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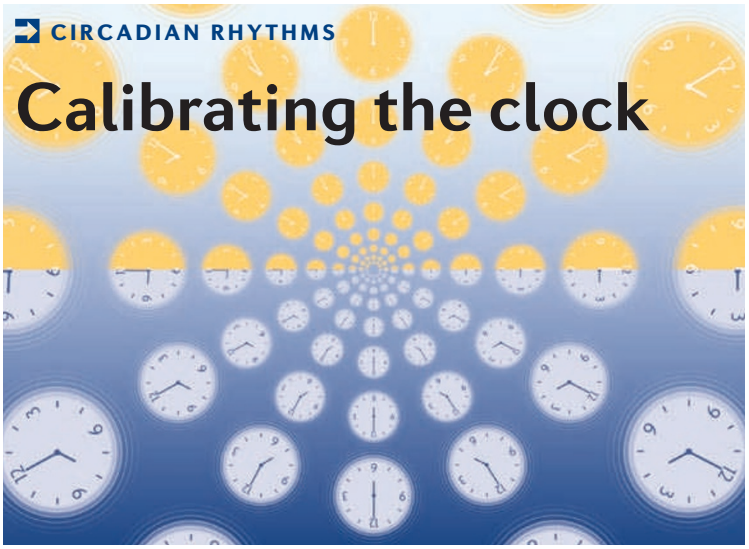
CIRCADIAN RHYTHMS

Calibrating the clock



> AMPK couples metabolic and circadian signals to calibrate the circadian clock by affecting the stability of the peripheral clock component CRY1.

Mammalian circadian rhythms, which govern activities such as sleeping and eating, are regulated by a light-controlled master clock in the brain. Mammals also have clocks in their peripheral organs, which are regulated by nutrient availability through an unknown molecular mechanism. Now, Lamia *et al.* show that circadian rhythm in mouse liver is controlled by the nutrient-responsive enzyme adenosine monophosphate-activated protein kinase (AMPK).

To calibrate the circadian clock, mammals rely on the oscillatory expression of genes encoding clock components, such as cryptochrome 1 (*Cry1*), and this expression depends on post-translational modifications. In preliminary studies the authors identified potential CRY1 phosphorylation sites and showed that phosphorylation of two amino acid residues (Ser71 and Ser280) destabilized this protein. Specifically, a phosphomimic mutation of one of these residues increased the affinity of CRY1 for the ubiquitin ligase FBXL3 (F-box and leucine-rich repeat protein 3), which ubiquitylates and therefore degrades CRY1. The two CRY1 residues correspond to AMPK phosphorylation target sites. To test whether AMPK phosphorylates these residues in CRY1, CRY1 was expressed in wild-type and *Ampk*$^{-/-}$ mouse embryonic fibroblasts, which were treated with an AMPK agonist that acts upstream of AMPK to phosphorylate it. CRY1 was phosphorylated only in wild-type cells, indicating that AMPK carries out this process *in vivo*. Phosphorylated CRY1 from wild-type cells was less stable than CRY1 from *Ampk*$^{-/-}$ cells, suggesting that AMPK-mediated phosphorylation leads to CRY1 degradation.

To confirm the importance of AMPK in transducing metabolic signals to the liver circadian clock, the authors examined the effects of glucose and AMPK on the rhythmic expression of genes encoding clock proteins. As predicted, glucose availability and AMPK expression affected the circadian expression of genes encoding clock proteins that are activated downstream of CRY1 in wild-type but not *Ampk*$^{-/-}$ cells. So how does AMPK integrate metabolic and circadian signals? Phosphorylation of AMPK substrates is higher during the subjective day than at night, suggesting that AMPK is expressed in a diurnal manner. This is mediated by the rhythmic expression of the AMPK regulatory subunit, which induces the nuclear localization of the catalytic subunit, and therefore of the whole AMPK protein, during the subjective day. Diurnal AMPK expression was also observed *in vivo*: mice deficient in liver kinase B1, which mediates AMPK activation, had significantly higher CRY1 levels than wild-type mice, particularly during the day, when AMPK levels are high.

Taken together, these findings reveal that AMPK couples metabolic and circadian signals to calibrate the circadian clock by affecting the stability of the peripheral clock component CRY1. However, as the communication of the nutritional status to the peripheral clocks is complex, other mechanisms are probably involved in this process.

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ORIGINAL RESEARCH PAPER Lamia, K. A. *et al.* AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* **326**, 437–440 (2009)
FURTHER READING Gallego, M. & Virshup, D. M. Post-translational modifications regulate the ticking of the circadian clock. *Nature Rev. Mol. Cell Biol.* **8**, 139–148 (2007)