Communication

Multitissue Circadian Expression of Rat period Homolog (rPer2) mRNA Is Governed by the Mammalian Circadian Clock, the Suprachiasmatic Nucleus in the Brain*

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The period (per) gene, controlling circadian rhythms in Drosophila, is expressed throughout the body in a circadian manner. A homolog of Drosophila per was isolated from rat and designated as rPer2. The rPER2 protein showed 39 and 95% amino acid identity with mPER1 and mPER2 (mouse homologs of per) proteins, respectively. A robust circadian fluctuation of rPER2 mRNA expression was discovered not only in the suprachiasmatic nucleus (SCN) of the hypothalamus but also in other tissues including eye, brain, heart, lung, spleen, liver, and kidney. Furthermore, the peripheral circadian expression of rPER2 mRNA was abolished in SCN-lesioned rats that showed behavioral arrhythmicity. These findings suggest that the multitissue circadian expression of rPER2 mRNA was governed by the mammalian brain clock SCN and also suggest that the rPER2 gene was involved in the circadian rhythm of locomotor behavior in mammals.

Circadian rhythms in physiology and behavior are governed by the endogenous clock (1, 2). Many circadian rhythms have been described in a diverse range of species, from bacteria to human (3). However, the common molecular mechanism of the circadian clock in diverse species is totally unknown. In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus has been shown to be the circadian pacemaker (1, 2). Much effort is being directed to identify the master genes that control the circadian rhythm in the SCN. One of the strong candidates is the clock gene, because a mutation in the clock gene results in arrhythmic locomotor behavior (4, 5). The period (per) gene in Drosophila, which is expressed throughout the body in a circadian manner, regulates the circadian locomotor rhythm (6, 7). Recently two different homologs of Drosophila per gene were reported for mouse and human (8–11). Though the two mammalian per homologs show circadian mRNA oscillation in the mouse SCN, their functional involvement in the circadian locomotor activity has not yet been reported.

To examine whether a mammalian per homolog is involved in the circadian rhythm of locomotor behavior, we cloned a rat per homolog and monitored its circadian expression rhythms in peripheral tissues of SCN-lesioned rats that showed arrhythmic locomotor activity.

EXPERIMENTAL PROCEDURES

Animals—Adult male Wistar rats (10 weeks old; 300–350 g) were obtained from Clea Japan, Inc. (Tokyo) and were housed in a 12 h light–12 h dark cycle (LD12:12; lights on at zeitgeber time (ZT) 0) for at least 1 week before the day of the experiment. A white fluorescent lamp was used as a source of light during the day (150–200 lux at the level of the cages). In this study, we killed rats in accordance with institutional guidelines.

In Situ Hybridization—Animals used for in situ analysis were anesthetized with pentobarbital and were perfused from the left ventricle with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). Tissues were fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4) for 1 h at room temperature. The tissues were embedded in Tissue-Tek OCT compound (Miles). Eight-μm cryosections were cut. Digoxigenin-labeled RNA probes were generated from a rPer2 cDNA fragment (bases: 1–5453) using a DIG RNA labeling Kit (Boehringer Mannheim, catalog no. 1175 025). Hybridization and detection of the probes were carried out as described (12, 13).

Northern Blot Analysis—Rats were decapitated, and tissues were dissected, quickly frozen, and kept in liquid nitrogen until used. When we prepared tissues from individuals kept under constant darkness (DD) or constant light (LL) conditions, the animals were housed in LD12:12 and transferred to DD or LL. They were killed on the 3rd day after the transition. Total RNA was isolated from tissues by using isogen (a guanidine HCl/phenol procedure; Nippon Gene Co., Ltd., Japan) and separated on a 1% agarose, 0.7 M formaldehyde gel. Each lane contained 20 μg of total RNA from each tissue or 10 μg of total SCN RNA. A 32P-labeled random primed probe was generated from a rPer2 cDNA fragment (bases: 1–1892; GenBank accession no. AB016532) and hybridized and detected as described (14).

SCN Lesion—Rats were anesthetized with pentobarbital of 100 mg/kg of body weight and placed in a stereotaxic instrument. Bilateral electrolytic lesions of the SCN were made. Alternating current was passed for 20 s at 2.5 mA. The tip of the electrode to make a lesion was placed 1.3 mm anterior to bream, 0.3 mm lateral to the midline, and 10 mm below the dura. We monitored the locomotor behavior of the SCN-lesioned rats in constant darkness for at least 3 weeks with food and water available ad libitum. The locomotor activity of animals was detected by using the recording device comprising an infrared beam sensor (Omuron, Tokyo). Activity was defined as the number of interruptions of the infrared light beam by a moving rat within 8-min intervals.

RESULTS AND DISCUSSION

To establish a protocol for efficient analysis of genes encoding large proteins, we constructed a series of strictly size-fractionated cDNA libraries from human brain, where the average insert size of cDNA clones ranged from 3.3 to 10 kilobases.

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In the process of screening the cDNA libraries, we isolated a human per-like gene (16), designated as KIAA0347 (KIAA is an acronym used by the genome project of Kazusa DNA Research Institute). The amino acid sequence of KIAA0347 was different from that of another human per-like gene hPer1 (8, 9).

We noted the existence of a per-like gene family in the human gene, as in the mouse (8–11). Then we attempted to clone a rat homolog of KIAA0347 and succeeded in isolating such a clone (GenBank accession no. AB016532; Fig. 1). Amino acid identities of this rat clone with mouse per homologs, mPER1 and mPER2, were 39 and 95%, respectively. Thus we concluded that this clone corresponded to the per2 gene of the rat and designated it as rPer2. We also found that KIAA0347 was human per2. These findings suggest the existence of a per-like

![Fig. 1. Comparison of the predicted amino acid sequence of the rPER2 protein (GenBank accession no. AB016532) with mPER1 and mPER2 (per homologs from the mouse; GenBank accession numbers are AB002108 and AF036893, respectively). Identical and conserved amino acid residues are shaded in blue and yellow, respectively. The PAS A, PAS B, and the PAC motifs (10, 22) are underlined, and a potential basic helix-loop-helix (bHLH) motif is indicated by a dotted line. The putative nuclear localization signal (NLS) is indicated by a dotted line above the rPER2 sequence.](image-url)
gene family in mammals.

We first carried out histochemical in situ hybridization to determine the spatial and temporal expression pattern of rPer2 mRNA in the brain. The rPer2 mRNA was detected throughout the SCN of the hypothalamus (Fig. 2). The mRNA level was low at day and high at night. As in the SCN, a diurnal oscillation of rPer2 mRNA expression was detected in the pineal gland and cortex, but the mRNA level was constant in the hippocampus (data not shown).

To determine whether the diurnal oscillation of rPer2 mRNA level in the SCN is really circadian in nature, we compared the expression pattern under three light conditions: a light-dark cycle (LD), constant darkness (DD), and constant light (LL), by Northern blot analysis (Fig. 3). In LD, the highest expression occurred at ZT 14 in the night, and a similar rhythmic pattern was also observed in DD. The diurnal rhythm was found even in LL. These findings suggest that the rhythmic expression of rPer2 mRNA was circadian in nature.

Then we observed the expression pattern of rPer2 mRNA in tissues other than the SCN. Northern blot analysis was carried out on total RNAs from eye, brain excluding the SCN, heart, lung, spleen, liver, and kidney (Fig. 4c). To our surprise, all the tissues examined showed a rhythmic expression of rPer2 mRNA, although the night-to-day ratio was different between the tissues. In the mouse, the circadian expression of mPer1

and mPer2 has been examined so far only in the SCN, pars tuberalis, and eye (8–11). The rPer2 gene of the rat showed an expression pattern similar to that of the Drosophila period gene in that the circadian mRNA expression with peaks at night was observed throughout a wide variety of tissues in the body (7).

As the SCN is considered to be the circadian clock pacemaker in mammals, we next tested whether the multiple tissue circadian expression of rPer2 mRNA is affected by an SCN lesion. We prepared eight SCN-lesioned rats. Their locomotor behavior was monitored for at least 3 weeks in DD (Fig. 4a), and the locomotor activity was analyzed using a chi-square periodogram (Fig. 4b). Consequently, seven rats of eight showed arrhythmic behavior as a result of the lesion. The lesion was also confirmed histochemically (data not shown). Three rats were sacrificed during the day (ZT 6) and the other three at night (ZT 18). Surprisingly enough, the rhythmic multitissue expression of rPer2 mRNA was abolished by the SCN lesion (Fig. 4d). The multiple tissue expression of rPer2 was therefore under the control of the SCN. This is the first report suggesting that the multitissue circadian expression of a mammalian per-like gene was governed by the mammalian brain clock, the suprachiasmatic nucleus. These findings imply that some signals from the SCN coordinated the rPer2 rhythm in the whole body. An SCN transplantation study has also suggested the existence of humoral factors from the SCN to regulate the circadian locomotor activity (17, 18). Such humoral factors from the SCN might be involved in generating the circadian expression of the rPer2 gene in peripheral tissues. In Drosophila, several lines of evidence from genetically engineered flies also suggest that some specific neurons and glia cells in the brain may be the pacemakers (19–21).

The data also suggest that the rPer2 gene was involved in the circadian rhythm of locomotor behavior in mammals, because
the circadian expression of rPer2 mRNA in the whole body vanished simultaneously with the disappearance of the circadian locomotor activity. To clarify this possibility, we require further experiments using transgenic animals of the rPer2 gene. Monitoring the rhythmic expression of rPer2 in tissues might be useful to analyze harmonized circadian rhythm generation in the body of mammals.

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Note Added in Proof—Circadian expression of mPer2 mRNA in peripheral tissues has been shown independently in a report by Zylka et al. (Zylka, M. J., Shearman, L. P., Weaver, D. R., and Reppert, S. M. (1998) Neuron 20, 1103–1110).

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