

When the *Clock* stops ticking, metabolic syndrome explodes

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Circadian control depends on oscillating transcription factors, master switches synchronized by stimuli such as light and feeding. Recent studies show that altering circadian rhythmicity also results in pathophysiological changes resembling the metabolic syndrome.

Many physiological processes display day-night rhythms. Feeding behavior, lipid and carbohydrate metabolism and blood pressure control are only a few examples of processes subject to daily variation. This biological oscillation is controlled by the circadian clock, an endogenous self-sustained machinery of rhythmically acting transcriptional loops. These self-regulated feedback loops balance each other; in mammals, transcription factors like *Clock* and *Bmal1* regulate the production of proteins like *Per* and *Cry*, which in turn regulate the production of *Bmal1*. Owing to this arrangement, the levels of *Bmal1*, *Clock*, *Per* and *Cry* oscillate rhythmically¹.

Various input signals (light and various paracrine and endocrine signals) can modulate these transcriptional pathways and 'reset the clock.' In addition, the circadian clock dictates the rhythmic production of output regulators, other transcription factors such as *DBP*, *HLF* and *TEF*. These transcription factors, in turn, regulate downstream target genes involved in different biochemical pathways, including metabolism of glucose and lipids, synthesis of cholesterol and bile acids, and others (Fig. 1).

In any given tissue, three to ten percent of transcripts show circadian rhythmicity. Many of them participate in common metabolic pathways, such as metabolism of glucose, cholesterol and fatty acids and mitochondrial oxidative phosphorylation^{2,3}. Dysregulation of these pathways owing to, for instance, a sedentary lifestyle and high caloric intake, results in the development of cholesterol abnormalities (dyslipidemia), insulin resistance, obesity and hypertension. These disorders often occur simultaneously and have therefore been grouped under the term 'metabolic syndrome' (Fig. 2). Recent studies show that deletion of the *Clock* and *Bmal1* genes results not only in circadian disturbances, but also in metabolic abnormalities of lipid and glucose homeostasis—a phenotype resembling the metabolic syndrome^{4,5}.

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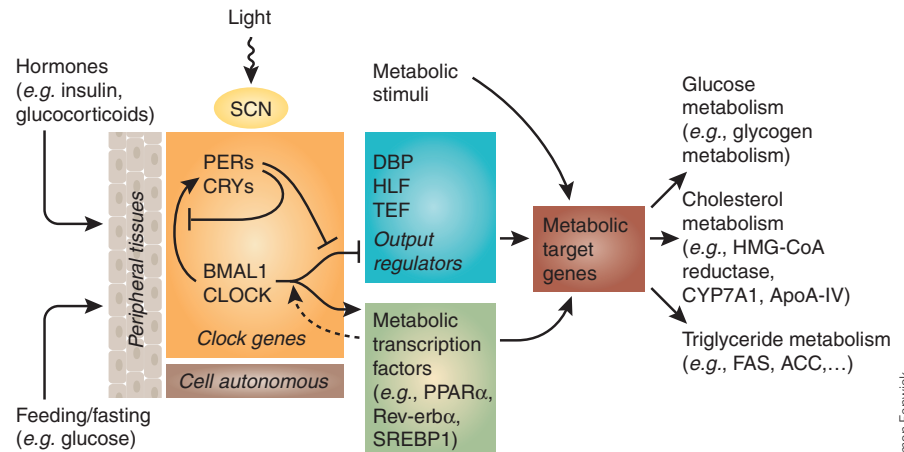


Figure 1 Transcriptional control of metabolic pathways by circadian oscillators. The molecular circadian oscillator is composed of two coupled feedback loops—*Per*-*Cry* and *Clock*-*Bmal1*—that regulate each other rhythmically. These feedback loops also control the expression of downstream transcription factors such as *DBP*, *HLF* and *TEF*. The circadian oscillator can be modulated by light, which acts on the suprachiasmatic nucleus (SCN) in the brain, and by metabolic stimuli such as hormones and nutritional status, which act on peripheral tissues. The transcription factors of the *Clock* machinery also regulate genes involved in metabolic control in peripheral tissues such as liver and adipose tissue.

Turek *et al.*⁴ showed that mice homozygous for a loss-of-function mutation in the *Clock* gene have altered patterns of food intake: they eat too much, become obese and develop hyperglycemia and dyslipidemia. These *Clock* mutant mice develop adipocyte hypertrophy and excessive accumulation of fat in the liver (hepatic steatosis), hallmarks of the metabolic syndrome. Rudic *et al.*⁵ found that mutations in *Bmal1* and *Clock* not only modified the diurnal variation in levels of plasma glucose and triglycerides, but also influenced the development of glucose intolerance and insulin resistance in response to a high-fat diet. The observed metabolic phenotypes in these mutant mice are fairly modest, indicating that the role of the *Clock* genes on metabolism might be modulatory and not obligatory.

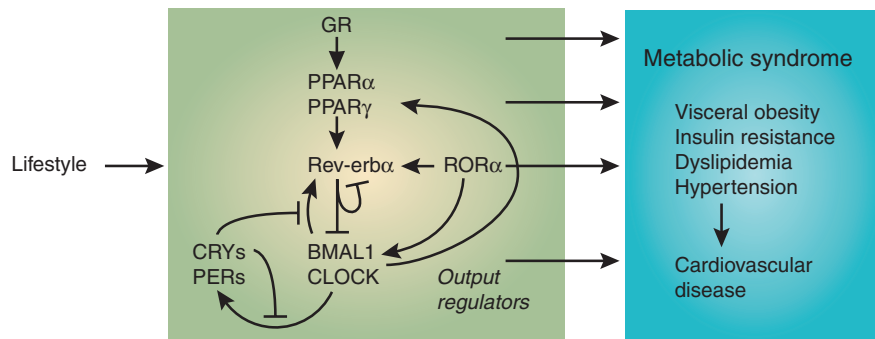
The *Clock* genes also show patterns of rhythmic expression in peripheral organs, such as liver and adipose tissue. In obese and diabetic animals, these rhythms are disturbed, as well as the rhythmic expression of adiponectin, resistin, visfatin and leptin, adipokines that control energy homeostasis, glucose and lipid metabo-

lism⁶. Interestingly, *Bmal1* is induced during and promotes adipocyte differentiation, possibly by activating the downstream adipogenic transcription factors *Rev-erbα* and *SREBP1* (ref. 7). These alterations in adipocyte differentiation and adipokine expression profiles might also occur in the liver and may contribute to the phenotype of the *Clock* mutant mice.

An important question is whether the phenotype of the *Clock* mutant mice results from the alterations in circadian rhythmicity *per se* or from other unknown activities of the *Clock* gene. To answer this question, it will be of interest to characterize the phenotype of mutant mice for other genes of the circadian clock and to create tissue-specific knockout mice for the different *Clock* genes.

Another important question is to establish the pathways downstream of the clock genes that ultimately influence glucose and lipid metabolism. It is likely that these pathways involve well-known clock output regulators, such as *DBP*, *Dec1* and *Dec2*, as well as other transcription factors known to regulate metabolic state, such

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Figure 2 Interplay between transcription factors of the Clock machinery and the nuclear receptor superfamily in metabolic control. The glucocorticoid receptor (GR), peroxisome proliferator-activated receptors (PPAR) α and γ , Rev-erb α and Clock-Bmal1 all regulate metabolic processes such as adipogenesis, cholesterol synthesis and catabolism and lipogenesis. Disruption of these pathways leads to the phenotypic characteristics of the metabolic syndrome.

as SREBP1 or the nuclear receptors ROR α , Rev-erb α and PPAR α . For example, expression of hepatic PPAR α , which is activated by fatty acids, shows circadian rhythmicity directly mediated by Clock and Bmal1 (ref. 8) and modulated by glucocorticoids⁹ (Fig. 1). Similarly, transcriptional regulation of CYP7A1, a rate-limiting enzyme in bile acid synthesis, a pathway long known to show circadian rhythmicity, is regulated by DBP and Dec2, and by nuclear receptors including PPAR α ^{10–12}. Along the same line, Rev-erb α , a nuclear receptor that regulates hepatic and adipose lipid metabolism through peripheral actions, is also expressed in the suprachiasmatic nucleus, a brain region crucial for circadian rhythmicity, where it has an important role in the control of the *Clock* genes¹². Moreover, PPAR α and PPAR γ regulate hepatic and adipose Rev-erb α expression, respectively^{14,15}. Finally, recent data indicate that ROR α and Rev-erb α regulate lipid metabolism and participate in the control of *Clock* and *Bmal1* expression^{13,16–18}. So, it appears that the Clock and nuclear receptor pathways may converge to

integrate metabolic and circadian signals from the environment. As nuclear receptors are putative drug targets for combating the metabolic syndrome, studies on these interacting pathways may provide clues for optimal therapeutic management.

Plasma cholesterol (high- and low-density lipoprotein), triglycerides and coagulation factors (fibrinogen and platelets) show circadian variations¹⁹. Moreover, the incidence of cardiovascular diseases such as myocardial infarction, sudden cardiac death, stroke, unstable angina and high blood pressure appears to occur at higher frequency at specific moments of the day¹⁹. Acute jet lag, shift work and night eating, syndromes related to changes in circadian rhythmicity, occur together with alterations in one or more of the pathological components of the metabolic syndrome^{20,21}. The finding that *Clock* genes impact metabolism provides a potential molecular basis for these pathophysiological conditions and may therefore suggest possibilities for new therapeutic approaches that take circadian rhythms into account. This is the basis

of chronotherapeutics, which, despite being in its infancy, has already proven to be promising for improving the therapeutic index of several drugs. An example relates to the circadian variations in cholesterol synthesis through its rate-limiting enzyme HMG-CoA reductase, the expression of which shows diurnal rhythmicity, being highest during the night. For this reason, statins, widely prescribed cholesterol-lowering drugs that inhibit this enzyme, are most efficient when taken before bedtime. Thus, the observation that *Clock* genes control energy homeostasis may lead to the development of more finely tuned, and hopefully more optimal, treatments of obesity and its related complications.

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Author's Comments

Timing and serendipity had a major role in our studies on clocks and metabolism. The work began shortly after I arrived at Northwestern University as junior faculty, when Joe Takahashi and Fred Turek told me about possible body weight abnormalities in the *Clock* mutant mouse, a model primarily studied because of its dysregulated locomotor activity and sleep phenotypes. A powerful aspect of the *Clock* model was the molecular framework it had already built in studies of behavior, and I was excited to think that we might exploit the model to deepen our insight into interconnections between behavior and energy balance. Timing was important, as microarray studies had just indicated that metabolic transcriptional networks were a major output of the clock, and there were data that redox flux could directly affect activity of NPAS2, a *CLOCK* homolog. Our key experiment was to place mutant and isogenic littermate mice on a high-fat diet and to track changes in metabolism, in conjunction with detailed round-the-clock studies of metabolic markers (including glucose, insulin, leptin and glucocorticoids). The many all-night experiments made for close bench-partners of members of both circadian and metabolic labs and paved a new direction for our own research. At least two major questions have emerged from our original study. First, how are cell-autonomous clocks synchronized? Second, how does the clock network contribute to energy homeostasis? Understanding the pathways that link circadian activity, sleep, feeding and peripheral metabolic homeostasis, and delineating the impact of changes in each parameter on the whole organism, will hopefully bring us one step closer to a unified 'systems' map of metabolic disease.

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