Brief Communication

Mouse Period1 (mPER1) Acts as a Circadian Adaptor to Entrain the Oscillator to Environmental Light/Dark Cycles by Regulating mPER2 Protein

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***Mouse period1* (*mPer1*) and *mPer2* are mammalian homologs of the *Drosophila* clock gene *period* that show robust oscillation in the suprachiasmatic nucleus, the mammalian master clock, and have been implicated as essential components of the core clock mechanism. Gene-targeting studies have demonstrated that *mPer2* plays a dominant function in behavioral rhythm generation, although the role of *mPer1* has not been fully clarified. Here, we report that prolongation of the lighting period (4–16 h) induces a larger-delay phase shift of the behavioral rhythm in *mPer1*-deficient (*mPer1*$^{-/-}$) mice. During the light-elongation task, mPER2 protein decay in *mPer1*$^{-/-}$ mice is slower (~4 h) than in wild-type mice, which thereby causes larger behavioral phase delay. *mPer1*$^{-/-}$ mice could not adapt to environmental light/dark cycles in long complete photoperiods with dim light or in long skeleton photoperiods. These photoperiodic conditions mimic natural environmental changes present at high latitudes, indicating that *mPer1* could operate in the adaptation of the circadian clock of nocturnal mice to large seasonal changes of environmental light/dark cycles.**

Key words: **hypothalamus; suprachiasmatic; circadian; behavior; *mPer1*; *mPer2***

Introduction

Mouse period1 (*mPer1*) and *mPer2* are mammalian homologs of the *Drosophila* clock gene *period* (for review, see Dunlap, 1999) that show robust oscillation in the suprachiasmatic nucleus (SCN), the mammalian master clock (Klein et al., 1991), and have been implicated as essential components of the core clock mechanism. Gene-targeting studies have demonstrated that *mPer2* knock-out mice are arrhythmic in constant darkness (DD) (Zheng et al., 1999; Bae et al., 2001), whereas *mPer1* knock-out (*mPer1*$^{-/-}$) mice elicit a persistent rhythmicity with a slightly shorter period length (Bae et al., 2001; Cermakian et al., 2001; Zheng et al., 2001). Although it is demonstrated that *mPer1* has a crucial role for rhythm generation in peripheral clocks (Cermakian et al., 2001; Pando et al., 2002), its function in the central oscillator remains unclear.

Phase shifting by light is an essential feature of circadian rhythms (Daan and Pittendrigh, 1976a). The phase-shift profiles arising from short exposure to light do not show a difference between *mPer1*$^{-/-}$ and wild-type (*mPer1*$^{+/+}$) mice (Cermakian et al., 2001; Spoelstra et al., 2004), although a decrease in phase advances was noted just after the move to DD (Albrecht et al., 2001; Spoelstra et al., 2004). Because it is considered that the larger the duration of light exposure, the greater the phase shift that occurs (Daan and Pittendrigh, 1976b), in the present study, we adopted long light exposure as a task for revealing the role of *mPer1* to detect the difference between *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice. Here, we found that the magnitude of phase delays arising from long light in *mPer1*$^{-/-}$ mice was larger than that of wild-type mice, accompanying the delay of mPER2 protein decay without effecting a change at the mRNA level in the initial phase during the light exposure. The altered core clock machinery in these mice was evident in long complete photoperiods with dim light or in long skeleton photoperiods in which animals show free-running rhythms not adapting to environmental cycles.

Materials and Methods

Animals and behavioral rhythm monitoring. *mPer1*$^{+/+}$, heterozygous mutant (*mPer1*$^{+/-}$), and *mPer1*$^{-/-}$ mice (Cermakian et al., 2001) were bred and housed under light/dark (LD) 12 h cycles (fluorescent light, 200–300 lux). Locomotor activity was detected by passive (pyroelectric) infrared sensors (FA-05 F5B; Omron, Kyoto, Japan) (Shigeyoshi et al., 1997). Data were monitored and analyzed as described previously (Masubuchi et al., 2000) by Chronobiology kit (Stanford Software Systems, Stanford, CA). The experimental protocol of the current research was approved by the Committee for Animal Research at Kobe University.

In situ *hybridization.* *In situ* hybridization histochemistry using the free-floating sections was performed according to the method detailed

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previously (Shigeyoshi et al., 1997). We used ^{33}P-radiolabeled cRNA probes for *mPer2* (Takumi et al., 1998) and albumin gene D–site-binding protein (*dbp*) (Yamaguchi et al., 2000) for the *in situ* hybridization studies. The peak value of *mPer1* $^{+/+}$ mice was adjusted to 100, and relative RNA abundance was used.

Immunocytochemistry. Immunocytochemistry was performed with the avidin–biotin–peroxidase method applied to free-floating sections (Ban et al., 1997). Serial frontal sections (30 μm thick) from the rostral end to the caudal end of the SCN were incubated for immunostaining with 1 μg/ml anti-mPER2 (affinity-purified rabbit antisera; Alpha Diagnostic International, San Antonio, TX) (Matsuo et al., 2003), which was finally visualized brown with 3,3′-diaminobenzidine tetrahydrochloride. We counted the mean number of immunoreactive cells in three sections in the middle portion of the SCN. For each point, we used four animals.

Statistical analysis. The 24 h mRNA and protein variations were statistically tested by one-way ANOVA. The effects of genotypes on the behavioral rhythm onsets were tested by two-way ANOVA. A *post hoc* Bonferroni/Dunn test was used for the comparison between the values of *Per1* $^{+/+}$, *Per1* $^{+/-}$, and *Per1* $^{-/-}$ groups at the same time points. The effects of genotypes on the entrainment daily lighting tasks (complete and skeleton photoperiod) were tested by Fisher's exact probability test.

Results

Extended light exposure induces larger phase delay of behavioral rhythm in *mPer1* $^{-/-}$ mice

mPer1 $^{+/+}$, *mPer1* $^{+/-}$, and *mPer1* $^{-/-}$ mice (Cermakian et al., 2001) entrained to an LD cycle (12 h; fluorescent light, 200–300 lux) were exposed to 0, 4, 8, 12, and 16 h of light prolongation (LP) (task LP00, LP04, LP08, LP12, and LP16, respectively) from lights off [Zeitgeber time 12 (ZT12)] at the last day of LD. Afterward, mice were kept in DD. We defined the lights-off points of each task as L00 (corresponding to ZT12), L04 (ZT16), L08 (ZT20), L12 (ZT24), and L16 (ZT28, ZT4 of the next cycle), respectively. The phase-delay effect of the prolongation of light of the last day on the behavioral rhythm was evaluated using extrapolated eye-fitted lines made by >10 d of activity onsets. Only the switch from LD to DD does not shift the onset of the extrapolated rhythm in both in *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice, when there was no light prolongation (LP00) (Fig. 1*A*, left). The onset of the behavioral rhythm was delayed in proportion to the increase of the duration of the light exposure (LP04, LP08, LP12) in all three groups (*mPer1* $^{+/+}$, *mPer1* $^{+/-}$, and *mPer1* $^{-/-}$ mice; one-way ANOVA; $p < 0.0001$) (Fig. 1*B*). The phase-delay effect peaked at 12 h (LP12) and then decreased at 16 h (LP16). Interestingly, depending on the genotype, the elongation of light exposure differentially affects the magnitude of the phase delay. As shown in Figure 1*A*, when mice were exposed to light prolongation for 12 h (LP12) for 1 d, the onset of activity rhythm in *mPer1* $^{-/-}$ mice was shifted by ~12 h, strikingly different from *mPer1* $^{+/+}$ mice (~5 h). The effects of elongation of light exposure on the phase delay were significantly different between genotypes (two-way ANOVA; *mPer1* $^{-/-}$ vs *mPer1* $^{+/+}$, $p < 0.0001$; *mPer1* $^{-/-}$ vs *mPer1* $^{+/-}$, $p < 0.0001$; *mPer1* $^{+/+}$ vs *mPer1* $^{+/-}$, $p < 0.01$): the behavioral phase delay is much larger in *mPer1* $^{-/-}$ than *mPer1* $^{+/+}$ ($p < 0.0001$ in LP04, LP08, LP12, and LP16 tasks) and than *mPer1* $^{+/-}$ ($p < 0.0001$ in LP08, LP12, and LP16 tasks) and slightly larger in *mPer1* $^{+/-}$ than *mPer1* $^{+/+}$ ($p < 0.0001$ in LP16 task) (Fig. 1*B*). The prolongation of light exposure (4–16 h) produces larger phase delay of behavioral rhythm in *mPer1* $^{-/-}$ mice.

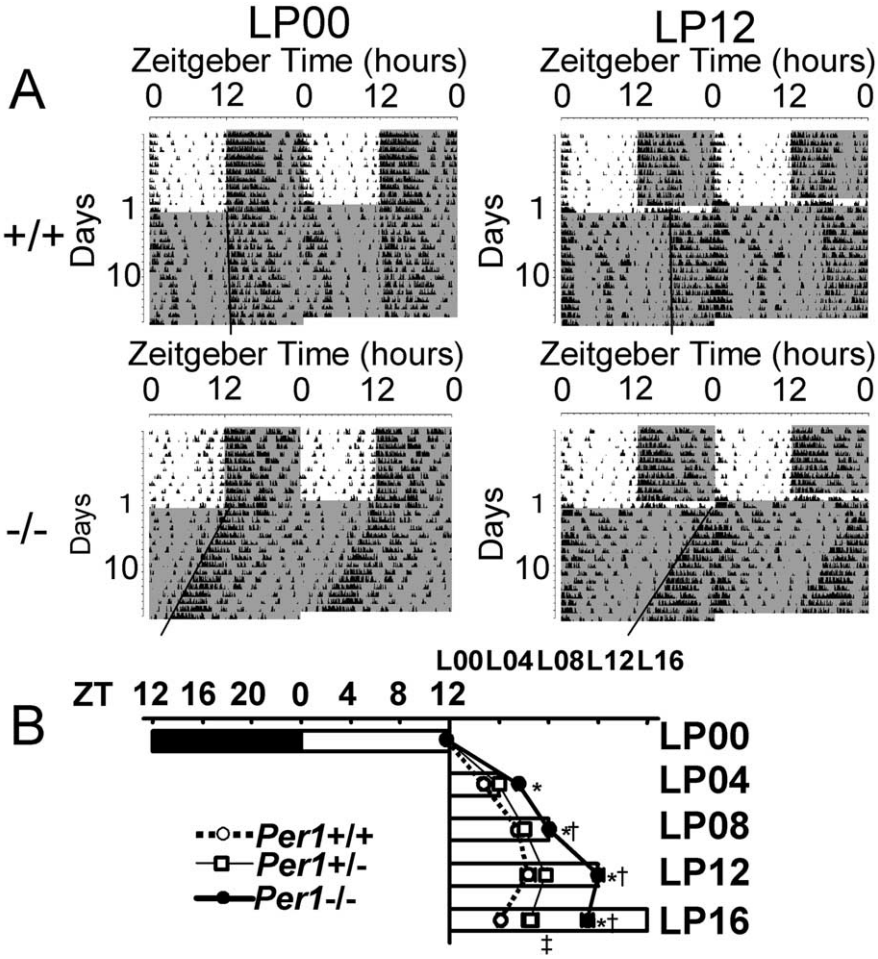

Figure 1. ***A***, Double-plotted locomotor activity rhythms of *mPer1* $^{+/+}$ mice (+/+) and *mPer1* $^{-/-}$ mice (−/−). Mice were moved to constant darkness after LD 12 h lighting cycle (LP00) and 12 h of light prolongation at the last day (day 1) of LD cycle (LP12). Locomotor activities were expressed in the histogram. Periods of darkness are indicated by gray backgrounds. ***B***, The effect of light tasks (LP00, LP04, LP08, LP12, LP16) to activity rhythms in *mPer1* $^{+/+}$, *mPer1* $^{+/-}$, and *mPer1* $^{-/-}$ mice. Extrapolated activity onsets of the first day are indicated by open circles (*mPer1* $^{+/+}$), open squares (*mPer1* $^{+/-}$), and filled circles (*mPer1* $^{-/-}$) (mean ± SEM). For each point, we used five or six animals. The filled bar is the dark phase of LD cycle; open bars are the light phase of LD cycle and light exposure time of each tasks. The values indicated by asterisks and daggers are statistically significant. *$p < 0.0001$, *mPer1* $^{+/+}$ versus *mPer1* $^{-/-}$; †$p < 0.0001$, *mPer1* $^{+/+}$ versus *mPer1* $^{+/-}$; ‡$p < 0.0001$, *mPer1* $^{-/-}$ versus *mPer1* $^{+/-}$ (Bonferroni/Dunn).

Extended light exposure does not yield differences in *mPer2* gene expression in the SCN of *mPer1* $^{-/-}$ mice

Because the SCN is the master clock that times rhythmicity at the systemic level (Schibler and Sassone-Corsi, 2002), the behavioral changes observed here must be a reflection of the alteration of the circadian core transcription/translation feedback loop within the SCN. Indeed, rapid resetting by photic cues was confirmed in the SCN *in vivo* (Best et al., 1999) and *in vitro* (Asai et al., 2001). Because *mPer2* is essential for the timing of the core feedback loop in the mammalian circadian clock (Hastings et al., 2003), we

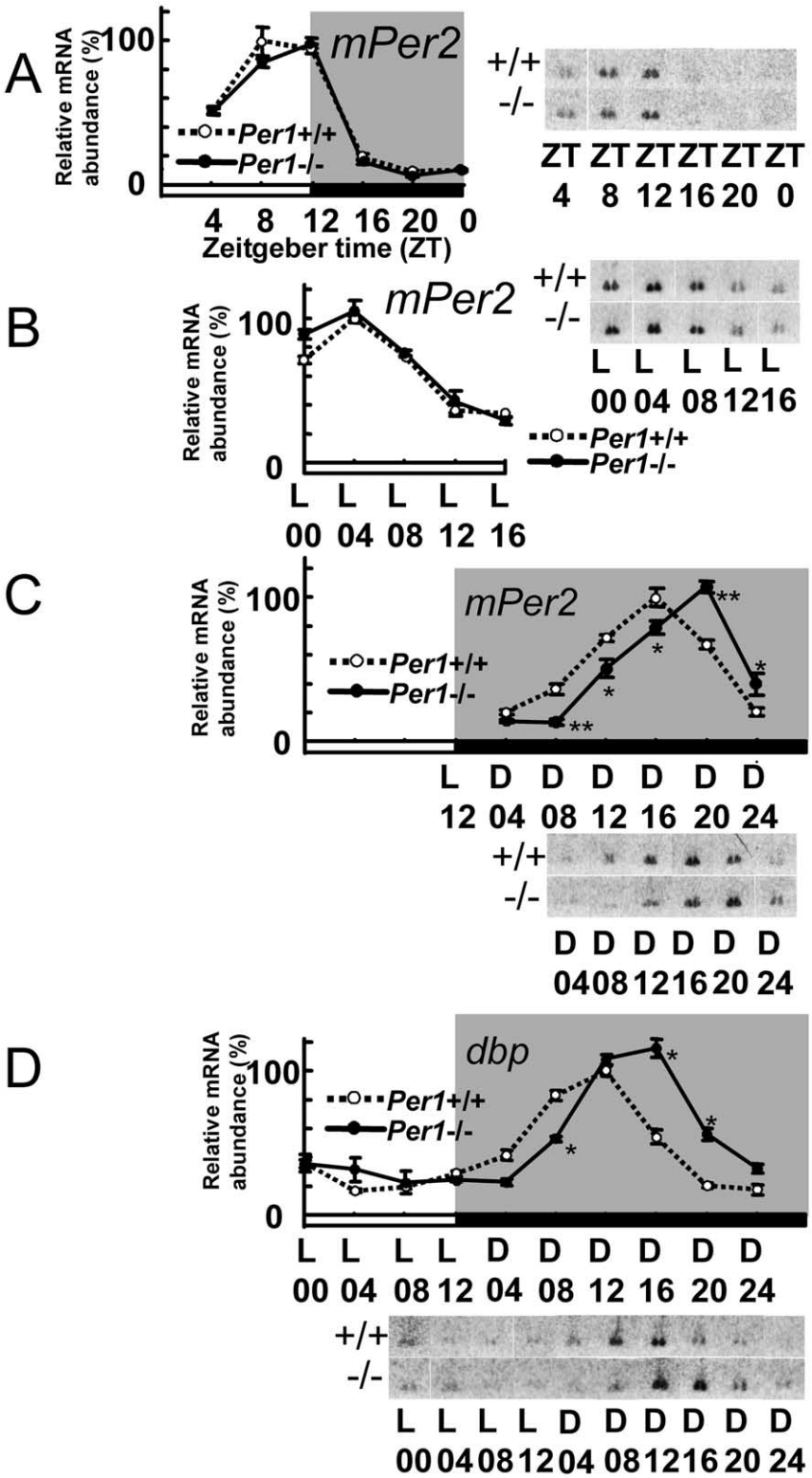

Figure 2. ***A***, Expression profiles of *mPer2* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice in LD. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *mPer2* mRNA (mean ± SEM; $n = 4$). ***B***, Expression profiles of *mPer2* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice during the prolongation of light for 16 h. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *mPer2* mRNA (mean ± SEM; $n = 4$). ***C***, Expression of *mPer2* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice after the 12 h prolongation of light. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *mPer2* mRNA (mean ± SEM; $n = 4$). The values indicated by asterisks are statistically significant. $^{**}p \leq 0.0005$; $^{*}p < 0.005$ *mPer1* $^{+/+}$ versus *mPer1* $^{-/-}$ (Bonferroni/Dunn). ***D***, Expression of *dbp* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice during and after the 12 h prolongation of light. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *dbp* mRNA (mean ± SEM; $n = 3$). The values indicated by asterisks are statistically significant. $^{*}p < 0.0001$, *mPer1* $^{+/+}$ versus *mPer1* $^{-/-}$ (Bonferroni/Dunn). A representative autoradiogram of the SCN is attached to each graph.

examined *mPer2* expression profiles in the SCN during the light-prolongation task. In the usual 12 h LD regimen (ZT0, ZT4, ZT8, ZT12, ZT16, ZT20), in both *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice, *mPer2* mRNA began to increase before dawn, peaked at ZT12, and then steadily decreased by ZT16–20 (two-way ANOVA; *mPer1* $^{-/-}$ vs *mPer1* $^{+/+}$; $p = 0.094$) (Fig. 2*A*). In 16 h of elongated light exposure (from L00 to L16) from lights off at ZT12 (L00), which induces a larger phase delay in *mPer1* $^{-/-}$ mice, *mPer2* transcript levels increased with 4 h of light prolongation (L04); thereafter, expression decreased during extended light exposure in both *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice in a time-dependent manner (one-way ANOVA; $p < 0.0001$) (Fig. 2*B*). Because *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice display analogous profiles of *mPer2* expression (two-way ANOVA; $p = 0.128$) (Fig. 2*B*), light-induced transient increase of *mPer2* transcription may occur similarly in these mice. These results indicated that the cause of the enhanced delay of behavioral onset by light prolongation in *mPer1* $^{-/-}$ mice was not attributable to changes at the *mPer2* transcription level.

Core clock oscillation and its output show a larger phase delay in the second cycle after extended light exposure in *mPer1* $^{-/-}$ mice

We then examined *mPer2* expression profiles after the end of a 12 h light prolongation (LP12 task) with 4 h intervals (D04, D08, D12, D16, D20, and D24). A LP12 task (12 h of light prolongation) was chosen because the magnitude of the behavioral phase shift was largest in both genetic backgrounds (Fig. 1*B*). *mPer1* $^{+/+}$ mice displayed an increase at D08 and a peak at D16 followed by a decrease (Fig. 2*C*). The increase of *mPer2* expression in *mPer1* $^{-/-}$ mice began at D12 and peaked at D20. Thus, in contrast to similar expression profiles observed during the long light prolongation in *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice (Fig. 2*B*), *mPer2* mRNA peaks 4 h later in *mPer1* $^{-/-}$ mice (two-way ANOVA; $p < 0.0001$) (Fig. 2*C*). The magnitude of the behavioral phase shift (4–6 h) did not correlate with the first *mPer2* mRNA expression profiles in light but correlated with the phases of *mPer2* expression in the next cycle. As an indicator of clock output levels, we next examined the expression profiles of a clock-controlled gene, *dbp*, the transcription of which is directly regulated by clock genes (Ripperger et al., 2000; Yamaguchi et al., 2000). As shown in Figure 2*D*, *dbp* expres-

sion was equivalent in *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice during the extended-light task (from L00 to L12; two-way ANOVA; $p = 0.2696$). However, after the task, *dbp* expression in *mPer1*$^{-/-}$ mice displayed a 4 h delay when compared with *mPer1*$^{+/+}$ mice (from D04 to D24; two-way ANOVA; $p < 0.0001$). These findings suggest that expression of core clock genes and clock-controlled genes did not reflect the behavioral phase shift seen during extended light exposure without *mPer1*. But after the task, the expression of these genes does reflect the behavioral rhythms; this occurs after the cessation of the lighting task.

Delayed disappearance of mPER2 protein in the SCN of *mPer1*$^{-/-}$ mice after the extended light exposure

Because there is a dissociation between clock gene expression during the extended-light task and the observed behavioral phase shifts, we examined the expression profile of clock protein levels to know whether there is a dissociation between protein expression patterns and behavioral rhythms. In the usual 12 h LD regimen, in both *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice, mPER2 protein began to increase at ZT8, peaked at ZT16, and then steadily decreased by ZT0 (Fig. 3*A*). We examined the expression of mPER2 protein in the SCN during (L00, L04, L08, and L12) and after light prolongation (D04, D08, and D12) (Fig. 3*B*, left). At L00 (ZT12), mPER2 protein levels were similarly high in both *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice. In *mPer1*$^{+/+}$ mice, mPER2 levels began to decrease at L08 and reached minimal expression at D08, to increase thereafter (D12) (Fig. 3*B*, left). In contrast, the high expression of mPER2 did not decrease until L08 in *mPer1*$^{-/-}$ mice, to reach minimal levels at L12 (4 h later than *mPer1*$^{+/+}$ mice) and then to steadily decrease until D12. From L04 to D08, mPER2 levels are clearly higher in *mPer1*$^{-/-}$ mice than that in *mPer1*$^{+/+}$ mice (two-way ANOVA; $p < 0.01$). We then examined mPER2 protein expression profiles in the next cycle at 4 h intervals (D16, D20, D24, D28, D32, and D36). In *mPer1*$^{+/+}$ mice, mPER2 at D16 steadily decreased until D32. In *mPer1*$^{-/-}$ mice, however, mPER2 protein increased until D24 and then decreased thereafter (Fig. 3*B*, right). Clear phase difference (4–8 h) of mPER2 expressions between *mPer1*$^{-/-}$ mice and *mPer1*$^{+/+}$ mice was observed in the second cycle (two-way ANOVA; $p < 0.0001$) (Fig. 3*B*, right). These findings reveal that *mPer1* regulates mPER2 protein levels without affecting *mPer2* transcription and that the alteration is likely to induce profound changes within transcription of core clock components and clock-controlled genes for the next cycle, which is then reflected at behavioral level.

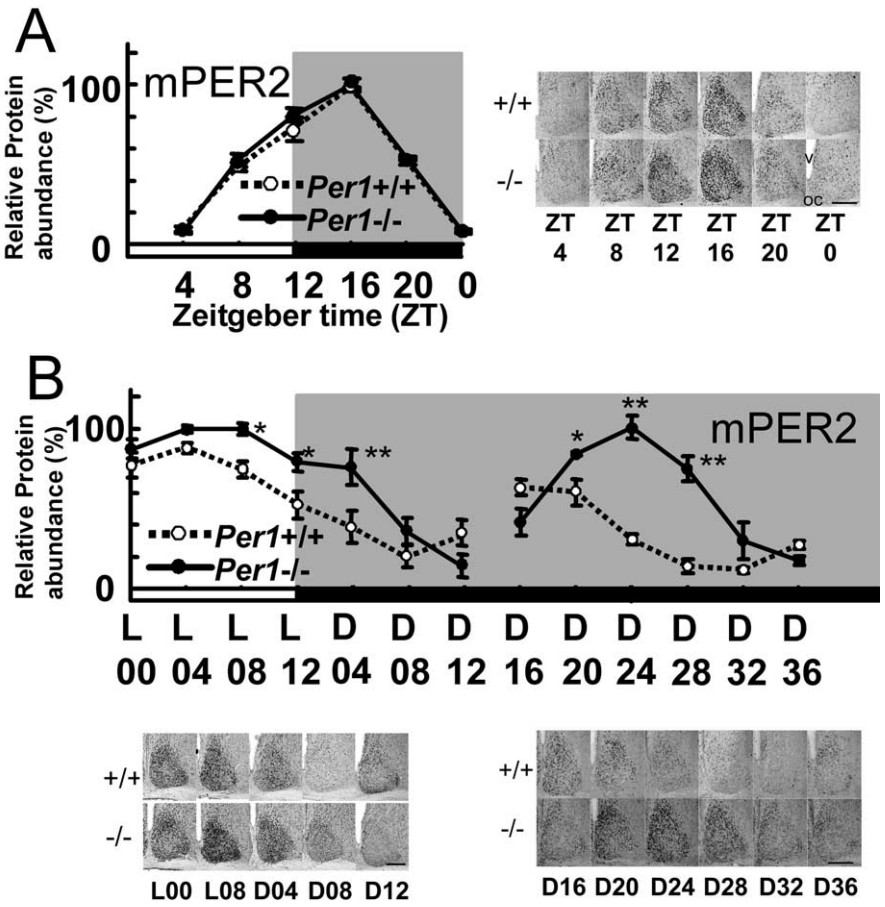

Figure 3. ***A***, Quantified immunostaining values of mPER2 in the SCN of *Per1*$^{-/-}$ and *Per1*$^{+/+}$ mice in LD (mean ± SEM; $n = 4$). ***B***, Quantified immunostaining values of mPER2 in the SCN of *Per1*$^{-/-}$ and *Per1*$^{+/+}$ mice during and after the 12 h prolongation of light (mean ± SEM; $n = 4$). Representative immunohistochemistry of the SCN is attached to each graph. Open circles (*mPer1*$^{+/+}$) and filled circles (*mPer1*$^{-/-}$) indicate relative number of mPER2-positive cells with the mean number of *mPer1*$^{-/-}$ at L00 being adjusted to 100. oc, Optic chiasma; v, third ventricle. Scale bar, 100 μm. The values indicated by asterisks are statistically significant. $^{**}p < 0.0005$; $^{*}p < 0.01$, *mPer1*$^{+/+}$ versus *mPer1*$^{-/-}$ (Bonferroni/Dunn).

mPer1-deficient mice cannot adapt to environmental light/dark cycles consisting of long complete photoperiods of dim light or long skeleton photoperiods

The larger phase resetting that we observed after several hours of light prolongation raised a possibility that *mPer1*$^{-/-}$ mice have a difficulty in entraining to a long-day environment. It is known that expression profiles of *Per* genes alter in response to photoperiodic change (Steinlechner et al., 2002). First, we put animals in a long-day schedule: we exposed *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice to LD 20/4 h (light on from ZT0 to ZT20; *mPer1*$^{-/-}$, $n = 14$; *mPer1*$^{+/+}$, $n = 12$; 200 lux illumination) (Fig. 4*A–C*) and LD 17/7 h (*mPer1*$^{-/-}$, $n = 8$; *mPer1*$^{+/+}$, $n = 8$; 200 lux illumination) (Fig. 4*D–F*). During the period of observation (3–10 weeks), all mice entrained to the both long-day tasks. Because this entrainability is also influenced by the intensity of light, we decrease the intensity of luminescence to ~0.3 lux, adding the long-day schedules in the first group (LD 20/4 h group). It is possible that low light intensity (0.3 lux) (Fig 4 *A–C*,*G*) conditions are more natural for nocturnal rodents than the classically high light intensity (200–300 lux) in which usual laboratory animals are housed, because nocturnal rodents live in dim light or darkness in daytime. Nine of 14 *mPer1*$^{-/-}$ mice could not entrain to the dim long-day photoperiod in 35 d (Fisher's exact probability test; $p < 0.005$) (Fig. 4*G*) by phase delaying (Fig. 4*A*) and advancing (Fig. 4*B*). In contrast, all *mPer1*$^{+/+}$ mice kept entrained to the long complete photoperiod for >35 d (Fig. 4*C*,*G*). However, low light conditions per se are not enough to entrain the rhythm, because all control mice (*mPer1*$^{-/-}$, $n = 8$; *mPer1*$^{+/+}$, $n = 6$) of the first group kept entrained to LD 12 h lighting cycle in 0.3 lux for >35 d (data not shown; Fisher's exact probability test; $p = 0.65$).

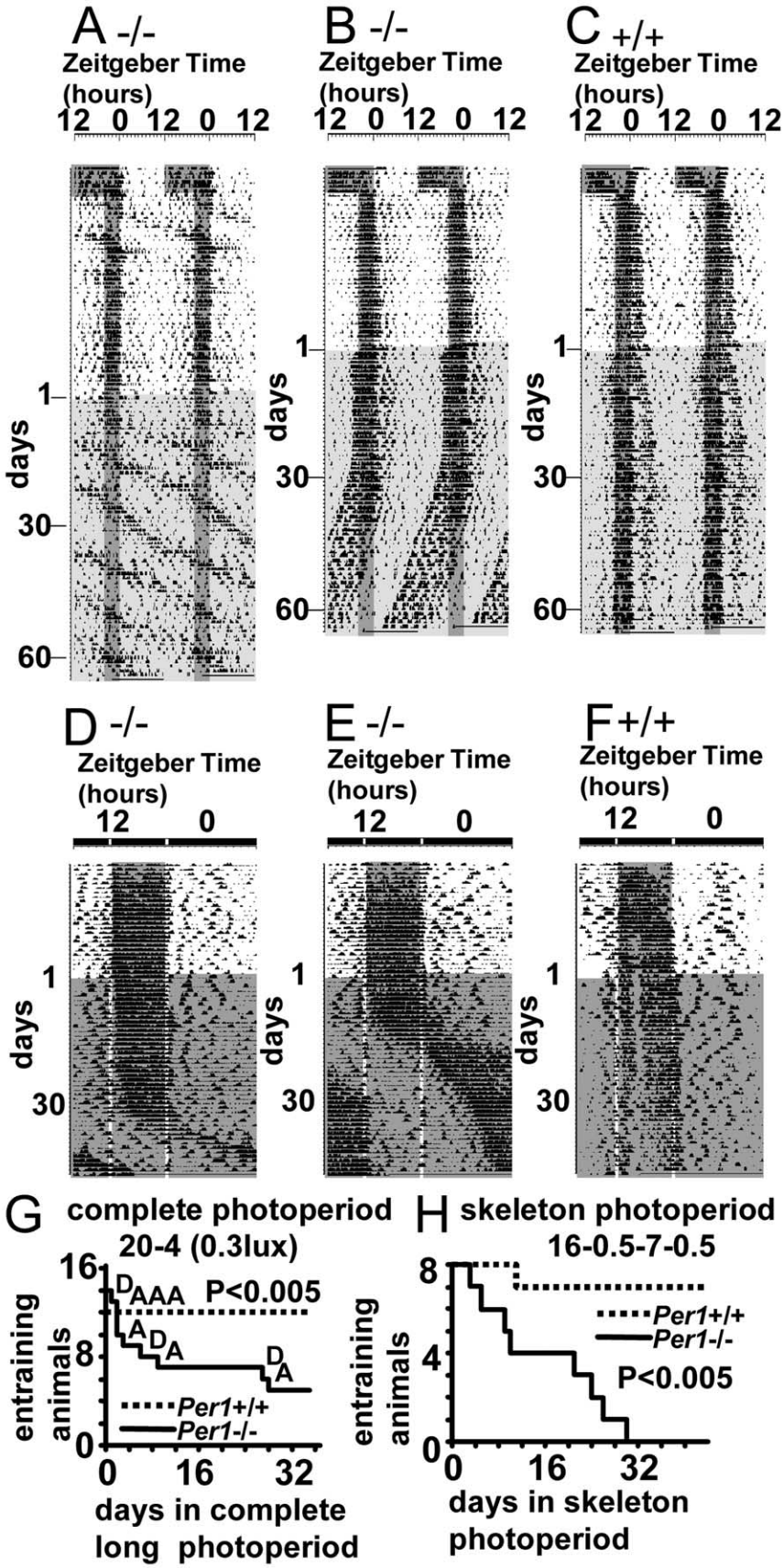

Figure 4. ***A–C***, Representative actograms of *Per1* $^{-/-}$ (***A***, ***B***) and *Per1* $^{+/+}$ (***C***) mice in complete photoperiod with dim light (double plot). Mice were moved (day 1, ZT12) to complete photoperiod with dim light (LD 20/4 h, 0.3 lux) after the same photoperiod with bright light (200 lux). Periods of dim light and complete darkness are indicated by light and dark gray backgrounds, respectively. ***D–F***, Representative actograms of *Per1* $^{-/-}$ (***D***, ***E***) and *Per1* $^{+/+}$ (***F***) mice housed in skeleton photoperiod (single plot). Mice were moved (day 1, ZT19) to skeleton photoperiod (L/D/L/D, 0.5/16/0.5/7 h) after complete photoperiod (LD 17/7 h). Periods of darkness are indicated by gray backgrounds. ***G***, Time course of the number of entrained animals in complete photoperiod. *mPer1* $^{-/-}$ mice desynchronized the activity rhythm from light cycle by phase delaying (D) or advancing (A). ***H***, Time course of the number of entrained animals in the skeleton photoperiod. The day of desynchronization is determined when the activity onsets began to advance or delay.

Next, a second group (LD 17/7 h group; light on, ZT19; lights off, ZT12) was exposed to skeleton photoperiods (Fig. 4*D–F*,*H*). Johnson et al. (2003) speculated that in the case of nocturnal animals, a skeleton photoperiod mimics natural seasonal lighting changes better than complete photoperiods. DeCoursey (1986) suggests that nocturnal rodents in nature reset their clocks by short light exposures. In her simulation experiments, nocturnal rodents (flying squirrels) housed in cages containing dark nest boxes returned to their nest during daytime, and therefore they reset their clocks by short periods (only several minutes per day) of daily light sampling. After entrainment to a LD 17/7 h lighting cycle for 3 weeks, mice were moved to skeleton photoperiods [L/D/L/D, 0.5/16/0.5/7 h; light on from ZT19 to ZT19.5 and from ZT11.5 to ZT12 (the beginning of the skeleton photoperiod being ZT19)]. As shown in Figure 4, *D* and *E*, all eight *mPer1* $^{-/-}$ mice could not entrain to the skeleton photoperiod during 30 d. In contrast, as shown in Figure 4*F*, seven of eight *mPer1* $^{+/+}$ mice kept entrained to the skeleton photoperiod for 44 d (Fisher's exact probability test; $p < 0.005$) (Fig. 4*H*). In this experimental condition, all desynchronized mice moved to another light interval by delaying the clock. In contrast, after entrainment to a LD 12 h lighting cycle for >3 weeks, control mice (*mPer1* $^{-/-}$, $n = 8$; *mPer1* $^{+/+}$, $n = 8$) of the second group were moved to a skeleton photoperiod [L/D/L/D, 0.5/11/0.5/12 h; light on from ZT0 to ZT0.5 and from ZT11.5 to ZT12 (the beginning of skeleton photoperiod being ZT0]. In this skeleton photoperiod, seven of eight *mPer1* $^{-/-}$ mice and all eight *mPer1* $^{+/+}$ mice remained entrained for 44 d (data not shown; Fisher's exact probability test; $p = 0.5$). Thus, whether synchronization occurs is a result of the difficulty of the task, and we speculate that *mPer1* $^{-/-}$ mice have weaker adaptation ability to environmental light/dark cycle.

Discussion

In the present study, we adopted a strong-lighting task to test the role of *mPer1* in clock resetting. The prolongation of the lighting period clearly induces a larger-delay phase shift of the behavioral rhythm in *mPer1*$^{-/-}$ mice. Compared with wild-type mice, in *mPer1*$^{-/-}$ mice, long light exposure initially changed mPER2 protein levels, although the expression profiles of *mPer2* at mRNA level was not altered. This result suggests that *mPer1* suppresses synthesis or enhances decay of light induced mPER2 in the SCN. In *mPer1*$^{-/-}$ mice, this increased mPER2 may cause larger behavioral phase delay, because *mPer2* mutant show attenuated phase delay by light pulse (Spoelstra et al., 2004). It is possible that desynchronization from the photoperiod (Fig. 4) is caused by the stability of mPER2 in *mPer1*$^{-/-}$ mice.

These findings raise the attractive possibility that mPER1 represses light-induced mPER2 at the protein level and attenuates phase resetting by light. Because this process is abolished in *mPer1* knock-out mice, environmental light information readily changes the phases of these animals. Although the molecular process involved is unknown at present, the dimerization of mPER1 and mPER2 (Zylka et al., 1998; Field et al., 2000; Yagita et al., 2000) may provide a likely explanation, because it could indeed influence the phosphorylation and/or ubiquitine proteasome-dependent mPER2 degradation.

mPer1$^{-/-}$ mice could not entrain to experimental long photoperiod with dim light or to skeleton photoperiods. These conditions (dim light, skeleton photoperiod) may mimic the natural lighting schedule for nocturnal rodents. From the present investigation, therefore, it is possible that a nocturnal animal that has *mPer1* has an advantage for entrainment to environmental light/dark cycles.

One essential issue is whether the duplication of clock genes in mammals has happened only to ensure functional redundancy or whether it has a physiological significance in the context of evolution. Our study underscores the importance of *mPer1* in entrainment to dim long photoperiods and long skeleton photoperiods, conditions that mimic seasonal changes of day/night (long-day condition) for nocturnal rodents living in nature. Our results demonstrate that *mPer1* appears to perform a function completely distinct from *mPer2*, because it is involved in the plasticity of the circadian system, allowing it to adapt to changing photoperiodic cycles.

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