular machinery drives the circadian clock both in C6 cells and in the SCN. The only exception was *Per1*, which was constitutively expressed at high levels for more than 48 h after exposure to Dex. This finding also has significance, however, in that it suggests that Dex can not only reset the C6 endogenous clock but also produce continuous changes in *Per1* transcript levels. In support of this, a recent report has demonstrated that the overt damping of circadian periodicity in NIH3T3 cells is due to both desynchrony among the cell population and damping of the oscillation in individual cells [23]. The sustained upregulation of *Per1* was only seen following Dex administration and we found that the most stable circadian oscillation pattern was evident following Dex treatment, compared with other stimulations (data not shown). It is possible that highly sustained *Per1* levels might contribute to the stability of the circadian rhythm in C6 cells and in the generation of the differences in the circadian period length between C6 and Rat-1 cells.

Serum stimulation and chemical treatments induced *Per1* in almost the same manner in C6 cells as in Rat-1 cells, with the exception of the calcium ionophore, IM. IM induced *Per1* and *Per3* in Rat-1 but not in C6 cells and a number of previous studies have suggested that *Per1* expression during the night is the principal event that produces the phase shift that resets the circadian clock [24–28]. The differences between Rat-1 and C6 cells in the response to calcium influx thus suggest that there are tissue-specific differences in the manner in which resetting of the circadian clock will occur. It was earlier reported that astrocytes sense neuronal activity and are involved in signal transmission between neurons [29,30]. Neuronal stimulation has also been shown to trigger electrophysiological and/or Ca^{2+} responses in astrocytes. The unresponsiveness of *Per1*/*Per3* genes in astrocytes after Ca^{2+} upregulation suggests that the internal circadian oscillator

in these cells is unresponsive to the Ca^{2+} mobilization generated by the electrical excitation of adjacent neurons. This lack of response to Ca^{2+} might contribute to the stability of the circadian oscillation in the SCN.

Acknowledgments

This work was supported by a High-Tech Research Center Grant and a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

References

[1] D.C. Klein, R.Y. Moore, S.M. Reppert, Suprachiasmatic Nucleus: The Mind's Clock, Oxford University Press, New York, 1991.
[2] D.K. Welsh, D.E. Logothetis, M. Meister, S.M. Reppert, Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms, Neuron 14 (1995) 697–706.
[3] J.C. Dunlap, Molecular bases for circadian clocks, Cell 96 (1999) 271–290.
[4] S. Panda, J.B. Hogenesch, S.A. Kay, Circadian rhythms from flies to human, Nature 417 (2002) 329–335.
[5] H. Tei, H. Okamura, Y. Shigeyoshi, C. Fukuhara, R. Ozawa, M. Hirose, Y. Sakaki, Circadian oscillation of a mammalian homologue of the *Drosophila* period gene, Nature 389 (1997) 512–516.
[6] T. Takumi, C. Matsubara, Y. Shigeyoshi, K. Taguchi, K. Yagita, Y. Maebayashi, Y. Sakakida, K. Okumura, N. Takashima, H. Okamura, A new mammalian period gene predominantly expressed in the suprachiasmatic nucleus, Genes Cells 3 (1998) 167–176.
[7] K. Kume, M.J. Zylka, S. Sriram, L.P. Shearman, D.R. Weaver, X. Jin, E.S. Maywood, M.H. Hastings, S.M. Reppert, mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop, Cell 98 (1999) 193–205.
[8] S.M. Reppert, D.R. Weaver, Coordination of circadian timing in mammals, Nature 418 (2002) 935–941.
[9] S.M. Reppert, D.R. Weaver, Molecular analysis of mammalian circadian rhythms, Annu. Rev. Physiol. 63 (2001) 647–676.
[10] A. Balsalobre, F. Damiola, U. Schibler, A serum shock induces circadian gene expression in mammalian tissue culture cells, Cell 93 (1998) 929–937.
[11] D.J. Earnest, F.Q. Liang, S. DiGiorgio, M. Gallagher, B. Harvey, B. Earnest, G. Seigel, Establishment and characterization of adenoviral E1A immortalized cell lines derived from the rat suprachiasmatic nucleus, J. Neurobiol. 39 (1999) 1–13.
[12] G. Allen, J. Rappe, D.J. Earnest, V.M. Cassone, Oscillating on borrowed time: diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts, J. Neurosci. 21 (2001) 7937–7943.
[13] J.M. Gillespie, B.P. Chan, D. Roy, F. Cai, D.D. Belsham, Expression of circadian rhythm genes in gonadotropin-releasing hormone-secreting GT1-7 neurons, Endocrinology 144 (2003) 5285–5292.
[14] M. Akashi, E. Nishida, Involvement of the MAP kinase cascade in resetting of the mammalian circadian clock, Genes Dev. 14 (2000) 645–649.
[15] K. Oh-hashi, Y. Naruse, M. Tanaka, Intracellular calcium mobilization induces period genes via MAP kinase pathways in NIH3T3 cells, FEBS Lett. 516 (2002) 101–105.
[16] P.E. Chappell, R.S. White, P.L. Mellon, Circadian gene expression regulates pulsatile gonadotropin-releasing hormone (GnRH) secretory patterns in the hypothalamic GnRH-secreting GT1-7 cell line, J. Neurosci. 23 (2003) 11202–11213.
[17] U. Schibler, F. Naef, Cellular oscillators: rhythmic gene expression and metabolism, Curr. Opin. Cell Biol. 17 (2005) 223–229.
[18] D.K. Welsh, S.H. Yoo, A.C. Liu, J.S. Takahashi, S.A. Kay, Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression, Curr. Biol. 14 (2004) 2289–2295.
[19] H.R. Ueda, W. Chen, A. Adachi, H. Wakamatsu, S. Hayashi, T. Takasugi, M. Nagano, K. Nakahama, Y. Suzuki, S. Sugano, M. Iino, Y. Shigeyoshi, S. Hashimoto, A transcription factor response element for gene expression during circadian night, Nature 418 (2002) 534–539.
[20] A. Balsalobre, L. Marcacci, U. Schibler, Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts, Curr. Biol. 10 (2000) 1291–1294.
[21] L.M. Prolo, J.S. Takahashi, E.D. Herzog, Circadian rhythm generation and entrainment in astrocytes, J. Neurosci. 25 (2005) 404–408.
[22] F.H. Guldner, Numbers of neurons and astroglial cells in the suprachiasmatic nucleus of male and female rats, Exp. Brain Res. 50 (1983) 373–376.
[23] T.K. Sato, R.G. Yamada, H. Ukai, J.E. Baggs, L.J. Miraglia, T.J. Kobayashi, D.K. Welsh, S.A. Kay, H.R. Ueda, J.B. Hogenesch, Feedback repression is required for mammalian circadian clock function, Nat. Genet. 38 (2006) 312–319.
[24] Y. Shigeyoshi, K. Taguchi, S. Yamamoto, S. Takekida, L. Yan, H. Tei, T. Moriya, S. Shibata, J.J. Loros, J.C. Dunlap, H. Okamura, Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript, Cell 91 (1997) 1043–1053.
[25] L.P. Shearman, M.J. Zylka, D.R. Weaver, L.F. Kolakowski Jr., S.M. Reppert, Two period homologs: circadian expression and photic regulation in the suprachiasmatic nuclei, Neuron 19 (1997) 1261–1269.
[26] M.J. Zylka, L.P. Shearman, D.R. Weaver, S.M. Reppert, Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain, Neuron 20 (1998) 1103–1110.
[27] M. Akiyama, Y. Kouzu, S. Takahashi, H. Wakamatsu, T. Moriya, M. Maetani, S. Watanabe, H. Tei, Y. Sakaki, S. Shibata, Inhibition of light- or glutamate-induced mPer1 expression represses the phase shifts into the mouse circadian locomotor and suprachiasmatic firing rhythms, J. Neurosci. 19 (1999) 1115–1121.
[28] S.A. Tischkau, J.W. Mitchell, S.H. Tyan, G.F. Buchanan, M.U. Gillette, Ca2+/cAMP response element-binding protein (CREB)-dependent activation of Per1 is required for light-induced signaling in the suprachiasmatic nucleus circadian clock, J. Biol. Chem. 278 (2003) 718–723.
[29] R.D. Fields, B. Stevens-Graham, New insights into neuron-glia communication, Science 298 (2002) 556–562.
[30] C.G. Schipke, H. Kettenmann, Astrocyte responses to neuronal activity, Glia 47 (2004) 226–232.