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CIRCADIAN RHYTHMS:

Daily Watch on Metabolism

Takato Imaizumi, Steve A. Kay, and Julian I. Schroeder

The authors are in the Division of Biological Sciences, Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093–0116, USA

Takato Imaizumi: timaizumi@ucsd.edu

Most organisms enhance fitness by coordinating their development with daily environmental changes through molecular time-keepers known as circadian clocks. In eukaryotes, these clocks comprise interlocking loops of transcriptional feedback and protein turnover (1). This system of multiple connected loops increases the clock's robustness and provides numerous points of input and output to the clock. Many metabolic pathways are regulated by circadian clocks in plants and animals (2, 3). Two papers in this week's *Science Express*, by Dodd *et al.* (4) and Yin *et al.* (5), provide evidence that clock feedback mechanisms in plants and animals incorporate small metabolites and signaling molecules. This represents yet another complex layer of feedback regulation within circadian networks, and how the clock maintains metabolic homeostasis in response to external conditions.

In plant and animal cells, the concentration of intracellular free calcium ions ($[Ca^{2+}]_i$) shows a diurnal oscillation (6). Because Ca^{2+} is a signaling molecule in various physiological responses, its daily oscillation could encode circadian clock signaling information (7, 8). Analyses in the model plant *Arabidopsis thaliana* suggest that the extracellular Ca^{2+} -sensing receptor contributes to generating this oscillation. This pathway involves inositol 1,4,5,-trisphosphate (IP_3), which triggers Ca^{2+} release from intracellular stores (9). In animal cells, cyclic adenosine diphosphate ribose (cADPR) is another signaling molecule that induces Ca^{2+} release by binding to the ryanodine receptor present on intracellular stores (10). Although there is not yet an obvious ryanodine receptor counterpart in plant genomes, cADPR triggers $[Ca^{2+}]_i$ increase in plants as well (11, 12).

Dodd *et al.* determined that cADPR concentration peaks during the early hours of the day. This fluctuation was abolished in plants with defective clock function, indicating that the circadian clock regulates cADPR concentration. cADPR is synthesized from nicotinamide adenine dinucleotide by the enzyme ADP ribosyl cyclase (10). Nicotinamide, at 10 to 50 mM concentrations, inhibited ADP ribosyl cyclase and weakened circadian $[Ca^{2+}]_i$ oscillation in plant cells. Dodd *et al.* also found a correlation between the expression of circadian- and cADPR-regulated genes. Moreover, decreasing the cellular concentration of cADPR lengthened the period of circadian gene expression. The authors suggest that circadian-regulated cADPR-derived Ca^{2+} signaling may configure part of the feedback loop that controls the clock (see the figure).

The results of Dodd *et al.* raise interesting questions. The phytohormone abscisic acid, thought to lengthen the clock period (13), induces cADPR production (11), and cADPR gene expression overlaps with that of genes controlled by abscisic acid (14). Does abscisic acid affect the clock partly through cADPR-derived signals? Also, assuming that both IP_3 - and cADPR-dependent pathways are involved in generating circadian $[Ca^{2+}]_i$ oscillation, do they interact with each other? Dodd *et al.* found that a pharmacological inhibitor (U73122 at 1 μ M) of IP_3 production did not affect daily $[Ca^{2+}]_i$ oscillation. Because IP_3 concentrations were not analyzed, more research is needed to understand the relative roles of both of

cADPR and IP₃. In particular, identification of the plant genes that encode the enzymes that produce cADPR and the proteins that control Ca²⁺ release by cADPR and IP₃ are required to analyze the functions of these signaling molecules in plants.

The circadian clock also controls daily metabolic homeostasis in mammals. Indeed, mice with a dominant mutation in *Clock*, the gene that encodes a core clock component, develop various metabolic syndromes (15). Many enzymes that catalyze diverse metabolic reactions require heme as a cofactor. The circadian clock regulates the heme metabolic pathway partly by controlling expression of 5-aminolevulinic acid synthase, the rate-limiting enzyme in heme biosynthesis (3). Yin *et al.* show that the circadian clock may also monitor heme metabolism through the clock component REV-ERB α . Heme binds to REV-ERB α and regulates its function by promoting its assembly with two proteins that repress transcription—nuclear receptor co-repressor and histone deacetylase 3 complex.

Heme suppresses the expression of genes involved in gluconeogenesis in the liver. Yin *et al.* show that in the presence of heme, REV-ERB α decreased the expression of genes encoding phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, both of which control glucose production, in human hepatoma cells. Heme also augmented transcriptional repression of the core clock gene *Bmal1* by REV-ERB α . Therefore, REV-ERB α couples the circadian clock with glucose metabolism. It would be intriguing to study whether REV-ERB α -dependent regulation contributes to the transcriptional regulation of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase genes in *Rev-erba*-deficient mice.

At first glance, the studies by Dodd *et al.* and Yin *et al.* appear unrelated. However, they propose that both plant and animal clocks possess a mechanism for implementing cellular signaling or redox status in the fine-tuning of daily transcriptional regulation. Thus, a common theme emerges in which small molecules provide feedback mechanisms between the circadian clock network and clock-controlled metabolic pathways to maintain metabolic homeostasis.

References

1. Wijnen H, Young MW. *Annu Rev Genet.* 2006; 40:409. [PubMed: 17094740]
2. Harmer SL, et al. *Science.* 2000; 290:2110. [PubMed: 11118138]
3. Panda S, et al. *Cell.* 2002; 109:307. [PubMed: 12015981]
4. Dodd AN, et al. *Science.* Nov 15.2007 10.1126/science.1146757
5. Yin L, et al. *Science.* Nov 15.2007 10.1126/science.1150179
6. Imaizumi T, Schroeder JI, Kay SA. *Sci STKE.* 2007; 2007:pe32. [PubMed: 17565121]
7. Johnson CH, et al. *Science.* 1995; 269:1863. [PubMed: 7569925]
8. Love J, Dodd AN, Webb AA. *Plant Cell.* 2004; 16:956. [PubMed: 15031410]
9. Tang RH, et al. *Science.* 2007; 315:1423. [PubMed: 17347443]
10. Lee HC. *Physiol Rev.* 1997; 77:1133. [PubMed: 9354813]
11. Wu Y, et al. *Science.* 1997; 278:2126. [PubMed: 9405349]
12. Leckie CP, McAinsh MR, Allen GJ, Sanders D, Hetherington AM. *Proc Natl Acad Sci USA.* 1998; 95:15837. [PubMed: 9861057]
13. Hanano S, Domagalska MA, Nagy F, Davis SJ. *Genes Cells.* 2006; 11:1381. [PubMed: 17121545]
14. Sanchez JP, Duque P, Chua NH. *Plant J.* 2004; 38:381. [PubMed: 15086800]
15. Turek FW, et al. *Science.* 2005; 308:1043. [PubMed: 15845877]

Figure 1. Metabolic feedback to clocks

(Left) The plant circadian clock comprises interlocking loops of the clock components CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), PSEUDO-RESPONSE REGULATORs (PRRs), TIMING OF CAB EXPRESSION 1 (TOC1), and GIGANTEA (GI). The clock controls the concentration of cADPR, which in turn regulates circadian oscillation in the cytosolic free Ca^{2+} concentration. **(Right)** In the mammalian clock, the CLOCK/BMAL1 heterodimer regulates expression of PERIOD (PER), CRYPTOCHROME (CRY), RORA, and REV-ERB α . REV-ERB α is a heme sensor that forms a transcriptional repressor complex. Heme provides feedback to the circadian clock and influences gluconeogenesis.