Cell-autonomous circadian clock of hepatocytes drives rhythms in transcription and polyamine synthesis

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The circadian clock generates daily rhythms in mammalian liver processes, such as glucose and lipid homeostasis, xenobiotic metabolism, and regeneration. The mechanisms governing these rhythms are not well understood, particularly the distinct contributions of the cell-autonomous clock and central pacemaker to rhythmic liver physiology. Through microarray expression profiling in Met murine hepatocytes (MMH)-D3, we identified over 1,000 transcripts that exhibit circadian oscillations, demonstrating that the cell-autonomous clock can drive many rhythms, and that MMH-D3 is a valid circadian model system. The genes represented by these circadian transcripts displayed both cophasic and antiphasic organization within a protein–protein interaction network, suggesting the existence of competition for binding sites or partners by genes of disparate transcriptional phases. Multiple pathways displayed enrichment in MMH-D3 circadian transcripts, including the polyamine synthesis module of the glutathione metabolic pathway. The polyamine synthesis module, which is highly associated with cell proliferation and whose products are required for initiation of liver regeneration, includes enzymes whose transcripts exhibit circadian oscillations, such as ornithine decarboxylase and spermidine synthase. Metabolic profiling revealed that the enzymatic product of spermidine synthase, spermidine, cycles as well. Thus, the cell-autonomous hepatocyte clock can drive a significant amount of transcriptional rhythms and orchestrate physiologically relevant modules such as polyamine synthesis.

networks | chronobiology | resistance distance

Many aspects of mammalian physiology and behavior display circadian (~24-h) rhythms, including the sleep/wake cycle, blood pressure, heart rate, metabolism, and liver regeneration (1, 2). These rhythms are regulated by the circadian clock, which enables consolidation and coordination of physiological events to specific phases of the 24-h cycle in anticipation of daily environmental changes. Dysfunction of the clock is associated with serious human health conditions, including shift work syndrome, sleep disorders, increased risk of cancer, cardiovascular disease, and metabolic syndrome (1, 2).

The circadian clock is a self-sustaining, entrainable, cell-autonomous network of three interlocked transcriptional negative feedback loops (2). The primary loop consists of BMAL1/CLOCK transcriptional activators, which dimerize and turn on transcription of *Period* (*Per1*, *Per2*, and *Per3*) and *Crytochrome* (*Cry1* and *Cry2*) genes through E-box elements. PER and CRY proteins dimerize and feed back to inhibit BMAL1/CLOCK activation. Two associate loops interlock with the core loop: the ROR/REV-ERB element (RRE) loop composed of ROR activators (RORa, RORb, and RORc) and REV-ERB repressors (REV-ERBα and REV-ERBβ), which compete for RRE transcription factor binding sites (TFBS), and the D-box loop composed of the activator DBP and repressor E4BP4, which act through D-box TFBS (2).

In addition to internal regulation of clock genes, the clock also orchestrates circadian rhythms of output networks, which ultimately govern overt rhythms in physiology and behavior. Nearly all mammalian cell types contain a circadian clock, producing at the organismal level a multioscillator system in which systemic and local circadian signals may jointly regulate physiology. This system can be divided into two main classes of clocks: the central pacemaker and peripheral clocks. The central pacemaker resides in the suprachiasmatic nucleus (SCN) and receives light input directly from the retina, entraining it directly to the light/dark cycle (1). The SCN acts to synchronize peripheral clocks in other tissues through systemic signals, and orchestrates rhythms in physiology. In contrast, the role of peripheral clocks remains to be elucidated. Despite ~10% of the genome displaying circadian rhythms in gene expression in many tissues, little overlap of rhythmic genes exists across tissues, suggesting that tissue-specific regulatory networks generate rhythms in local physiology (3, 4). In mice, disrupting the local liver clock abolishes circadian rhythms in many liver genes, even in the presence of a functional central pacemaker, implying a significant role for the liver clock in hepatic gene expression (5).

Rhythmic feeding behavior also represents a major entrainment signal for the hepatic clock. Restricting food access to the middle of the light period induces phase inversion of the liver clock in wild-type (WT) mice (6), and rhythmic feeding alone can drive oscillations in hepatic gene expression (7). When food is plentiful, feeding behavior is synchronized with the light/dark SCN-driven activity cycle. However, in conditions of scarcity or restricted access to food, feeding rhythms can be driven by the food-entrainable oscillator (FEO), which is independent of SCN light entrainment and is believed to involve multiple regions of the central nervous system (8–10). It remains unclear how hepatocytes balance the respective roles of systemic circadian regulation applied by the SCN and FEO vs. cell-autonomous regulation from the hepatic clock to generate circadian rhythms in liver functions.

To address the role of the cell-autonomous circadian clock, systemic influences need to be removed while still maintaining the integrity of the circadian clock and its physiological outputs. We selected the immortalized mouse cell line Met murine hepatocytes (MMH)-D3 as a candidate model system. Derived from the 3-d-old liver of transgenic c-Met mice (11), MMH-D3 is immortalized but not transformed, and maintains a high level of differentiation upon induction (11, 12), providing a system that reflects to a significant extent an in vivo hepatocyte.

We combined multiple analytic methods for the identification of circadian rhythms in large datasets. Using this pipeline, we reveal that MMH-D3 hepatocytes contain a functional cell-autonomous

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