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PNAS published online May 29, 2007;
doi:10.1073/pnas.0703516104

This information is current as of May 2007.

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Lean gene and the clock machine

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Signs of an increased girth are everywhere across the “fattening” world. Attempts to countermand this epidemic can be gauged by the rise in bariatric surgery in both the United States and Europe and the continued search for as-yet-elusive anti-obesity therapies. Against this backdrop, an intensive effort to unravel the molecular underpinnings of feeding and weight regulation has been underway since the discovery in 1994 of the adiposity hormone leptin (from the Greek *leptos*, for thin) (1). The prevailing model holds that long-term body weight constancy is maintained by leptin and other molecular signals that communicate between peripheral sites of fuel storage and utilization and specific regions of brain involved in ingestive behavior and energy expenditure. This system is thought to provide a selective advantage by preserving nutrient sufficiency during times of food scarcity. In a fascinating twist, brain regions involved in energy balance also appear to control peripheral glucose homeostasis, although the molecular pathways relating CNS and peripheral metabolism remain obscure. Recently, an emerging line of research into the genetic underpinnings of circadian behavior has surprisingly implicated a role for internal molecular clock genes in the coordinate regulation of behavior, energy balance, and metabolism. Work reported by Green *et al.* (2) in this issue of PNAS points toward a molecular output of the clock, an RNA deadenylase, as an additional factor that may underlie the coregulation of circadian rhythms and metabolism.

Intracellular clock genes encode a transcription–translation feedback loop composed of activators and repressors that cycle together according to a 24-h period to match the rotation of the earth (see Fig. 1 and reviewed in refs. 3–5). In mammals, the master clock resides within neurons of the suprachiasmatic nucleus (SCN) and functions like a metronome, maintaining rhythmic synchrony between multiple clock oscillators located within other regions of brain and peripheral tissues. Resonance of multiple tissue circadian oscillators is thought to arise from the alignment of CNS and peripheral clocks and may be important in optimizing internal physiological processes with daily changes in the environment.

Insight into the genetic control of circadian physiology and behavior has begun to emerge with the identification of core mammalian clock genes (6). A number of studies have also shown that a large percentage (3–10%) of the mammalian transcriptome oscillates according to a 24-h rhythm, including both direct clock controlled genes (CCGs) and downstream outputs of these CCGs (7, 8). In a screen for differentially displayed retinal transcripts selectively increased at night in *Xenopus*, Green and Besharse (9) identified a novel RNA deadenylase, which they termed “nocturnin.” *Nocturnin* expression has subsequently been identified in a wide range of mammalian tissues, including liver, oocytes, and brown fat, in addition to retina [Genomics Institute of the Novartis Research Foundation (GNF) Gene Expression Atlas and ref. 10]. Intriguingly, expression of *Nocturnin* increases ≈ 100 -fold in the early evening hours in the liver, and mice that have mutations in the core circadian clock gene, *Clock*, have reduced levels of Nocturnin (11).

To address the question as to whether *Nocturnin* is a component of the core clock machinery or whether it is a downstream CCG output gene, Green *et al.* (2) ablated the *Nocturnin* gene in mice. Their observation that *Nocturnin*-null mice exhibit both normal circadian locomotor activity rhythms and normal expression of the canonical core clock genes demonstrates that *Nocturnin* is not a part of the core clock machinery but is rather a downstream CCG. Given the pronounced cyclic variation in the expression of *Nocturnin* in liver, as well as evidence for circadian oscillation of hepatic transcription networks involved in lipogenesis, lipid catabolism, bile acid synthesis, and gluconeogenesis, a series of studies were undertaken to investigate hepatic physiology in *Nocturnin*-knockout mice. Intriguingly, on a regular chow diet, *Nocturnin*-knockout mice have reduced lipid accumulation (steatosis) in liver compared with wild-type mice, and after high-fat feeding, they are resistant to obesity and steatosis. Leanness in *Nocturnin*-knockout mice indicates an abnormality in weight regulation that could result from defects in nutrient absorption, food intake, energy expenditure, or adipogenesis.

Surprisingly, Green *et al.* (2) have demonstrated that the *Nocturnin*-null animals have reduced activity, equivalent caloric intake, and similar metabolic rates compared with controls. However, it should be noted that slight alterations in energy intake and/or expenditure induced by the loss of *Nocturnin* may be too small to measure but still have an impact when summated over many days. Curiously, the observation that *Nocturnin*-knockout mice have a trend toward elevated free fatty acid levels suggests a possible abnormality in either lipogenesis or insulin sensitivity. However, the relative protection from hepatic lipid accumulation and the absence of overt hypertriglyceridemia in *Nocturnin*-null animals also suggests an abnormality in intestinal lipid absorption.

Interestingly, analysis of glycemic control in *Nocturnin*-null mice appears to reflect potential differential effects of the gene in multiple tissues because oral glucose tolerance is impaired, whereas insulin sensitivity is improved, in regular chow-fed *Nocturnin*-null mice. One interpretation of these results is that the *Nocturnin* mutation may promote insulin sensitivity in fat, muscle, and liver, but impair glucose responsiveness of the pancreas. Moreover, on a high-fat diet, *Nocturnin*-knockout and wild-type mice display equivalent glucose tolerance, consistent with the protection from steatosis in *Nocturnin*-null mice. It is important to note that the key role of the liver is to maintain glucose levels through hepatic gluconeogenesis during sleep; thus, *Nocturnin* may normally play an important role in the metabolic transition from wakefulness to sleep (i.e., the fed to the fasted condition). To further illuminate the role of *Nocturnin* in the circadian regulation of gluconeogenesis, it will be helpful to extend analyses of *Nocturnin* knockout mice to addi-

Author contributions: K.M.R. and J.B. wrote the paper.

Conflict of interest statement: J.B. has been a consultant for Amylin, Genentech, Merck, and Takeda Pharmaceuticals.

See companion article on page 9888.

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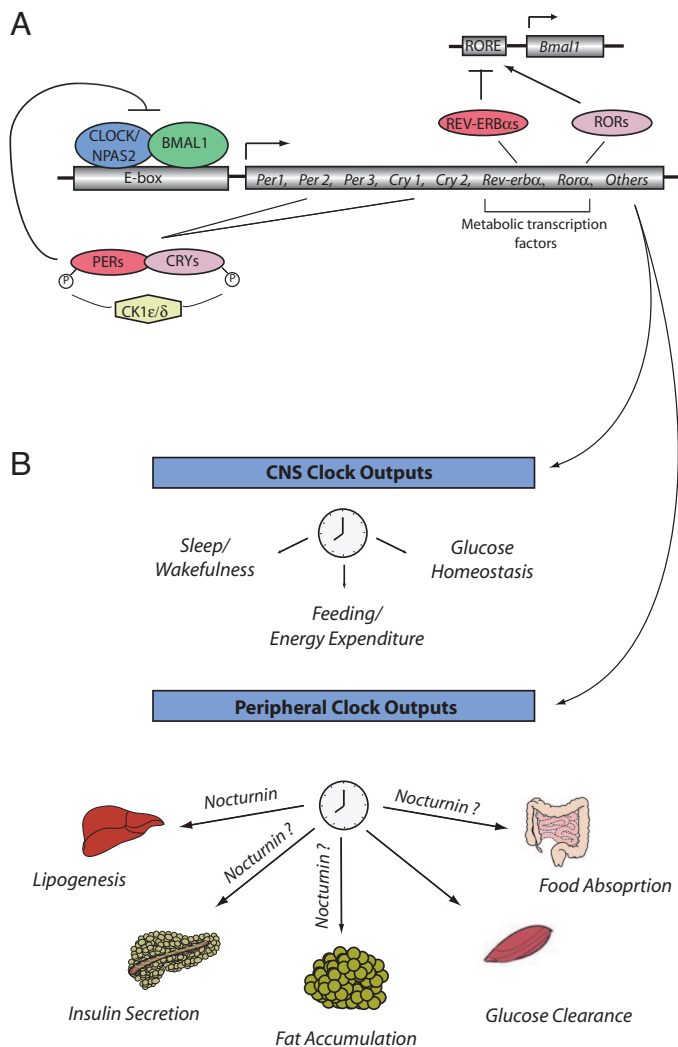


Fig. 1. Emerging studies continue to identify core elements and the physiologic functions of the intrinsic molecular oscillator. (A) Core molecular clock machinery. CLOCK/NPAS2 (circadian locomotor output cycles kaput/neuronal PAS domain protein 2) and BMAL1 (brain and muscle ARNT-like 1) constitute the positive limb of the core circadian network by activating downstream targets, including the *period* (*Per*), *cryptochrome* (*Cry*), *Ror*, and *Rev-Erb* genes. The major negative limb of the circadian network is mediated by PER and CRY, which inhibit CLOCK/BMAL1 activity. CK1 ϵ/δ (casein kinases I ϵ and δ) phosphorylates PER and CRY and triggers the degradation of PER, and the RORs and REV-ERBs, circadian-controlled metabolic transcription factors, regulate *Bmal1* transcription. (B) Integration of molecular clock and metabolic systems. Both direct and indirect transcriptional targets of the clock machinery participate in regulation of energy balance and metabolism at multiple levels within both the CNS and peripheral tissues. The CNS clock output involves signaling from the central pacemaker neurons of the suprachiasmatic nucleus (SCN), which synchronizes clock gene expression within extra-SCN regions of the brain and many peripheral metabolic tissues. Transcription of *Nocturnin*, a well characterized deadenylase, is highly rhythmic in several peripheral tissues and has now been identified as an important factor modulating the response to diet-induced obesity.

tional time points in the light–dark cycle and to test the response of these animals to a prolonged fast or calorie restriction. Future studies *in vitro* in isolated cells will also aid in distinguishing pancreatic islet, hepatic, and adipose effects of the mutation.

Analysis of mRNA expression in liver and adipose tissue could offer some clues to the effects of the *Nocturnin* mutation on metabolic regulation. Green *et al.* (2) observed that high-fat feeding does not result in an induction of *Srebp-1c* levels in livers of *Nocturnin*-null mice, whereas a robust increase in *Srebp-1c* levels is observed in livers of wild-type mice fed high-fat chow (12). Several lines of evidence indicate that *Srebp-1c* expression contributes to hepatic lipid accumulation in insulin-resistant conditions (13). Moreover, *Srebp-1c* expression is downstream of the insulin-responsive nuclear receptor LXR, and induction of *Srebp-1c* reflects continued insulin sensitivity in liver even in the setting of insulin resistance in adipose tissue and muscle (14). Because *Nocturnin* animals remain lean on the high-fat diet, the reduced level of *Srebp-1c* may be due to decreased lipid absorption and/or accumulation. An alternative explanation would be that diminished levels of *Srebp-1c* indicate insulin resistance at the level of liver in *Nocturnin*-knockout mice. A better understanding of the effects of *Nocturnin* on lipid absorption and/or hepatic lipogenesis will provide more detailed information concerning the interactions between circadian and metabolic gene regulatory networks.

Recognition that circadian gene expression is a feature of nearly all cells and that disruption of circadian genes gives rise to alterations in feeding and metabolism has spurred increased investigation into both physiologic and molecular function of clock genes and CCGs in brain and peripheral metabolic tissues. The results of Green *et al.* (2) provide a new example of the participation of CCGs in the susceptibility to diet-induced obesity and diabetes. Continued analysis of the tissue-specific effects of clock genes on metabolic systems ultimately may illuminate a mechanistic basis for links between disorders of timing and metabolism.

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