Cell Reports
Article

PER1 Phosphorylation Specifies Feeding Rhythm in Mice

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http://dx.doi.org/10.1016/j.celrep.2014.04.032

SUMMARY

Organization of circadian behavior, physiology, and metabolism is important for human health. An S662G mutation in *hPER2* has been linked to familial advanced sleep-phase syndrome (FASPS). Although the paralogous phosphorylation site S714 in PER1 is conserved in mice, its specific function in circadian organization remains unknown. Here, we find that the PER1^{S714G} mutation accelerates the molecular feedback loop. Furthermore, *hPER1*^{S714G} mice, but not *hPER2*^{S662G} mice, exhibit peak time of food intake that is several hours before daily energy expenditure peaks. Both the advanced feeding behavior and the accelerated clock disrupt the phase of expression of several key metabolic regulators in the liver and adipose tissue. Consequently, *hPER1*^{S714G} mice rapidly develop obesity on a high-fat diet. Our studies demonstrate that PER1 and PER2 are linked to different downstream pathways and that PER1 maintains coherence between the circadian clock and energy metabolism.

INTRODUCTION

The circadian clock orchestrates the daily rhythms in physiology and behaviors that allow organisms to anticipate regular environmental cycles and increase their adaptive fitness. The current mammalian clock model is composed of a transcriptional-translational feedback network that includes the Per-Amt-Sim-domain-containing helix-loop-helix transcription factors *Clock* and *Bmal1*, the *Period* genes (*Per1*, *Per2*, and *Per3*), and the *cryptochrome* genes (*Cry1* and *Cry2*). The CLOCK:BMAL1 complex activates the transcription of the *period* and *cryptochrome* genes by binding to E-boxes in their promoters, whereas the PER:CRY complex closes the negative feedback loop by repressing the activity of CLOCK:BMAL1 (Lowrey and Takahashi, 2004; Reppert and Weaver, 2002; Schibler and Naef, 2005). *Bmal1* and *Cry1* expression is also regulated by a secondary feedback loop comprised of the nuclear hormone receptors Rev-Erbα/Rev-Erbβ and the retinoid-related orphan receptors (Preitner et al., 2002; Sato et al., 2004; Ueda et al., 2005; Ukai-Tadenuma et al., 2011). Collectively, these complexes sustain the endogenous circadian oscillators.

Although the basic function of the molecular clock is conserved, mammals employ multiple paralogous clock genes. Analysis of *Per1*/*Per2*, *Cry1*/*Cry2*, and *Rev-erbα*/*Rev-erbβ* double knockout mice indicated that these family genes exhibited a certain degree of redundancy in sustaining rhythms; however, important differences among family genes do exist (Bae et al., 2001; Cho et al., 2012; van der Horst et al., 1999; Vitaterna et al., 1999). Clock mutations significantly affect many physiological processes and have been linked to previously unexpected physiological function, even in the members of a gene family (Ko and Takahashi, 2006; Liu et al., 2008), suggesting that the expansion of the clock gene members has acquired additional functions in complex mammalian physiology rather than simple redundancy to confer robustness. Posttranslational modifications significantly contribute to the precision of the mammalian clock. However, the specific roles of these posttranslational modifications in shaping the period, amplitude, and phase of the oscillator and the consequent effects on whole-animal physiology remain largely unexplored.

Phosphorylation of clock components determines the rate of protein accumulation, nuclear-cytoplasmic distribution, and their degradation, thus setting the robustness, pace, and phase of the circadian oscillator, which ultimately translates to change in the overt circadian rhythms. Among the PER proteins, individuals carrying an S662G mutation in hPER2 exhibit familial advanced sleep-phase syndrome (FASPS) (Jones et al., 1999). The S662 position of the hPER2 protein is the first of five serines spaced three amino acids apart, and the S662G mutation impedes sequential phosphorylation by casein kinase and alters the protein stability (Toh et al., 2001; Xu et al., 2007). The SXXS motif is highly conserved in mammalian PER proteins (Figure S1A; Toh et al., 2001), and this serial phosphorylation of the PER2 protein shows striking temporal changes (Xu et al., 2007). We thus hypothesize that (1) this motif arose before duplications to retain the essential time-keeping function and similar

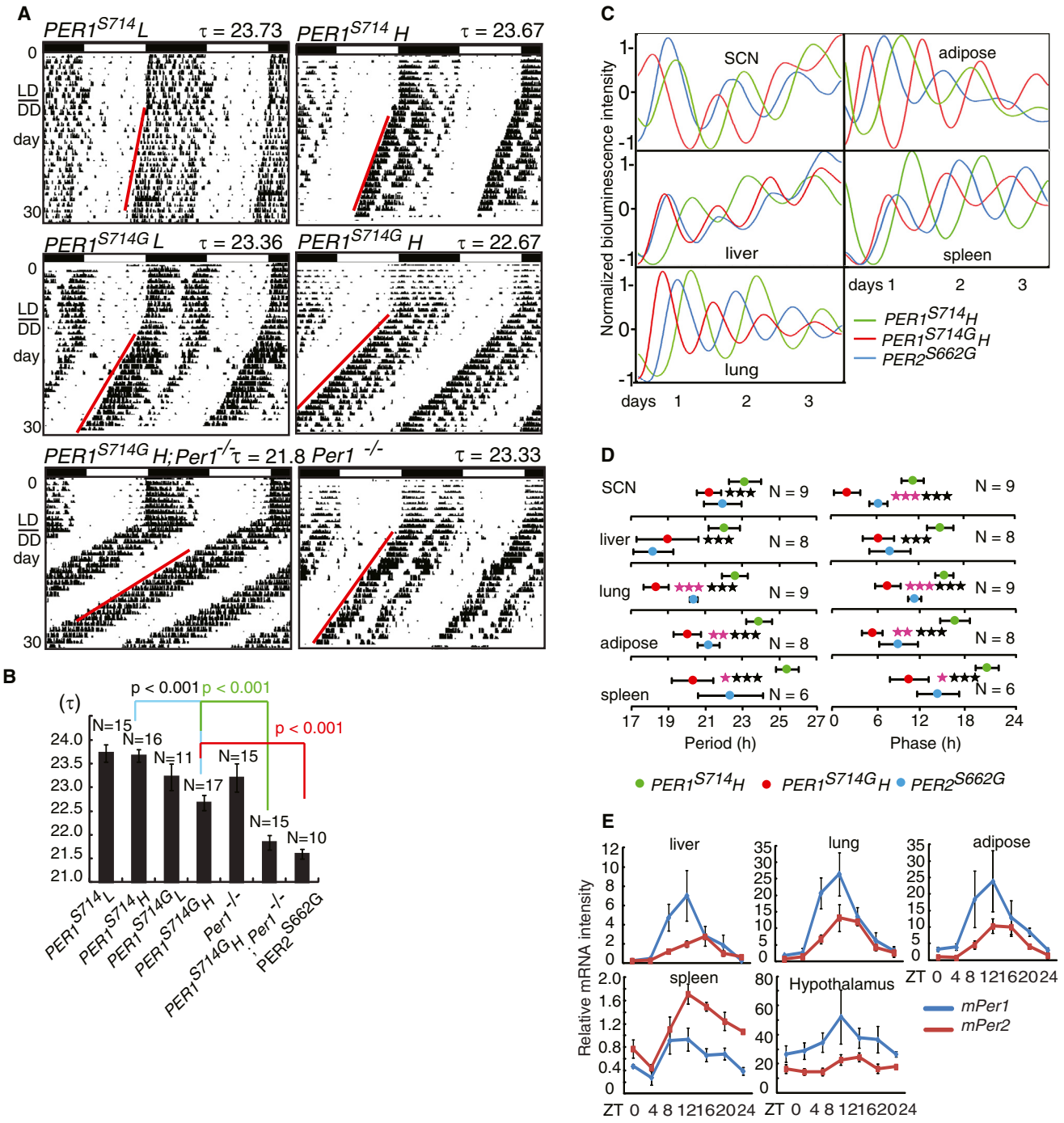

Figure 1. The PER1^{S714G} Mutation Impairs Normal Circadian Oscillator Function

(A) Representative actograms of locomotor activity in mice. The mice were entrained to a 12 hr light/12 hr dark (LD) cycle for 7–10 days and then maintained in constant darkness (DD). The red lines represent the phase of activity onset in constant darkness. The period length from days 8 and 21 in DD was calculated using ClockLab.

(B) Average period length (±SD) quantification of the indicated mouse lines. The indicated p value was determined using Student's t test.

(C) Representative *Per2*^{Luc} bioluminescence traces of explants from *hPER1*^{S714}*H* (green), *hPER1*^{S714G}*H* (red), and *hPER2*^{S662G} (blue) mice. The explants were prepared within 1 hr before dark onset. The indicated time windows were used to determine the period over 3 consecutive days.

(D) The period and phase in different tissues and genotypes are shown as mean ± SD. The indicated sample sizes (i.e., the number of rhythmic tissues) are from three mice for each genotype. One-way ANOVA indicated a significant difference between *hPER1*^{S714}*H* and *hPER1*^{S714G}*H* or *hPER2*^{S662G} mice. Black stars

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