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Per2 has time on its side

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The circadian clock runs with a period of about 24 h and therefore allows mammals to predict sunrise at the cellular level. Phosphorylation of the clock protein period 2 influences this process by varying the clock's period length.

Ask your friends about their bedtimes and you will be amazed at how different the answers can be. Though most people's sleep-wake schedule follows the solar cycle, some individuals are 'larks' whereas others are 'night owls'. From these tendencies, it seems that the internal body clock (circadian clock) is not running at the same speed in these people, thereby leading to altered clock phase. However, the mechanism for this change in speed or period length of a circadian cycle remains a mystery. Evidence for the involvement of specific genes that determine cycle length has come from the examination of lark families with early-morning awakening and sleep times (a short period), a condition termed familial advanced sleep phase syndrome (FASPS). The cause for this syndrome is a mutation (S662G) in the gene encoding period 2 (Per2), which leads to hypophosphorylation of the Per2 protein[1]. The same phenotype is caused by a mutation in either casein kinase Iε (CKIε) or CKIδ, which normally bind Per2 at phosphorylated Ser662 and then phosphorylate adjacent serine residues[2]. A new study now provides the missing link in this process by demonstrating that altering the FASPS site in the Per2 protein leads to a change in its phosphorylation state[3]. The study shows that this site is critical for regulating Per2 concentrations and leads to either a faster or slower circadian clock.

Whereas circadian period length in *Drosophila melanogaster* is directly connected to Per protein phosphorylation and stability[4], the molecular correlate for period length in mammals is not known. However, the clock mechanism is similar in the two organisms. At the core of the mammalian molecular clock are two transcriptional activators (Clock and Bmal1) and two factors that act negatively on them (Per and Cry). The heterodimer of Clock-Bmal1 binds to the Per and Cry promoters, leading to their expression. The proteins enter the nucleus and disrupt Clock-Bmal1 action, thereby suspending the transcription of their own genes. Although it is not understood how Per2 functions in this mechanism, it seems to be involved in modulating transcription indirectly (it cannot bind to DNA). Because the above-described mechanism does not exactly run on a 24-h schedule, it has to be adjusted daily by light to keep the clock in tune with the environment.

Given that FASPS is caused by hypophosphorylation of Per2, Xu *et al.*[3] imagined that there would be an important relationship between Per2 phosphorylation and circadian

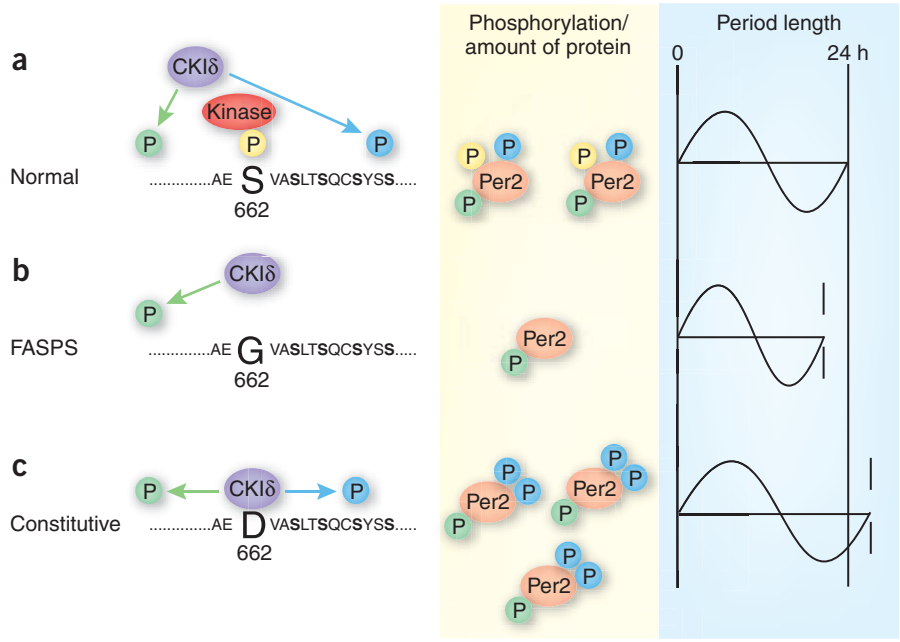

Figure 1 Differential phosphorylation of Per2 modulates period length of the circadian clock. (**a**) Amino acid sequence of normal human Per2 with significant serine residues in bold. Ser662 is phosphorylated (yellow) by an unknown kinase (red), which enables CKIδ to bind and phosphorylate adjacent serine residues (blue path). Independently of Ser662 phosphorylation, CKIδ phosphorylates other residues in Per2 (green path). This leads to intermediate concentrations of Per2 protein, which defines a period length of 24 h. (**b**) Sequence of Per2 with the FASPS amino acid change S662G. CKIδ phosphorylates Per2 only through the green path. This leads to low concentrations of Per2 protein and a shortening of the clock period. (**c**) The S662D mutation leads to constitutive binding of CKIδ and phosphorylation of adjacent serine residues (blue path). Phosphorylation on other sites still occurs (green path). This leads to elevated concentrations of Per2 and a long period length.

period length in general. They generated transgenic mice harboring either normal human Per2 (Ser662), FASPS Per2 (S662G), or a Per2 version that mimics phosphorylation charge at Ser662, thus permitting constitutive binding of CKIδ (S662D). They observed that this mutation leads to hyperphosphorylation of Per2 (**Fig. 1**). By assessing the locomotor activity of these mice in darkness, they found that the three transgenes are associated with different period lengths. Mice with the normal transgene had a period length of about 24 h (**Fig. 1a**), whereas the S662G transgenic mice (FASPS mutation) had a period shorter than that of the normal mice (**Fig. 1b**), thereby recapitulating the syndrome as observed in humans. In contrast, mice bearing the S662D transgene had a period longer than that of normal mice (**Fig. 1c**). In additional experiments measuring protein abundance, the authors discovered that, interestingly, phosphorylation influences the abundance of the Per2 protein. Whereas the S662G mutants (short period) displayed low levels of Per2, the nuclei of liver cells in mice with the S662D mutation (long period) had elevated Per2 concentrations relative to liver cells of normal mice (**Fig. 1**). The authors concluded that protein abundance determines the circadian period.

After having modulated phosphorylation of Per2 by changing the Ser662 residue (which is important for the initial phosphorylation event and thus also for binding of CKIδ), Xu *et al.*[3] generated mice expressing different amounts of CKIδ. Changing CKIδ dosage further modulated the phenotypes of both Ser662 mutants (to increasingly shorter and longer periods), but not that of normal mice. To explain these findings, the authors propose that CKIδ interacts with Per2 not only at the Ser662 position but also at other sites. In this model, CKIδ acts on the adjacent serine residues when Ser662 is phosphorylated (blue path in **Fig. 1**), but CKIδ also acts independently of Ser662 phosphorylation elsewhere (green path in **Fig. 1**). In the absence of Ser662 phosphorylation, as is the case with S662G, CKIδ phosphorylates Per2 only through the green path (**Fig. 1b**), targeting it for degradation and resulting in a short period. Only with the normal protein can the balance between the two paths be maintained, which results in a 24-h period.

The fact that perturbation of the Per2 phosphorylation pattern can lead to opposite period phenotypes has also been observed in a recent *in vitro* study[5], which strongly supports the findings of Xu *et al.*[3]. Though the two studies agree on the effects of distant phosphorylated residues (increased Per2 degradation), they disagree on the roles of the phosphorylation of Ser662 and proximal residues (higher Per2 concentrations through increased nuclear retention[5] versus increased mRNA transcription[3]). Because the Per2 protein contains at least 21 phosphorylated residues[5], regulation of stability and localization of this protein is probably more complex than highlighted here. Characterization of these sites should uncover additional kinases involved in Per2 regulation and their contribution to clock function. Most importantly, the identification of the kinase that initially phosphorylates Ser662 (**Fig. 1**) is of great interest because its activity is necessary to allow CKIδ to bind and exert its function. Another important line of research is the elucidation of the mechanism by which Per2 changes transcriptional activity. Because Per2 does not bind to DNA itself, it must mediate this function through other proteins. Hence, Per2 might be viewed as a transcriptional coactivator or corepressor depending on its binding partner. Identification of such partners might explain why Per2 can have both a positive and a negative role in transcriptional regulation.

Circadian rhythms are established through autoregulatory feedback loops involving transcriptional and post-transcriptional mechanisms. The study by Xu *et al.*[3] demonstrates the value of analyzing circadian mutants in elucidating the details of this biological process. And although this report has brought us a step closer to understanding the regulation of the clock, the many remaining mysteries in this field are likely to keep researchers working late, whether they are 'larks' or not.

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