AKT keeps the beat in CLOCK’s circadian rhythm

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Masayuki Noguchi1, Noriyuki Hirata, and Futoshi Suizu
From the Division of Cancer Biology, Institute for Genetic Medicine, Hokkaido University, 060-0815, N15 W7 Kita-Ku, Sapporo, Japan

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The circadian clock plays a critical role in physiology and medicine by cyclically regulating the expression of numerous genes. The core components of the clock are thought to be controlled by several regulatory mechanisms, including post-translational modifications, but the full extent of this regulation is not known. In this issue, Sessa and co-workers demonstrate that the transcription factor CLOCK is a direct substrate of the kinase AKT. This phosphorylation controls the nuclear–cytosolic translocation of CLOCK and thus its ability to regulate circadian gene expression in peripheral tissues.

The circadian rhythm is a biological cycle that coordinates cellular and organismal energy metabolism, including regulation of heart rate, stress, growth hormones, and immunity as well as glucose, lipid, bone, and drug metabolism (1, 2). This cycle, which exists in plants, mammals, and even cyanobacteria, is orchestrated by a circadian clock consisting of several proteins that use biochemical oscillations to keep time. Defects in the circadian clock cause deregulation of metabolic physiology, resulting in metabolic disorders and mental illness, such as neurodegeneration-linked dementia, increased risk of cancer, diabetes mellitus, Alzheimer’s disease, schizophrenia, Down syndrome, obesity, and coronary heart disease (3, 4). Thus, understanding the various components of the clock as well as the inputs and regulatory mechanisms that influence oscillations is both academically interesting and clinically important.

A new study by Sessa and colleagues (5) advances our knowledge on this front, providing the first direct evidence that a core intracellular survival regulator acts on a central clock component, impacting its function. These new data help to solidify our comprehension of clock regulation and point to intriguing new connections between nutrient sensing and biological timekeeping.

The circadian clock in mammals requires the core clock gene CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle ARNT-like 1). These transcription factors heterodimerize to facilitate binding to E-box elements on DNA and transcriptional activation of relevant genes, including those for repressor proteins such as PER and CRY and transcriptional activators such as Dbp and Rorα (1). The PER and CRY proteins accumulate with time, causing them to dimerize and translocate into the nucleus to repress their own transcription by directly interacting with CLOCK/BMAL1, forming a cell autonomous negative feedback loop. However, the regulation of this circuit goes beyond protein–protein interactions. For example, CRY appears to target acetylated histone H3, introducing cross-talk with chromatin modification pathways (1, 6), and the PER–CRY repressor complex is targeted for degradation by specific E3 ubiquitin ligase complexes, resulting in reactivation of CLOCK/BMAL1 for a subsequent round of the transcriptional feedback loop (2, 6). Intracellular localization and DNA-binding affinity of the clock proteins also play a role in the transcription-based feedback mechanisms of circadian rhythm (2). Finally, post-translational modifications, and specifically phosphorylation, are thought to influence clock proteins (7, 8). For example, casein kinase 1ε and glycogen synthase kinase 3 phosphorylate PER and CRY proteins, impacting their transit to the nucleus and degradation. Evidence suggests that BMAL1 is phosphorylated by AKT, which leads to its retention in the cytosol. Phosphoproteomics studies have identified CLOCK as differentially phosphorylated at Ser-845 upon AKT knockout or other stimuli, but as casein kinase 2, protein phosphatase 1, and protein phosphatase 2A (kinases and phosphatases upstream of AKT) and glycogen synthase kinase 3 (a known AKT substrate) have also been implicated in regulating the clock, the relevance of these AKT data sets is not known (7).

To explore the possibility of direct involvement by AKT in regulating CLOCK, Sessa and coworkers (5) tested the reaction in vitro and in cells. They observed that AKT phosphorylates CLOCK at Ser-845 in mice (836 in humans) (8); phosphorylation is diminished by AKT inhibition or mutation of Ser-845 and induced by stimulation of the AKT signaling pathway. The authors next explored the consequences of Ser-845 phosphorylation, finding that the modification did not influence protein synthesis or degradation but did substantially influence CLOCK localization, with only half as much WT protein in the nucleus as the S845A mutant. The phosphorylation site is within the consensus 14–3–3 protein binding motif RxRxS/TxP, which could explain how the phosphorylated protein is sequestered in the cytosol. Indeed, immunoprecipitation experiments showed that the WT sequence was associated with 14–3–3 proteins more than the mutant.

Tests with CLOCK S845A knock-in mice indicated the animals were essentially like WT mice, with normal central circadian rhythms and hemodynamics, but with altered metabolic preferences, favoring fatty acid utilization and diminished expression of clock target genes in skeletal muscle and in the liver during the circadian cycle. Since the most affected genes

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1 To whom correspondence should be addressed: Division of Cancer Biology Institute for Genetic Medicine Hokkaido University 060-0815 N15 W7, Kita-Ku, Sapporo, Japan. E-mail: m_noguchi@igm.hokudai.ac.jp.
were those that have E-boxes such as Dbp, the authors investigated this interaction in more detail. Surprisingly, a reduction in Dbp levels is associated with reduced H3K9ac at the E-boxes where CLOCK binds, despite there being no change in total CLOCK levels.

The current study demonstrates a novel biological function of AKT in phosphorylating CLOCK at Ser-845 in mice and thus controlling circadian gene expression in peripheral tissues, possibly through chromatin modification of histone acetylation. The authors propose that CLOCK phosphorylation by AKT regulates its nuclear translocation by stimulating 14-3-3 protein binding in the cytosol, impacting the expression levels of certain core circadian genes in two insulin sensitive tissues (Fig. 1). This in turn would ensure the proper alignment of behavioral states through peripheral metabolism in the liver, muscle, adipose tissue, and pancreas (3, 4).

The article also raises additional questions for future study. For example, there are two AKT homologues, AKT1 and AKT2, involved in different cellular processes, and multiple 14-3-3 proteins that could be interacting with CLOCK in the cytosol. Further clarifying which specific partners are involved in this process could help to elucidate the physiological role of this new step. In addition, it will be interesting to gain more insights into the connection between CLOCK phosphorylation, Dbp expression, and histone H3 acetylation, as well as the extent of histone modifications caused. Finally, the AKT–mTOR pathways influence multiple inputs related to nutrient influx, endocrine signaling, and cellular energy balance (8, 9). Given that the activities of AKT and TOR pathways underlie metabolic disorders and/or cancers (10), the current study supports the view that altered circadian activity by AKT-mediated CLOCK phosphorylation might be causative of human diseases (2, 8). We look forward to new insights in this field in due time.

References
1. Takahashi, J. S. (2017) Transcriptional architecture of the mammalian circadian clock. Nat. Rev. Genet. 18, 164–179 CrossRef Medline
2. Reddy, A. B., and Rey, G. (2014) Metabolic and nontranscriptional circadian clocks: Eukaryotes. Annu. Rev. Biochem. 83, 165–189 CrossRef Medline
3. Huang, W., Ramsey, K. M., Marcheva, B., and Bass, J. (2011) Circadian rhythms, sleep, and metabolism. J. Clin. Invest. 121, 2133–2141 CrossRef Medline
4. Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E., and Hogenesch, J. B. (2014) A circadian gene expression atlas in mammals: Implications for biology and medicine. Proc. Natl. Acad. Sci. U.S.A. 111, 16219–16224 CrossRef Medline
5. Luciano, A. K., Zhou, W., Santana, J. M., Kyriakides, C., Velazquez, H., and Sessa, W. C. (2018) CLOCK phosphorylation by AKT regulates its nuclear accumulation and circadian gene expression in peripheral tissues. J. Biol. Chem. 293, 9126–9136 CrossRef Medline
6. Partch, C. L., Green, C. B., and Takahashi, J. S. (2014) Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 24, 90–99 CrossRef Medline
7. Gallego, M., and Virshup, D. M. (2007) Post-translational modifications regulate the ticking of the circadian clock. Nat. Rev. Mol. Cell Biol. 8, 139–148 CrossRef Medline
8. Zheng, X., and Sehgal, A. (2010) AKT and TOR signaling set the pace of the circadian pacemaker. Curr. Biol. 20, 1203–1208 CrossRef Medline
9. Manning, B. D., and Toker, A. (2017) AKT:PKB signaling: Navigating the network. Cell 169, 381–405 CrossRef Medline
10. Noguchi, M., Hirata, N., and Suyzu, F. (2014) The links between AKT and two intracellular proteolytic cascades: Ubiquitination and autophagy. Biochim. Biophys. Acta 1846, 342–352 CrossRef Medline