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CIRCADIAN RHYTHMS

Cycling with cAMP

The suprachiasmatic nucleus (SCN) is the main mammalian timekeeper: it coordinates circadian rhythms in gene expression throughout the body. The traditional description of its pacemaker activity includes an intracellular negative-feedback loop through which the products of the clock genes period (*Per*) and cryptochrome suppress their own expression in the SCN. However, recent studies indicated that this description is incomplete, and Hastings and colleagues have now identified cyclic AMP signalling as a major component of the central oscillator.

The authors showed that cAMP levels oscillate in a circadian fashion in tissue slices of mouse SCN. In addition, they observed circadian expression of a luciferase reporter gene that contained a cAMP-response element (CRE). These findings could indicate that cAMP expression is regulated by circadian pacemaker activity; however, further experiments suggested that cAMP is itself part of the pacemaker.

Using the activity of a luciferase reporter gene that was under the control of the *Per* promoter as a measure of circadian gene transcription, the authors showed that reducing cAMP levels dampened circadian transcription in SCN slices in a dose-dependent manner. Prolonged reduction of cAMP levels also caused the cycles of circadian transcription in individual cells in an SCN slice, which are normally synchronized, to become desynchronized. In addition, elevating cAMP concentrations acutely increased circadian gene expression, and prolonged cAMP elevation reduced its rhythmicity.

When cAMP levels were simultaneously elevated in different SCN slices by pharmacological treatment, the slices' transcription cycles were synchronized. This effect disappeared with sustained cAMP elevation, but if cAMP was subsequently reduced by washing out the drugs, the circadian transcription cycles resynchronized. This suggests that acute changes in cAMP signalling affect transcriptional oscillation. Finally, slowing cAMP synthesis slowed the oscillations of circadian transcription in SCN neurons. Together, these findings show that not only are cAMP levels regulated by the circadian pacemaker, they also influence the activity of the pacemaker, indicating that cAMP is an integral part of the central oscillator.

In vivo experiments showed that slowing cAMP synthesis lengthened the period of circadian transcription in peripheral tissues and lengthened circadian activity (wheel running) patterns, revealing that the actions of cAMP in the central pacemaker also influence peripheral cells.

The authors also investigated the downstream factors through which cAMP sustains circadian transcriptional rhythms. Blocking hyperpolarization-activated cyclic-nucleotide-gated K^+ (HCN) channels and inhibiting the guanine nucleotide exchange factors EPAC1 and EPAC2 decreased circadian gene expression in the SCN, whereas inhibiting cAMP-dependent protein kinases had no effect. cAMP-induced activation of EPACs in turn activates CRE-binding proteins, which are transcription factors that bind to CRE sequences on *Per* genes.

Recent studies have shown that the intracellular signalling molecules Ca^{2+} and cyclic adenosine diphosphate-ribose are involved in circadian pacemaking in *Drosophila melanogaster* and plants, respectively. Together with this study, they provide increasing evidence that an interaction between intracellular signalling molecules and transcriptional feedback loops forms an evolutionarily conserved mechanism for timekeeping.

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ORIGINAL RESEARCH PAPER O'Neill, J. S. *et al.* cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science* **320**, 949–953 (2008)
FURTHER READING Herzog, E. D. Neurons and networks in daily rhythms. *Nature Rev. Neurosci.* **8**, 790–802 (2007)

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