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REVIEWS

Spotlight

Reconstitution of Organismal Liver Clock Function Requires Light

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Recently Koronowski *et al.* (*Cell*, 2019) showed that reconstitution of the liver circadian clock in otherwise genetically arrhythmic mice revives the daily program of the nicotinamide adenine dinucleotide salvage pathway and glycogenesis *in vivo*. This liver-autonomous metabolic cycling cannot be sustained under constant darkness, revealing the importance of the daily light cycle to restore liver function.

The circadian clock is an endogenous daily pacemaker of our life. The fundamental adaptive advantage of the endogenous clock system is that it allows for predictive, rather than entirely reactive, homeostatic regulation of daily physiologies. These include homeostatic regulation of energy metabolisms in the liver that occurs in anticipation of a regular feeding–fasting cycle.

The work by Koronowski *et al.* [1] provides unprecedented evidence that the circadian clock within the liver is sufficient to drive circadian glycogen synthesis and nicotinamide adenine dinucleotide (NAD^+) production rhythm in the liver under physiological light–dark (LD) cycle conditions. Notably, circadian clock functions outside the liver are all dispensable for giving rise to circadian oscillation of glycogen and NAD^+ in the liver (Figure 1).

At the organismal level, the principal circadian pacemaker governing daily rhythms in behavior and physiology resides in the suprachiasmatic nucleus (SCN) of the hypothalamus. Nevertheless, tissues outside the SCN, including liver, also contain local clocks (so-called peripheral clocks), and their rhythms are synchronized, harmoniously, by an array of direct or indirect signals emanating from the SCN. Thus, the SCN lies at the top of a hierarchical, multioscillator system distributed across the body. The work by Koronowski *et al.* [1] revised this view: at least under physiological LD conditions, the liver clock retains its autonomy over the relevant metabolic cycling. This happens without relying on the SCN and other peripheral clock functions.

For all tissues and cell types, Bmal1 is an essential component of the circadian clock [2]. To investigate the functional independence of the liver from different tissues, Koronowski *et al.* took advantage of a new mouse model, in which *Bmal1* is expressed in liver hepatocytes in its endogenous locus, in an otherwise *Bmal1*-deficient mouse [1]. Using a conditional gene trap approach, Bmal1-Stop-FL (stop-floxed) mice were generated; these mice are devoid of Bmal1 organism-wide, but Bmal1 expression can be restored upon expression of Cre [1,3]. Subsequent crossings with mice expressing Cre recombinase under the control of a modified albumin promoter (Alfp-Cre) create

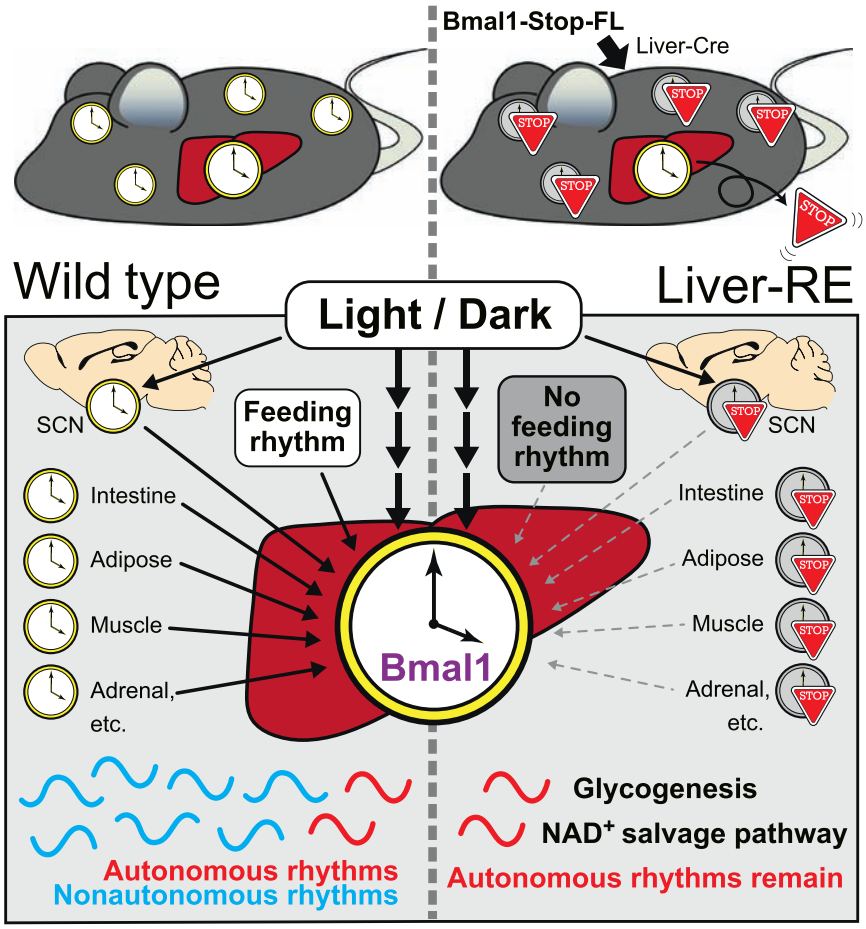

Figure 1. Liver-RE Mice Identify Liver-Autonomous Molecular Rhythms that Require a Daily Light–Dark Cycle to Operate. Liver-RE mice are animals with reconstituted circadian expression of Bmal1 exclusively in the liver. Abbreviations: NAD^+, nicotinamide adenine dinucleotide; SCN, suprachiasmatic nucleus.

mice with a specific reintroduction of a Bmal1-dependent clock in the liver (Liver-RE). The unique feature of this model is that it allows the restored expression of *Bmal1* to be under regulation of the endogenous clock. How does the liver clock behave when Bmal1 is completely absent in the rest of the body? Liver-RE mice will reveal which processes are under liver-autonomous circadian control and which require external cues from other clocks.

High-throughput transcriptomics and metabolomics show that, in the liver of wild-type (WT) mice, 2010 coding transcripts and 757 annotated metabolites cycle in abundance [1]. Surprisingly, however, reconstituted livers were only capable of restoring ~10% of transcript and ~20% of metabolite oscillations. This means that a large fraction of hepatic molecular oscillations were not autonomous, despite reconstitution of liver Bmal1.

At the molecular level, the liver Bmal1 displays characteristic circadian oscillations in expression and post-translational modifications (phosphorylation and acetylation), is properly recruited to chromatin at target genes, and restores rhythmic expression of other clock genes in Liver-RE, implying that the fundamental circadian clock function is rescued. However, the impact of the liver clock is limited. For example, most WT oscillating lipid and xenobiotic metabolites are not restored in Liver-RE mice. Insulin signaling is also defined as a nonautonomous pathway. As for mRNA profiles, mTOR and insulin pathways display mixed patterns: some regained oscillation in Liver-RE, such as *Pik3r1* and *Mtor*, while others did not, like *Deptor* and *Insr*. These pathways are likely influenced by systemic inputs from other metabolic tissues like the intestine, muscle, adipose, and pancreas, which are not functioning properly in Liver-RE mice. Also, consistent with the absence of adrenal clock, the rhythms in hepatic corticosterone level are not restored: other clocks appear to distally influence Bmal1 liver clock function and its oscillatory output.

In stark contrast with the above nonautonomous pathways, genes and metabolites related to glycogenesis and the NAD^+ salvage pathway remain rhythmic in Liver-RE mice [1]. Crucially, Liver-RE mice do not show significant circadian feeding/fasting behavior in LD, which resembles that of global *Bmal1*-knockout (KO) mice. These animals lack, accordingly, circadian cycles of oxygen consumption and energy expenditure. The rhythms in the Liver-RE cannot be due to signals derived from a pattern of feeding or its associated metabolism. Rather, those are likely *bona fide* autonomous rhythms by the liver.

The underlying regulatory mechanism of the autonomous rhythms involve Bmal1-induced transcriptional regulation of key enzymes for glycogen synthesis (glycogen synthase 2, Gys2) and the salvage pathway for NAD^+ synthesis (nicotinamide phosphoribosyltransferase, Nampt). Critically, these enzymes constitute the rate-limiting step of the relevant biosynthetic pathways and therefore their circadian regulations are highly effective and efficient [4–6].

The archetypal use of the term 'autonomy' in the field refers to the self-sustained, cell-autonomous oscillation of the molecular clock in single cells. In this sense, attention is required because although the liver clock expresses its autonomy from the rest of the clock network, a physiological LD cycle is required for maintaining this liver-autonomous circadian metabolic cycling; in constant darkness, no significant oscillations remain [1]. There are several pathways through which light information can reach the liver; for example, (i) sympathetic innervation via the paraventricular nuclei [7], (ii) sympathetic nervous system-mediated induction of corticosterone from the adrenal [8], and (iii) a not-direct route through the light-masking effect of locomotor activity [1,3]. Yet, the exact modes-of-action of light on liver metabolism will need further investigation. Equally, a potential direct effect of forced feeding–fasting cycle on the nonautonomous pathways would also merit further investigation for both fundamental and clinical research.

Besides addressing the tissue-independence, the Liver-RE system will be useful to reveal a degree of governance over other tissues. The liver clock may have a role in long-range signaling, however, the mechanisms through which the liver may signal distal sites, such as the brain, remain unexplored. *Bmal1*-KO mice not only lose behavioral rhythmicity but also exhibit other phenotypes, such as decreased body weight and shortened life span [9]. Skeletal muscle-specific transgenic rescue of Bmal1 expression ameliorates these phenotypes [10]; the failure of the Liver-RE to rescue the early ageing phenotype [1] prompts investigation of skeletal muscle-RE mice. Simultaneous reconstitution of multiple tissues, including a combination of liver and SCN, and other metabolic tissues, would promote identification of organ-to-organ clock communication pathways that influence timekeeping organism-wide. Let's expect to see RE-RE-RE mice in future.

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Spotlight

Antagonism between Antiviral Signaling and Glycolysis

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RIG-I-like receptor (RLR)-mediated interferon production is critical for antiviral responses. A recent study (Zhang *et al*., *Cell*, 2019) uncovered a reciprocal inhibition between RLR signaling and glycolysis: lactate produced by glycolysis inhibits RLR signaling by binding to RLR signaling component mitochondrial antiviral-signaling (MAVS), whereas RLR activation suppresses glycolysis through inhibiting glycolysis enzyme hexokinase.

Type I interferons (IFNs), such as IFN-α and IFN-β, not only are central to host innate and adaptive immunity against viral infection, but also can exert antitumor and immunomodulatory activities [1]. Type I IFNs are induced following the recognition of pathogen-associated molecular patterns by different pattern recognition receptors, such as retinoic-acid-inducible gene I (RIG-I)-like receptors (RLRs) [2]. RIG-I is an RLR that senses cytosolic viral double-strand RNAs (dsRNAs) to initiate downstream signaling pathways, including the mitochondrial antiviral-signaling (MAVS)-TANK binding kinase 1 (TBK1)-interferon regulatory factor 3 (IRF3) signaling axis, culminating in IRF3-mediated type I IFN transcription. The RIG-I-MAVS interaction occurs on mitochondria, the 'powerhouses of the cell', indicating a potential crosstalk between this antiviral signaling and cellular metabolism; however, whether cellular metabolism plays any role in regulating this crucial host defense signaling pathway has remained elusive.

To investigate potential crosstalk between RLR signaling and cellular metabolism, Zhang *et al.* [3] carried out an untargeted metabolomic analysis to capture alterations in cellular metabolism following RLR activation. This analysis revealed a significant decrease of most metabolites involved in glycolysis and the tricarboxylic acid (TCA) cycle following the initial RLR activation, suggesting that RLR signaling inhibits glycolysis. Once imported into cells, glucose is first converted to glucose-6-phosphate by hexokinase, followed by a series of glycolytic reactions that break down glucose into pyruvate [4]. Since RLR activation increased intracellular glucose levels but decreased most other glycolytic intermediates downstream of hexokinase, the authors reasoned that hexokinase might be the crucial step regulated by RLR signaling. Further analyses indeed revealed a decrease in hexokinase activity during the initial stages of RLR activation. Mechanistically, the authors showed that RLR activation hampers mitochondrial localization of hexokinase (which is required for its activation) [5] by inhibiting hexokinase interaction with MAVS, a mitochondria-localized adaptor protein critical for RLR signaling [2]. Upon recognition of dsRNA by RIG-I, a conformational switch in RIG-I promotes RIG-I interaction with MAVS and causes the displacement of hexokinase from MAVS and therefore from mitochondria, resulting in impaired hexokinase activation and decreased glycolysis.

The authors further speculated that the altered glucose metabolism upon RLR activation may in turn regulate RLR signaling. In support of this hypothesis, the authors found that, upon RLR activation, cells cultured in low-glucose media or treated with 2-deoxy-glucose (a glycolysis inhibitor) exhibited increased type I IFN production and decreased viral replication compared with cells cultured in high-glucose media or treated with vehicle. Importantly, similar results were confirmed in fasting mice treated with low glucose compared with those treated with high glucose. These results suggest that the downregulation of glucose metabolism promotes RLR signaling.

Pyruvate, the end product of glycolysis, has two major fates. Under aerobic conditions, pyruvate is transported into mitochondria and converted to acetyl-CoA, which then enters the TCA cycle, followed by electron transport and oxidative phosphorylation to completely oxidize glucose for ATP production. Under anaerobic conditions, pyruvate is converted to lactate by lactate dehydrogenase (LDHA), and lactate is subsequently exported out of cells, a process called anaerobic glycolysis [4]. (Although it is much less efficient in producing ATP, anaerobic glycolysis allows cells to regenerate NAD^+ needed for the early steps in glycolysis in the absence of oxygen, whereas under aerobic conditions NAD^+ can be regenerated through the electron transport chain in which oxygen serves as the final electron acceptor.) Using a series of approaches to switch between these two metabolic routes downstream of pyruvate, the authors elegantly demonstrated that it is the lactate generated by the LDHA reaction that negatively regulates RLR signaling. Further analyses using mice treated with an LDHA inhibitor