Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 301 (2003) 1–7
BBRC
www.elsevier.com/locate/ybbrc

Structural and functional analysis of 3′ untranslated region of mouse *Period1* mRNA

Shihoko Kojima,[a,b] Matsumi Hirose,[a] Katsushi Tokunaga,[b] Yoshiyuki Sakaki,[a,c] and Hajime Tei[a,*]

[a] *Laboratory of Functional Genomics, Human Genome Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan*
[b] *Department of Human Genetics, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyou-ku, Tokyo 113-0033, Japan*
[c] *Human Genome Research Group, Genomic Sciences Center, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan*

Received 3 December 2002

Abstract

In order to investigate the post-transcriptional regulation of *Period1* (*Per1*), the 3′-untranslated region (3′UTR) of mouse *Per1* (m*Per1*) mRNA was characterized. In addition to high similarity between human and mouse *Per1* 3′UTRs, AU-rich element and differentiation control element were found in both species. Transient transfection assays using *luc*::m*Per1* 3′*UTR* fusion genes revealed that the m*Per1* 3′UTR repressed its own expression in a post-transcriptional manner. The region critical for this translational down-regulation was confined to nucleotide positions 322–517. These results suggest that the m*Per1* 3′UTR could be involved in the generation of time lag between the transcriptional and translational products of m*Per1* in the suprachiasmatic nucleus.

Keywords: Circadian rhythms; Mouse *Period1* mRNA; 3′-Untranslated region; Post-transcriptional regulation

Circadian oscillations of biological rhythms are generated by endogenous clocks, and the principal mammalian circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus [1,2]. Circadian clocks regulate the daily fluctuations of biochemical, physiological, and behavioral rhythms [3]. The rhythms persist under constant conditions and are predominantly entrained by the environmental light–dark cycles [4].

The mammalian *Period1* (*Per1*) gene has been identified as a structural homologue of *Drosophila* circadian clock gene, *period* (*per*) [5]. The expression of mouse *Per1* oscillates in the SCN and other peripheral tissues such as liver, lung, and skeletal muscle under both 12 h light–12 h dark (LD) and constant darkness (DD) conditions [5–7]. The transcription of *Per1* is activated by the Clock–*Bmal1* heterodimer through its binding to E-box sequences located in the *Per1* promoter region [8,9] and repressed by either Cry1 or Cry2 [10]. Indeed, both the behavioral rhythms and the oscillation of *Per1* were severely disrupted in *Per1* mutant mice as in the case of mutant mice of the *Per2*, *Cry1*, *Cry2*, *Clock*, and *Bmal1* genes [11–16]. These findings clearly demonstrate that the circadian oscillation of *Per1* expression is essential for the regulation of mammalian circadian clocks. The oscillation of *Per1* mRNA was at its peak circadian time (CT)/zeitgeber time (ZT) 4–6 and at its minimum CT/ZT 16. Whereas the amount of Per1 protein peaks around CT/ZT 12 and becomes minimum at CT/ZT 20 in the mouse SCN, 6–8 h delay is observed between the transcriptional and translational products of m*Per1* [17]. Similar time lag (4–6 h) between the expressions of *per* mRNA and Per protein had also been observed in the brain of *Drosophila* [18–20]. At present, the precise function and mechanisms underlying this long delay observed in both animals remain to be elucidated.

The 3′-untranslated regions (3′UTRs) in many kinds of mRNAs play crucial roles in the stability, localization,

* Corresponding author. Fax: +81-3-5449-5445.
E-mail address: tei@ims.u-tokyo.ac.jp (H. Tei).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(02)02938-8

and translational efficiency of its transcripts [21,22]. For example, AU-rich element (ARE) and cytoplasmic polyadenylation element (CPE) located in 3′UTRs can control the stability and translational efficiency of mRNA, respectively [21,23].

In this study, in order to elucidate the structure and function of the 3′UTR of m*Per1*, we determined the sequence of the m*Per1* 3′UTR and compared it with the human *Per1* (h*Per1*) 3′UTR. We found that the m*Per1* 3′UTR participates in the post-transcriptional regulation. Moreover, the region critical for such a post-transcriptional regulation within the m*Per1* 3′UTR was also determined by transient expression assays using a series of deletion mutants of *luc*::m*Per1* 3′UTR reporter genes.

Materials and methods

Isolation and sequencing of mPer1 3′UTR. The m*Per1* 3′UTR was cloned from mouse brain cDNA by a nested-PCR method. The first PCR was performed at 98 °C for 30 s as the initial step and then for 28 cycles of 94 °C for 15 s, 55 °C for 20 s, and 68 °C for 1 min, followed by a final extension of 1 cycle of 68 °C for 1 min. Then the second PCR was performed using 1/50 of the first PCR products at 98 °C for 30 s, and 23 cycles of 94 °C for 15 s, and 60 °C for 20 s, followed by a final extension of 1 cycle of 68 °C for 1 min. The sequences of primer pairs were: 5′-GCT CTA GAG CTA GAC TCC ATT TTG GG-3′ (forward primer for the first PCR), 5′-GCT CTA TCC ATT TTG GGG CCG CTT AC-3′ (forward primer for the second PCR), and *Bam*T (ACG) 5′-GGA TCC TTT TTT TTT TTT TTT V-3′ (V = one of A, C, or G) (reverse primers for the first and second PCRs). The m*Per1* 3′UTR was analyzed by sequencing using a Dye Terminator Cycle Sequencing kit (ABI) and an ABI PRISM 377 sequencer (ABI).

Construction of reporter genes and mutagenesis. pPLS containing the m*Per1-luc*-SV40 reporter gene was used in this study, which was reported previously [9]. A fragment covering a 6.7-kb region upstream of the translation–initiation codon of m*Per1* was ligated to the second codon of the *luciferase* gene flanked by the poly(A) signal of the SV40 late poly(A) signal in the pGL3-Basic vector (Promega). pPL3 was constructed by replacing the SV40 polyadenylation [poly(A)] signal in pPLS with the *Nhe*I/*Sal*I fragment of the m*Per1* 3′UTR.

pELSB was constructed by inserting the *Nhe*I/*Bam*HI fragment of pGL3-Basic vector harboring the luciferase ORF and SV40 late poly(A) signal form pGL3-Basic vector into pIND (Invitrogen). pEL3B was generated by replacing the m*Per1* 3′UTR with the SV40 late poly(A) signal fragment flanked by the *Xba*I/*Apa*I fragment in pELSB. Point mutations into the ARE of pEL3B were introduced by an inverse PCR-based method. pEL3B was amplified with mutated primer pairs [forward: 5′-TGA TGT AGT TGG GAG AGA CAG-3′ (mutated nucleotides are underlined), reverse: 5′-GTG CAG CCC CAA CCC TGC-3′] and KOD plus DNA polymerase (Toyobo). Then *Dpn*I treated PCR products were transformed into *Escherichia coli* DH5α. The resultant mutations in the ARE were verified by sequencing.

Transient expression assay. Mouse NIH3T3 cells (JCBR0615) obtained from Health Science Research Resources Bank (HSRRB) were grown in Dulbecco's minimum essential medium (Nissui Co.) with 10% fetal bovine serum (JRH Biosciences) at 37 °C with 5% CO_2. Cells were seeded in 12-well plates at a density of 1×10^5 cells per well 1 day prior to transfection. Transfection was carried out using FuGENE6 (Roche) according to manufacturer's instruction. Cells were washed with phosphate-buffered saline (PBS) and harvested in 150 μl of passive lysis buffer (Promega) 48 h after the transfection. For induction of ecdysone-mediated transcription, muristerone A (5 μM) (Invitrogen) was added to the culture medium 20 h prior to harvesting the cells. Luciferase activities were assayed with the Dual-Luciferase Reporter Assay System (Promega) using Turner Designs Model 20 luminometer (Turner). The *Renilla* luciferase plasmid was cotransfected to normalize each transfection assay. In the experiments examining the m*Per1*-driven expression, each transfection mixture contained 10 ng pPLS or pPL3, 0.5 ng pRL-CMV, 165 ng each of pCI-neo-m*Clock* and pCI-neo-m*Bmal1* expression vectors, and pCI-neo (Promega) to obtain a total amount of 670.5 ng DNA per well. In the case of the experiments utilizing an ecdysone inducible system, cells were transfected with 10 ng pELSB, pEL3B, or several deletion mutants of pEL3B, 0.5 ng pRL-CMV, 10 ng pVgEcR-RXR, and pCI-neo to obtain a total amount of 500 ng of DNA per well.

Real-time RT-PCR analysis. NIH3T3 cells were transfected as described above. Total RNA was extracted with TRIZOL reagent (Gibco/BRL), followed by DNaseI treatment twice (Takara). A TaqMan EZ RT-PCR kit (Applied Biosystems) was used for reverse transcription of 3 μg RNA. The levels of firefly and *Renilla* luciferase mRNA were examined by TaqMan Real-Time PCR (ABI PRISM 7900) (Applied Biosystems) using a SYBR Green PCR kit (PE Biosystems). The following primers were designed using a PrimerExpress software (ABI): pGL3-lucF, 5′-AAC ATA AAG AAA GGC CGG C-3′; pGL3-lucR, 5′-GCC TTA TGC AGT TGC TCT CCA-3′; pRL-lucF, 5′-ACA TGG TAA CGC GGC CTC TT-3′; and pRL-lucR, 5′-TGC CCA TAC CAA TAA GGT CTG GTA-3′. The amount of the firefly luciferase mRNA was normalized against that of the *Renilla* luciferase mRNA, and the relative mRNA abundance was calculated using the comparative delta-Ct method according to manufacturer's instruction.

Results

Structural comparison between human and mouse Per1 3′UTR

The 3′UTR of the m*Per1* mRNA was analyzed by a nested-PCR method. Sequence analyses of PCR products showed that the length of m*Per1* 3′UTRs was 601 nt long (Fig. 1) and that the 3′UTR was followed by poly(A) stretch, which was not encoded in the correspondent genomic sequences [9]. Based on these findings, we concluded that the m*Per1* 3′UTR we determined here contained the entire sequence.

The identity of the 3′UTR sequences between m*Per1 and* h*Per1* [24] was 78.0%, clearly indicating the structural conservation of the *Per1* 3′UTRs in both species (Fig. 1). In addition, a database search (http://bighost.area.ba.cnr.it/BIG/UTRHome/) revealed that the presence and absence of signals which may be involved in post-transcriptional regulations were also conserved between the human and mouse *Per1* 3′UTRs. Particularly, ARE, which regulates mRNA degradation, was located approximately 340 nt downstream from the stop codon in both the human and mouse *Per1* transcripts (Fig. 1). In addition, 15-LOX-DICE, which regulates the stability and translation of mRNA through its interaction with hnRNP E1 and hnRNP K [25], was also found approximately 180 nt

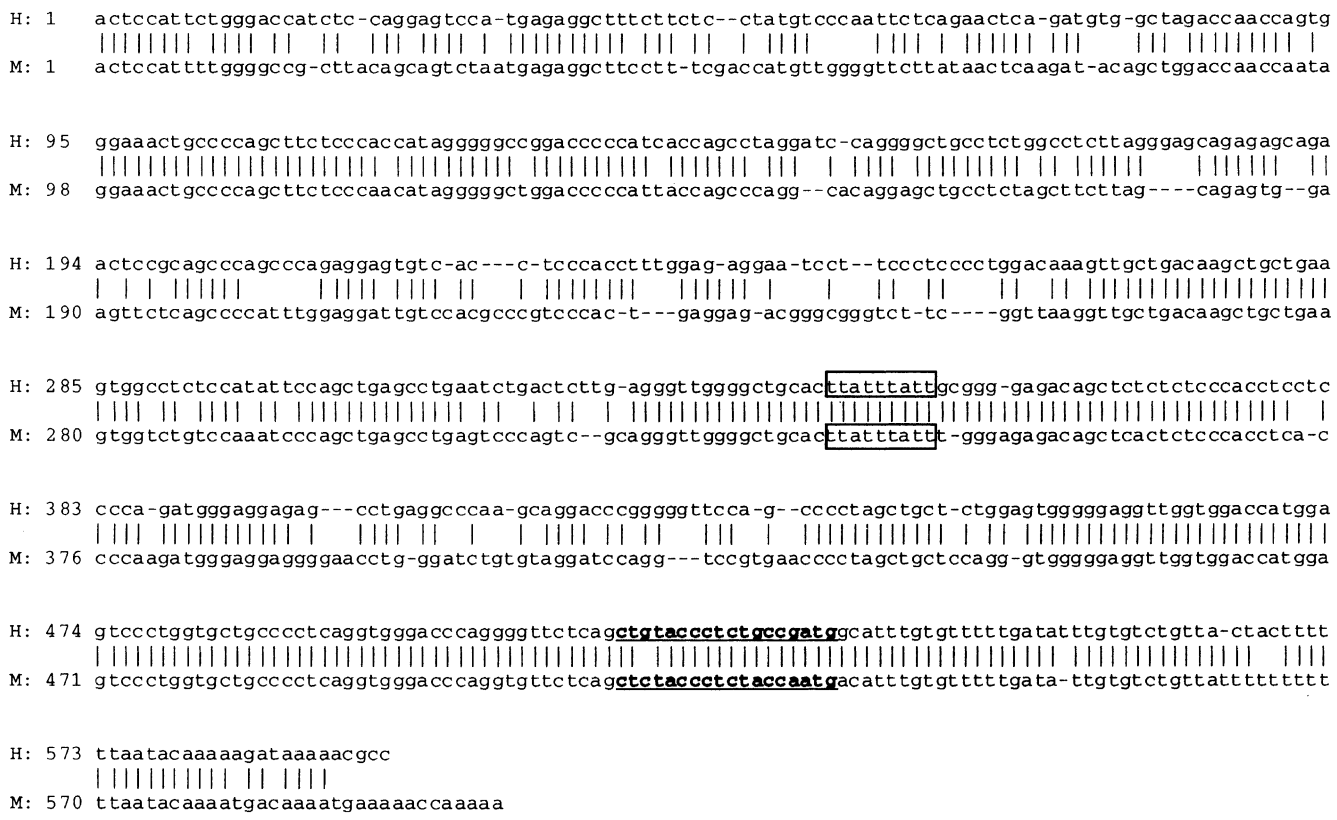

Fig. 1. Sequence alignments of the human and mouse *Per1* 3′UTRs. H and M stand for the human and mouse sequences, respectively. AREs are represented by boxes and 15-LOX-DICEs are indicated by underlines.

downstream of the ARE in both 3′UTRs (Fig. 1). The m*Per1* 3′UTR contained neither CPE nor typical polyadenylation signal (AAUAAA) as in the h*Per1* 3′UTR [24].

Post-transcriptional regulation by mPer1 3′UTR

In order to examine the function of the m*Per1* 3′UTR, a fusion gene possessing the m*Per1* promoter and the *luciferase* reporter [9] was used in this study. The expression of pPLS is induced by Clock and Bmal1, and repressed by either Cry1 or Cry2 in vitro [9], and exhibits a robust circadian oscillation in vivo [7]. pPL3 was constructed by replacing the SV40 poly(A) signal in pPLS with the entire 3′UTR of m*Per1* gene.

When pPLS and pPL3 plasmids were transfected into mouse NIH3T3 cells, the level of luciferase activity of pPL3 was significantly lower than that of pPLS in the presence and absence of mClock and mBmal1. When the luciferase activity level of pPLS was given as 100%, the luminescence level of pPL3 attained 13.0–15.0%, regardless of the transcriptional induction mediated by mClock and mBmal1 (Fig. 2B). The amounts of the fusion gene transcripts were determined by a real-time quantitative RT-PCR method. Interestingly, no significant difference of the mRNA levels between pPL3 and pPLS was detected in the presence and absence of the induction by mClock and mBmal1 (Fig. 2C). These results clearly indicate that the m*Per1* 3′UTR in pPL3 is involved in the suppression of the reporter gene expression at the stage of post-transcription, rather than mRNA degradation.

To eliminate the interference of the m*Per1* 5′UTR with the expression of reporter genes in both of the fused gene transcripts, another pair of reporter plasmids was constructed and studied further. The *luciferase* genes linked to either the m*Per1* 3′UTR or the SV40 poly(A) signal were inserted between a segment of the heat shock protein minimal promoter, which contains five ecdysone/glucocorticoid-responsive elements (E/GREs) and the bovine growth hormone (BGH) poly(A) signal in the pIND vector. These reporter genes were designated as pELSB (ecdysone-Luc-SV40-BGH) and pEL3B (ecdysone-Luc-m*Per1* 3′UTR-BGH), respectively (Fig. 3A). The activation of transcription of the reporter genes in NIH3T3 cells completely depends on both the expression of the ecdysone receptor (pVgEcR-RXR) gene and the treatment with ecdysone (muristerone A). Accordingly, the luciferase activity was not induced without muristerone A treatment in both pELSB and pEL3B (data not

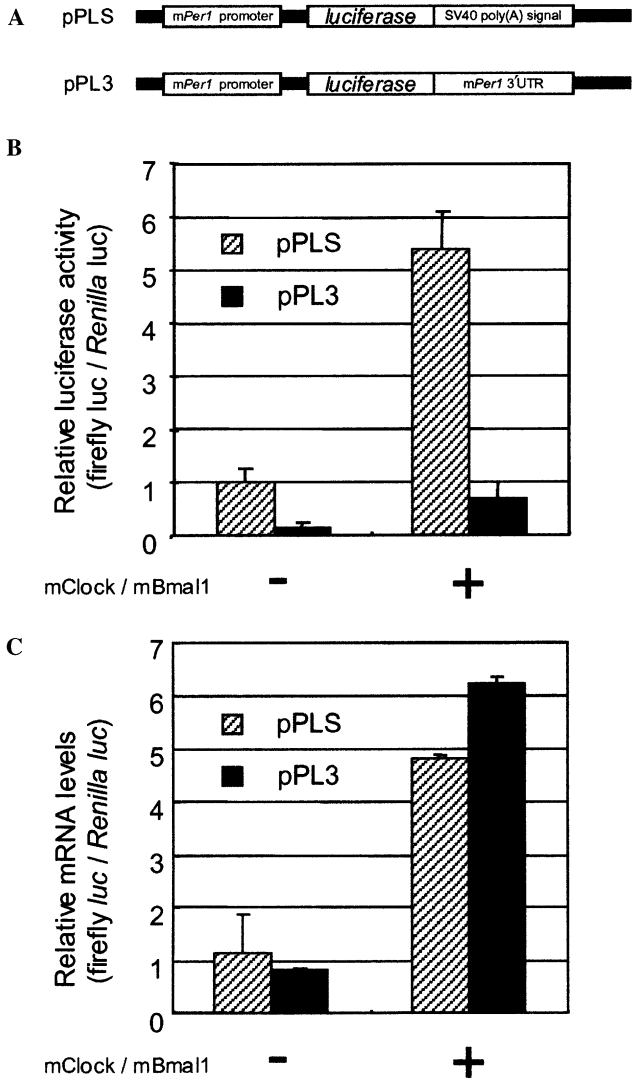

Fig. 2. Association of m*Per1* 3′UTR with the post-transcriptional regulation examined by pPLS and pPL3. (A) Schematic representation of chimeric reporter genes. (B) Relative luciferase activities of pPLS and pPL3. NIH3T3 cells were transiently transfected with either pPLS or pPL3 and dual-luciferase assay was carried out. *Renilla luciferase* reporter gene (pRL-CMV) was cotransfected as a control. The results (means ± SEM) are presented as ratios of firefly luciferase activity to *Renilla* luciferase activity. The experiments in the absence and presence of mClock/mBmal1 are indicated by − and +, respectively. (C) Relative levels of reporter mRNAs. The amounts of firefly and *Renilla* luciferase transcripts were measured by a TaqMan RT-PCR. The data (means ± SEM) are expressed as a ratio of firefly *luc* mRNA/*Renilla luc* mRNA.

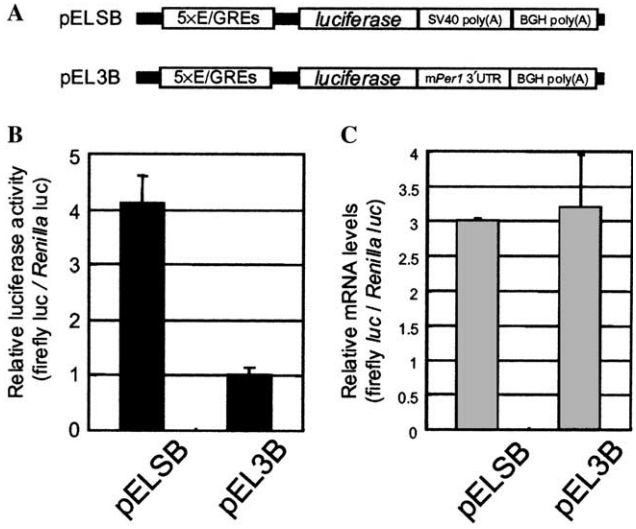

Fig. 3. Association of m*Per1* 3′UTR with the post-transcriptional regulation examined by pELSB and pEL3B. (A) Schematic representation of chimeric reporter gene. (B) Relative luciferase activities of pELSB and pEL3B. NIH3T3 cells were transiently transfected with either pELSB or pEL3B, and dual-luciferase assay was carried out. *Renilla luciferase* reporter gene (pRL-CMV) was also transfected as a control. The results (means ± SEM) are presented as ratios of firefly luciferase activity to *Renilla* luciferase activity. (C) Relative levels of reporter mRNAs. The amounts of firefly and *Renilla* luciferase transcripts were measured by a TaqMan RT-PCR. The data (means ± SEM) are expressed as a ratio of firefly *luc* mRNA/*Renilla luc* mRNA.

shown). As shown in Fig. 3B, pEL3B harboring the m*Per1* 3′UTR represented approximately 24.2% of the luciferase luminescence level as compared with the provisional 100% of the luciferase luminescence level in pELSB. In contrast, two reporter genes expressed almost equal amounts of their transcripts. These observations are consistent with the data obtained in Fig. 2. Consequently, these data confirm the idea that the m*Per1* 3′UTR contributes to the post-transcriptional regulation of its transcripts.

Functional analysis of mPer1 3′UTR for post-transcriptional repression

To define the segment in the 3′UTR of the m*Per1* mRNA that ensures the post-transcriptional repression, we constructed systematic deletion mutants of the pEL3B (Fig. 4A). These mutants were then tested for their abilities to suppress the expression of the reporter genes (Fig. 4A). The attachment of the BGH poly(A) signal to the 3′ termini of all the reporter genes guarantees polyadenylation of their transcripts deprived of the potential poly(A) signal(s) in the m*Per1* 3′UTR. We found that the reporter transcripts fused to nucleotides 1–601 (pEL3B), nucleotides 144–601 (I), and nucleotides 322–601 (II) of the m*Per1* 3′UTR reduced the luciferase activity to 24.2–28.9% (Fig. 4A) of that obtained in pELSB. In contrast, the reporter transcripts fused to nucleotides 509–601 (III) restored the luciferase expression (66.8% of pELSB). The critical region for the post-transcriptional control was further delimited from the 3′ terminus of the m*Per1* 3′UTR. The luciferase activity expressed from the reporter transcript fused to nucleotides 1 to 517 (IV) remained at a low level, and showed a sharp contrast with the high activities observed in the

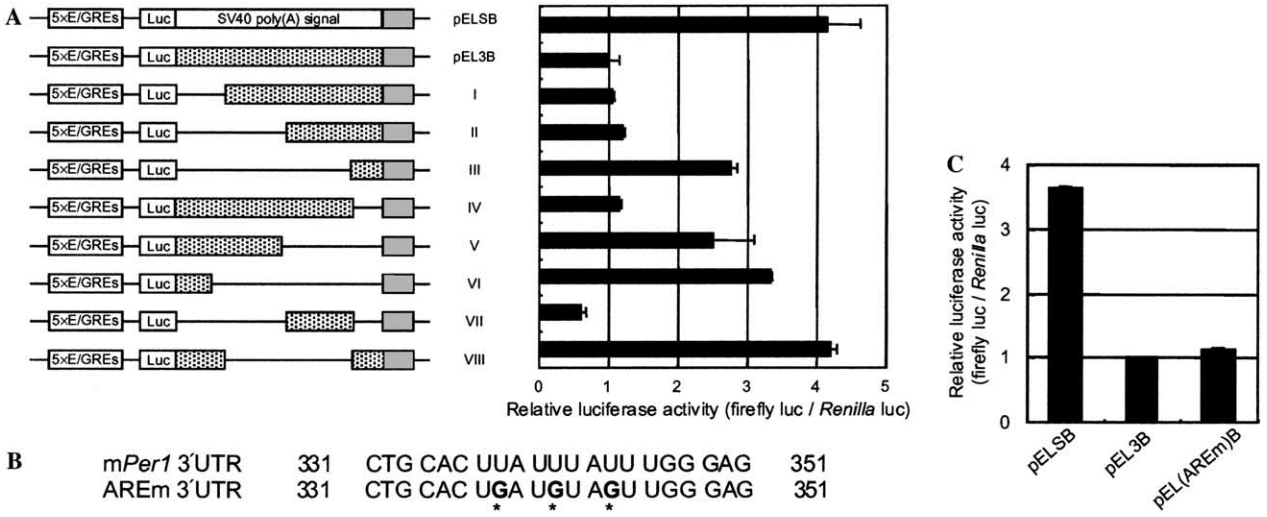

Fig. 4. *Cis*-element in the m*Per1* 3′UTR associated with the post-transcriptional regulation. (A) Luciferase activities of a series of m*Per1* 3′UTR deletion mutants. The m*Per1* 3′UTR deletion constructs containing firefly *luciferase* gene (Luc) driven by the minimal promoter containing five E/GREs are schematically shown on the *left*. Dotted and gray boxes represent the m*Per1* 3′UTR and BGH poly(A) signal, respectively. The luciferase acivities of the constructs in NIH3T3 cells are shown in the *right* graph. The firefly luciferase luminescence was normalized by the *Renilla* luciferase activities (pRL-CMV). The data (means ± SEM) are represented as rations of firefly luciferase activity to *Renilla* luciferase activity. (B) Schematic representation of mutated ARE in the m*Per1* 3′UTR. Asterisks indicate the position of mutated nucleotides (U to G). (C) Luciferase activities of ARE-mutated reporter gene. pEL(AREm)B with the mutated ARE as well as pELSB or pEL3B were cotransfected with pRL-CMV in NIH3T3 cells. The luciferase assay was carried out as in Fig. 3B. The relative luciferase activities are shown as means ± SEM.

reporter transcripts fused to nucleotides 1–308 (V) and nucleotides 1–105 (VI). In addition, the reporter transcript fused to nucleotides 322–517 (VII) efficiently reduced the level of enzymatic activity to 14.6%, whereas the reporter lacking nucleotides 143–517 (VIII) remained unaffected. These results demonstrated that the 196-nt segment from nucleotide positions 322–517 in the m*Per1* 3′UTR was sufficient for the post-transcriptional repression in NIH3T3 cells.

We observed that one ARE was included in the 196-nt segment associated with the post-transcriptional repression, and that not only the sequence but also its position was conserved between the human and mouse *Per1* 3′UTRs (Fig. 1). Since ARE contributes to the deadenylation and turnover of mRNA [26,27], we examined whether ARE contributed to the repression of the reporter gene expression using ARE mutant of pEL3B, pEL(AREm)B (Fig. 4B). As a result, there was no significant difference in the luciferase activity between the wild type (pEL3B) and mutant type of ARE [pEL(AREm)B], indicating that ARE is not necessary for the post-transcriptional repression (Fig. 4C). Taking all the data together, these observations indicate that the 196-nt segment other than ARE in the m*Per1* 3′UTR has a critical role in the post-transcriptional repression. Putative regulator(s) such as RNA-binding protein(s) would interact with the 196-nt region to suppress the m*Per1* expression post-transcriptionally. It would be of great interest to verify such regulatory molecule(s) in both NIH3T3 cells and SCN.

Discussions

In all organisms where the circadian clock has been examined at the molecular level, oscillating molecules in a circadian manner appear to form the basis for generating rhythms. In this study, we characterized the m*Per1* 3′UTR and compared it with the h*Per1* 3′UTR. The 3′UTR structure of *Per1* was highly conserved in both the human and mice species. In addition, analyses using the *luc*::m*Per1* 3′UTR fusion genes revealed that the m*Per1* 3′UTR contained *cis*-element(s) for the post-transcriptional repression of the reporter gene expression in NIH3T3 cells. The sequence responsible for the post-transcriptional regulation was present in the 196-nt segment (nucleotides 322–517) of the m*Per1* 3′UTR. Because rapid turnover of the transcript carrying the m*Per1* 3′UTR was not observed, the degradation of the transcripts was hardly associated with the post-transcriptional control.

In *Drosophila*, the daily fluctuations of the transcriptional and translational products of *per* in the brain are indispensable for the maintenance of circadian rhythms. In addition, the time lag (4–6 h) has been observed between the circadian cycling of the *per* mRNA and Per protein in the brain, with the peak of the *per* mRNA at CT/ZT 12–16 and that of the Per protein at about CT/ZT 18–22 [18–20]. Indeed, the expression of d*per* is regulated by post-transcriptional mechanisms [28,29] and the circadian cycling of dPer has been revealed to be dependent on the 3′UTR of the d*per*

mRNA, in spite of the fact that the signals for the post-transcriptional regulation within the d*per* 3′ UTR have not been determined [28].

Similar circadian control at translation has also been observed in the expression of the luciferin-binding protein (*lbp* expression of *Gonyaulax*. LBP synthesis might be regulated by the 22-nt RNA segment in the *lbp* 3′UTR and this 22-nt segment contains an UG-containing sequence which serves as a binding site for regulatory proteins in a circadian manner [30].

Recent studies of *Arabidopsis*, *Drosophila*, and mouse using DNA chips or microarrays have determined that the transcription of several genes encoding RNA-binding proteins is under the control of circadian clocks [31–34]. These data indicate that the post-transcriptional regulation of gene expression by these RNA-binding proteins might be involved in the mammalian circadian clock system, although little is known about the roles for these RNA-binding proteins in the translational regulation. RNA-binding proteins that activate or repress the gene expression at the stage of translation often bind to the UTRs of mRNA. Proteins that interact with the 196-nt segment of m*Per1* 3′UTR may be involved in circadian control of the expression. Therefore, the isolation and identification of the RNA-binding protein, which binds to 196-nt segment of the m*Per1* 3′UTR, may shed further light on the role of the translational regulation in the mammalian circadian system and remain to be elucidated.

Acknowledgments

We thank Drs. Naoyuki Tsuchiya, Hirohiko Hohjoh, and Jun Ohashi for helpful discussions. This work was partially supported by research grants from the Japanese Ministry of Education, Science, Sports and Culture, and the Japanese Ministry of Health and Welfare.

References

[1] R.Y. Moore, V.B. Eichler, Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat, Brain Res. 42 (1972) 201–206.
[2] A.N. van den Pol, F.E. Dudek, Cellular communication in the circadian clock, the suprachiasmatic nucleus, Neuroscience 56 (1993) 793–811.
[3] C.S. Pittendrigh, Temporal organization: reflections of a Darwinian clock-watcher, Annu. Rev. Physiol. 55 (1993) 16–54.
[4] J.S. Takahashi, Molecular neurobiology and genetics of circadian rhythms in mammals, Annu. Rev. Neurosci. 18 (1995) 531–553.
[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] Z.S. Sun, U. Albrecht, O. Zhuchenko, J. Bailey, G. Eichele, C.C. Lee, *RIGUI*, a putative mammalian ortholog of the *Drosophila period* gene, Cell 90 (1997) 1003–1011.
[7] S. Yamazaki, R. Numano, M. Abe, A. Hida, R. Takahashi, M. Ueda, G.D. Block, Y. Sakaki, M. Menaker, H. Tei, Resetting central and peripheral circadian oscillators in transgenic rats, Science 288 (2000) 682–685.
[8] N. Gekakis, D. Staknis, H.B. Nguyen, F.C. Davis, L.D. Wilsbacher, D.P. King, J.S. Takahashi, C.J. Weitz, Role of the CLOCK protein in the mammalian circadian mechanism, Science 280 (1998) 1564–1569.
[9] A. Hida, N. Koike, M. Hirose, M. Hattori, Y. Sakaki, H. Tei, The human and mouse *Period1* genes: five well-conserved E-boxes additively contribute to the enhancement of m*Per1* transcription, Genomics 65 (2000) 224–233.
[10] 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.
[11] K. Bae, X. Jin, E.S. Maywood, M.H. Hastings, S.M. Reppert, D.R. Weaver, Differential functions of m*Per1*, m*Per2*, and m*Per3* in the SCN circadian clock, Neuron 30 (2001) 525–536.
[12] B. Zheng, U. Albrecht, K. Kaasik, M. Sage, W. Lu, S. Vaishnav, Q. Li, Z.S. Sun, G. Eichele, A. Bradley, C.C. Lee, Nonredundant roles of the m*Per1* and m*Per2* genes in the mammalian circadian clock, Cell 105 (2001) 683–694.
[13] N. Cermakian, L. Monaco, M.P. Pando, A. Dierich, P. Sassone-Corsi, Altered behavioral rhythms and clock gene expression in mice with a targeted mutation in the *Period1* gene, EMBO J. 20 (2001) 3967–3974.
[14] G.T. van der Horst, M. Muijtjens, K. Kobayashi, R. Takano, S. Kanno, M. Takao, J. de Wit, A. Verkerk, A.P. Eker, D. van Leenen, R. Buijs, D. Bootsma, J.H. Hoeijmakers, A. Yasui, Mammalian *Cry1* and *Cry2* are essential for maintenance of circadian rhythms, Nature 398 (1999) 627–630.
[15] M.H. Vitaterna, D.P. King, A.M. Chang, J.M. Kornhauser, P.L. Lowrey, J.D. McDonald, W.F. Dove, L.H. Pinto, F.W. Turek, J.S. Takahashi, Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior, Science 264 (1994) 719–725.
[16] M.K. Bunger, L.D. Wilsbacher, S.M. Moran, C. Clendenin, L.A. Radcliffe, J.B. Hogenesch, M.C. Simon, J.S. Takahashi, C.A. Bradfield, Mop3 is an essential component of the master circadian pacemaker in mammals, Cell 103 (2000) 1009–1017.
[17] M.D. Field, E.S. Maywood, J.A. O'Brien, D.R. Weaver, S.M. Reppert, M.H. Hastings, Analysis of clock proteins in mouse SCN demonstrates phylogenetic divergence of the circadian clockwork and resetting mechanisms, Neuron 25 (2000) 437–447.
[18] P.E. Hardin, J.C. Hall, M. Rosbash, Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels, Nature 343 (1990) 536–540.
[19] I. Edery, L.J. Zwiebel, M.E. Dembinska, M. Rosbash, Temporal phosphorylation of the *Drosophila* period protein, Proc. Natl. Acad. Sci. USA 91 (1994) 2260–2264.
[20] H. Zeng, P.E. Hardin, M. Rosbash, Constitutive overexpression of the *Drosophila* period protein inhibits period mRNA cycling, EMBO J. 13 (1994) 3590–3598.
[21] E.A. Grzybowska, A. Wilczynska, J.A. Siedlecki, Regulatory functions of 3′UTRs, Biochem. Biophys. Res. Commun. 288 (2001) 291–295.
[22] D. St Johnston, The intracellular localization of messenger RNAs, Cell 81 (1995) 161–170.
[23] G. Seydoux, Mechanisms of translational control in early development, Curr. Opin. Genet. Dev. 6 (1996) 555–561.
[24] D. Taruscio, G.K. Zoraqi, M. Falchi, F. Iosi, S. Paradisi, B. Di Fiore, P. Lavia, V. Falbo, The human *per1* gene: genomic organization and promoter analysis of the first human orthologue of the *Drosophila* period gene, Gene 253 (2000) 161–170.
[25] I. Reimann, A. Huth, H. Thiele, B.J. Thiele, Suppression of 15-lipoxygenase synthesis by hnRNP E1 is dependent on repetitive nature of LOX mRNA 3′-UTR control element DICE, J. Mol. Biol. 315 (2002) 965–974.

[26] A.M. Zubiaga, J.G. Belasco, M.E. Greenberg, The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation, Mol. Cell. Biol. 15 (1995) 2219–2230.
[27] N. Xu, C.Y. Chen, A.B. Shyu, Modulation of the fate of cytoplasmic mRNA by AU-rich elements: key sequence features controlling mRNA deadenylation and decay, Mol. Cell. Biol. 17 (1997) 4611–4621.
[28] Y. Chen, M. Hunter-Ensor, P. Schotland, A. Sehgal, Alterations of per RNA in noncoding regions affect periodicity of circadian behavioral rhythms, J. Biol. Rhythms 13 (1998) 364–379.
[29] W.V. So, M. Rosbash, Post-transcriptional regulation contributes to *Drosophila* clock gene mRNA cycling, EMBO J. 16 (1997) 7146–7155.
[30] M. Mittag, D.H. Lee, J.W. Hastings, Circadian expression of the luciferin-binding protein correlates with the binding of a protein to the 3′ untranslated region of its mRNA, Proc. Natl. Acad. Sci. USA 91 (1994) 5257–5261.
[31] S. Panda, M.P. Antoch, B.H. Miller, A.I. Su, A.B. Schook, M. Straume, P.G. Schultz, S.A. Kay, J.S. Takahashi, J.B. Hogenesch, Coordinated transcription of key pathways in the mouse by the circadian clock, Cell 109 (2002) 307–320.
[32] K.F. Storch, O. Lipan, I. Leykin, N. Viswanathan, F.C. Davis, W.H. Wong, C.J. Weitz, Extensive and divergent circadian gene expression in liver and heart, Nature 417 (2002) 78–83.
[33] M.J. McDonald, M. Rosbash, Microarray analysis and organization of circadian gene expression in *Drosophila*, Cell 107 (2001) 567–578.
[34] R.A. Gutierrez, R.M. Ewing, J.M. Cherry, P.J. Green, Identification of unstable transcripts in *Arabidopsis* by cDNA microarray analysis: rapid decay is associated with a group of touch- and specific clock-controlled genes, Proc. Natl. Acad. Sci. USA 99 (2002) 11513–11518.