

HIGHLIGHTED TOPIC | *The Role of Clock Genes in Cardiometabolic Disease*Rev-erb- $\alpha$ : an integrator of circadian rhythms and metabolismHélène Duez<sup>1,2,3,4</sup> and Bart Staels<sup>1,2,3,4</sup><sup>1</sup>University Lille Nord de France, <sup>2</sup>Institut National de la Santé et de la Recherche Médicale, U545, <sup>3</sup>UDSL, and <sup>4</sup>Institut Pasteur de Lille, Lille, France

Submitted 27 May 2009; accepted in final form 13 August 2009

**Duez H, Staels B.** Rev-erb- $\alpha$ : an integrator of circadian rhythms and metabolism. *J Appl Physiol* 107: 1972–1980, 2009. First published August 20, 2009; doi:10.1152/jappphysiol.00570.2009.—The endogenous circadian clock ensures daily rhythms in diverse behavioral and physiological processes, including locomotor activity and sleep/wake cycles, but also food intake patterns. Circadian rhythms are generated by an internal clock system, which synchronizes these daily variations to the day/night alternance. In addition, circadian oscillations may be reset by the time of food availability in peripheral metabolic organs. Circadian rhythms are seen in many metabolic pathways (glucose and lipid metabolism, etc.) and endocrine secretions (insulin, etc.). As a consequence, misalignment of the internal timing system vs. environmental zeitgebers (light, for instance), as experienced during jetlag or shift work, may result in disruption of physiological cycles of fuel utilization or energy storage. A large body of evidence from both human and animal studies now points to a relationship between circadian disorders and altered metabolic response, suggesting that circadian and metabolic regulatory networks are tightly connected. After a review of the current understanding of the molecular circadian core clock, we will discuss the hypothesis that clock genes themselves link the core molecular clock and metabolic regulatory networks. We propose that the nuclear receptor and core clock component Rev-erb- $\alpha$  behaves as a gatekeeper to timely coordinate the circadian metabolic response.

circadian rhythm; metabolic disorders; biological clock; metabolic syndrome

## INTRODUCTION: CIRCADIAN RHYTHMS ARE DAILY FLUCTUATIONS IN BEHAVIORAL AND PHYSIOLOGICAL PROCESSES

CIRCADIAN RHYTHMS ARE DAILY fluctuations with a period of approximately 1 day (“circa diem”) observed in many physiological processes and behavior. They are driven by an endogenous clock and are defined as cycles persisting when organisms are isolated from environmental cues (“free-running”), the subjected circadian “night” and “day” being, in this case, predicted by the endogenous oscillator. Obvious circadian rhythms are the sleep and wake alternance, as well as daily variations in body temperature, blood pressure, and heart rate. In humans, sleep onset is accompanied by a fall in body temperature, while blood pressure and heart rate start to increase in the late dark phase to prepare for the awakening. Along the same line, catabolic functions are turned on during the active awaken phase, whereas anabolic processes usually take place during the rest phase. These rhythms allow the organism to anticipate, adapt, and optimize its metabolic, hormonal, and locomotor activity to predictable environmental daily changes imposed by the rising and setting of the sun (27).

In mammals, a central “master” clock residing in the supra-chiasmatic nuclei of the hypothalamus integrates information from light and synchronizes our physiology to the day/night alternance (Fig. 1). In turn, it coordinates many “slave oscillators” in the rest of the body through still poorly defined neuroendocrine signals. These peripheral oscillators convey output signals from the master clock and integrate additional circadian cues, such as food availability, to drive local circadian rhythms in intermediary metabolites (such as NAD<sup>+</sup>/NADH) or in key, often rate-limiting, enzymes involved in local physiology, such as in metabolic pathways. Indeed, many metabolic functions are subjected to circadian variations, ranging from lipid and carbohydrate metabolism, hormone (insulin, leptin, cortisol, growth hormone) secretion, and, less intuitive, although primordial, gene transcription (58). This ensures that nutrient transporters and metabolic enzymes are produced at appropriate times (i.e., stimulation of catabolic pathways during the wake period to match fuel demands) and allows temporal separation of divergent processes, such as glycolysis and gluconeogenesis. Thus there is a strong interaction between circadian control and metabolism, a concept that is further supported by the observation that circadian disruptions induced either by phase shift (as observed in shift workers) or due to genetic alteration in the clock machinery (sequence polymorphisms present in certain clock genes or genetically induced mutations) are associated with increased incidence of metabolic disorders in humans and murine models.

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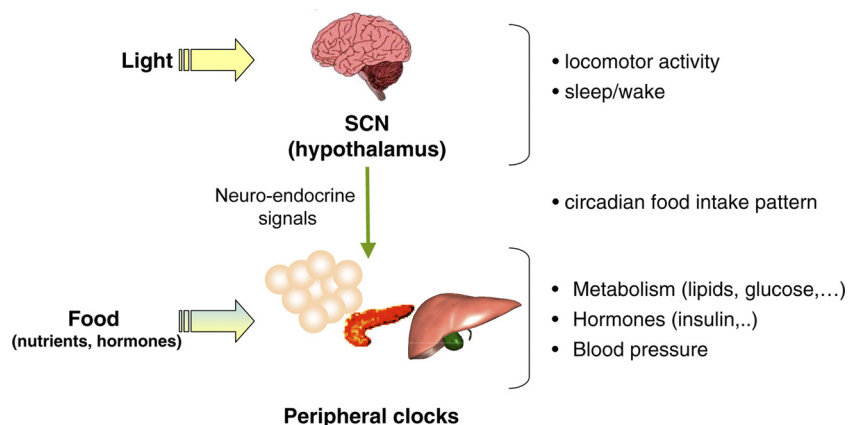


Fig. 1. The “master” clock residing in the suprachiasmatic nucleus (SCN) of the hypothalamus drives rhythmic behavior (sleep/wake, locomotor activity, circadian feeding pattern) and synchronizes many peripheral clocks, which are thought to be important for local rhythmic activity, for instance of hepatic metabolism, cardiovascular function, and the endocrine response. Food is also an important time cue for peripheral clocks. Transcriptome analysis in peripheral organs found 3–20% rhythmic genes (58), including genes involved in lipid and glucose metabolism and most of the transcription factors of the nuclear receptor family (86), suggesting a direct interaction between circadian behavior and metabolic transcriptional networks.

### MOLECULAR BASIS OF CIRCADIAN CONTROL

The central clock and peripheral oscillators share a common molecular architecture, conserved between species, and consist of transcriptional/(post)translational events generating and sustaining circadian rhythms. In mammals, CLOCK (circadian locomotor output cycles kaput), Bmal1 (brain and muscle ARNT like protein 1), and the CLOCK paralog NPAS2 (78) activate transcription of various target genes, including the *Per* (*Period*) and *Cry* (*Cryptochrome*) genes (Fig. 2). In turn, the proteins *Per* and *Cry* repress CLOCK/Bmal1-mediated gene transactivation, thereby shutting down their own transcription. This allows a new cycle to start. An additional feedback loop, thought to improve the robustness of the former one, involves the nuclear receptors Rev-erb- $\alpha$  and retinoic acid-related orphan receptor (ROR)- $\alpha$ . CLOCK/Bmal1 activates Rev-erb- $\alpha$  transcription, resulting in daily fluctuations of Rev-erb- $\alpha$ , which, in turn, represses *Bmal1* (60). ROR- $\alpha$ , another nuclear receptor and component of the clock machinery, com-

petes with Rev-erb- $\alpha$  for the binding to the *Bmal1* promoter ROR element (RORE)/RevRE site, and activates its transcription (1, 28, 69). Therefore, the two nuclear receptors, Rev-erb- $\alpha$  and ROR- $\alpha$ , act as a regulatory loop, and occupancy of *Bmal1* promoter by either Rev-erb- $\alpha$  or ROR- $\alpha$  is crucial for a proper timing of the core clock machinery. More recently, the nuclear receptor cofactor and metabolic regulator peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) coactivator (PGC)-1 $\alpha$ , whose expression cycles in liver and muscle has been suggested to potentiate ROR- $\alpha$  activation of Rev-erb- $\alpha$  and *Bmal1* transcription and, as such, to be involved in circadian rhythm regulation (46).

Several reports have challenged the concept of transcriptionally mediated cellular rhythmicity. Using an inhibitor of transcription, Dibner and colleagues (17) show that inhibition of RNA polymerase II-dependent transcription only diminished the amplitude, but did not abolish gene expression circadian oscillations in

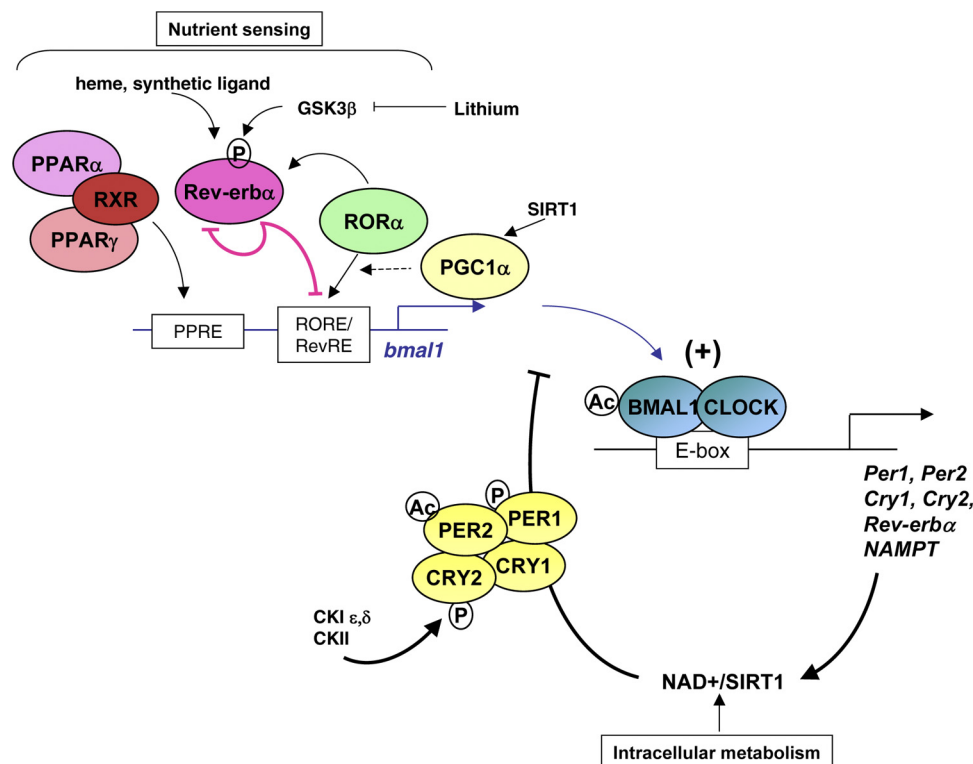


Fig. 2. Generation and maintenance of circadian rhythms require a complex interplay of transcriptional/translational feedback regulatory loops. The CLOCK/BMAL1 heterodimer activates transcription of the *Per* and *Cry* genes via E boxes in their promoter. PER and CRY form a repressive complex, which inhibits CLOCK/BMAL1 transcriptional activity, leading to their own repression. An interlocked regulatory loop is formed by Rev-erb- $\alpha$  and ROR- $\alpha$ , which compete for the binding to a RORE/RevRE and subsequent regulation (repression vs. activation) of *Bmal1*. Rhythmic expression of Rev-erb- $\alpha$  drives cyclic occupancy of this RORE. Posttranslational modification (phosphorylation, ubiquitination, acetylation, and degradation by the proteasome) plays an important role for the proper timing of the clock. Intracellular metabolism (through NAD<sup>+</sup>/SIRT1) and nutrients (through binding to nuclear receptors) impinge on the clock machinery. NAMPT, nicotinamide phosphoribosyltransferase; CK, casein kinase; Ac, acetylated; P, phosphorylated. See text for definition of all other acronyms.

cultured fibroblasts, highlighting the importance of nontranscriptional events in the regulation of the clock machinery. Along the same line, *Bmal1* oscillations are not required for the circadian clock function (45), and phosphorylation, independently of any transcriptional event, is a crucial step for circadian rhythm generation and amplitude in cyanobacteria (56).

Beside the core set of clock genes described above, post-translational modifications, such as phosphorylation, sumoylation, and acetylation of the clock components, are important for an appropriate timing to nearly 24 h. Protein phosphorylation by the casein kinase (CK)-1 $\epsilon$  (20) and CKII (44, 79, 80) influences *Per* and *Cry*, as well as *Bmal1* protein stability and activity, and thus the circadian period (24). For instance, missense mutation of *CK-1 $\epsilon$*  results in a circadian period shortened to 20 h in the *tau* hamsters (47). Inhibition of glycogen synthase kinase (GSK)-3 $\beta$ , which stabilizes the Rev-erb- $\alpha$  protein, with lithium leads to rapid proteasomal degradation of Rev-erb- $\alpha$  and activation of *Bmal1* (88). GSK-3 $\beta$ -mediated stabilization of Rev-erb- $\alpha$  appears to be a crucial event for circadian rhythm initiation, maintenance, and synchronization after serum shock (88). Additionally, SUMOylation has been shown to affect *Bmal1* activity, protein ubiquitination, and degradation, thereby being essential for proper circadian rhythmicity (12, 40). Along the same line, acetylation of histones and the chromatin compaction state participate in the regulation of the clock machinery (21). CLOCK itself was shown to function as an acetyltransferase on both histones (18) and *Bmal1* (29). Finally, Yin and Lazar (87) have demonstrated that Rev-erb- $\alpha$  regulates *Bmal1* activity after recruiting the nuclear corepressor (NCoR)/histone deacetylase 3 (HDAC3) complex to the RevRE/RORE present in its promoter.

#### PERIPHERAL CLOCKS AND METABOLISM

The discovery that clock genes are expressed *in vivo* in organs, such as the liver, adipose tissue, aortas, etc., has been instrumental in the identification of clock machineries in peripheral organs (3, 58, 68, 90). In the liver, 3–20% of the transcripts are cyclic, the majority of which are involved in key metabolic events (58, 75). Although the observation that clock genes cycle in periphery does not necessarily prove the existence of peripheral clock systems, it can be hypothesized that, at least in some tissues, peripheral pacemakers are able on their own to drive and orchestrate local circadian variations in metabolic transcription clusters. Indeed, disruption of the hepatic molecular clock via overexpression of the clock machinery component Rev-erb- $\alpha$  specifically in the liver, leaving the central pacemaker intact, results in nearly complete (90%) dampening of circadian variations of the hepatic transcriptome (34). However, 10% of the transcriptome, *i.e.*, 31 transcripts, including *Per2*, still cycle, indicating that rhythmic expression of these transcripts can be driven by both systemic cues and local oscillators. Interestingly, Lamia *et al.* (35) have described the metabolic phenotype of total vs. liver-specific *Bmal1*-deficient mice. Although some transcripts still cycle in the liver of hepato-specific *Bmal1*-*l*- mice, indicating again that systemic cues are important in driving local oscillations, liver-specific, but not whole body, *Bmal1*-deficient mice suffer from hypoglycemia and display altered liver circadian expression of genes important in the glucose metabolic pathway, supporting the concept that the lack of a functional liver clock, in the presence of an intact central oscillator, leads to impaired glucose homeostasis.

Food availability is a dominant “zeitgeber” for peripheral clocks, and changes in the time of food availability resynchronize peripheral clocks independently of the master suprachiasmatic nuclei clock (15, 75) (Fig. 1). Interestingly, food-derived, lipid-soluble nutrients and hormones can activate transcription factors of the nuclear receptor superfamily, which comprises numerous regulators of metabolic pathways, such as Rev-erb- $\alpha$  and ROR- $\alpha$ , and also the PPARs, or the glucocorticoid receptor, among many others. A gene expression analysis has revealed that 28 out of 49 of them display tissue-specific circadian expression in liver, white and brown adipose tissue, and skeletal muscle (86), highlighting the possibility that nuclear receptors may trigger circadian expression of their target genes (see below).

#### CIRCADIAN MISALIGNMENT AND METABOLIC DISORDERS

From the above data, it appears that a proper alignment of the circadian clock with environmental “zeitgebers” is required for normal, “healthy” metabolic regulation. Martino and colleagues (50) have recently reported that circadian desynchrony in CK-1 $\epsilon$  mutant hamsters (due to misalignment between the endogenous clock period, which is shortened by 4 h, and environmental day/night cues) induced cardiomyopathy and renal disease, and that adjusting the light/dark cycle to the shortened endogenous circadian period of these animals normalizes their behavioral and locomotor patterns and reverses this abnormal phenotype. In humans, a progressive desynchrony protocol was applied to 10 adults by subjecting them to 28-h days for 10 days, with a 14:14-h sleep-wake alternance (70). Circadian misalignment led to decreased leptin throughout the entire cycle, increased glucose, despite increased insulin, suggesting decreased insulin sensitivity, and increased blood pressure. Even more striking was the observation that forced desynchrony resulted in exacerbated postprandial glucose excursion, with a maximal disturbance during maximal misalignment (*i.e.*, 180° phase shift obtained after 3 extended days).

Along the same line, numerous association studies have demonstrated that chronic desynchronization experienced by shift workers increases the risk to develop obesity, type 2 diabetes, hyperlipidemia, high blood pressure, and cardiovascular disease (16, 32, 76, 77). In addition, shift workers display an altered postprandial response during the nighttime work period, characterized by exaggerated increase in glucose, insulin, and triglyceride levels following a test meal (48).

Altogether, these observations reflect the now recognized relationship between the circadian clock and metabolism and emphasize that disruption of circadian rhythms leads to metabolic complications and higher risk to obesity and type 2 diabetes, with its constellation of adverse cardiovascular events.

#### MOLECULAR EVIDENCES THAT THE MOLECULAR CLOCK SYSTEM IMPINGES ON METABOLIC PATHWAYS, AND RECIPROCALLY

##### *Core Clock Genes Connect to Metabolism*

Clock genes cycle and thus might regulate circadian rhythms in many tissues, including peripheral metabolic tissues (such as liver, adipose tissue, skeletal muscle, and pancreas), and in central nuclei known to be involved in nutrient sensing and



food entrainment (such as the mediobasal nucleus of the hypothalamus). The concept that clock genes themselves participate in metabolic regulation stemmed from a study from Turek and colleagues (81) published in 2005, which revealed that *clock* <sup>$\Delta$ 19</sup> mutant mice are hyperphagic, become obese, and develop hyperlipidemia and hyperglycemia upon high-fat feeding (Table 1). Interestingly, clock mutant mice on a Jcl-ICR background are resistant to diet-induced obesity, likely due to impaired dietary lipid absorption (57). In addition, *Bmal1*<sup>-/-</sup> mice display altered circadian plasma glucose oscillations, as well as glucose intolerance (67). *Bmal1* expression is induced during the differentiation of 3T3L1 preadipocytes into mature adipocytes and seems to participate in the control of adipogenesis (72).

In 2008, Lamia and colleagues (35) reported the metabolic phenotype of mice with a liver-specific *Bmal1* deletion. Interestingly, these mice have normal locomotor activity and feeding behavior, compared with total *Bmal1* deficiency, which results in loss of circadian locomotor and feeding pattern. Liver-specific *Bmal1* knockout mice have altered circadian glucose homeostasis and altered circadian expression of genes involved in glucose homeostasis, especially GLUT-2, neoglucogenic genes (glucose-6-phosphatase-translocase and phosphoenolpyruvate carboxy kinase), and L-pyruvate kinase, resulting in hypoglycemia during the fasting period (35). These data suggest that the hepatic clock plays a role in maintaining adequate circadian glucose availability. Alternatively, and because whole body *Bmal1* deficiency results in normal fasting glycemia but glucose intolerance, desynchronization between hepatic and other endogenous clocks may have resulted in an inappropriate or desynchronized hepatic glucose response.

Emerging evidence demonstrates that mutations or polymorphisms in clock genes are associated with features of the

metabolic syndrome in humans as well. Clock genes are expressed in human tissues and cells (6, 26) and are linked to the metabolic syndrome. Several *Clock* polymorphisms have been identified that are associated with body weight and increased susceptibility to obesity (71, 73). *Bmal1* has also been associated with type 2 diabetes and hypertension in humans (85).

#### SIRT1/NAD<sup>+</sup>

Asher et al. (5) and Nakahata et al. (54) have shown that the NAD<sup>+</sup>-dependent histone deacetylase sirtuin (SIRT)1, whose activity and/or expression level cycles, counterregulates CLOCK and drives cyclic expression of *Bmal1*, *Per2*, and *Cry1*. Nakahata et al. (55) and Ramsey et al. (63) went on by demonstrating that, in turn, Clock/*Bmal1* regulates the expression of the nicotinamide phosphoribosyltransferase, the rate-limiting enzyme of the NAD<sup>+</sup> synthetic pathway, also known as visfatin. Interestingly, SIRT1 plays a crucial metabolic role, and SIRT1 protein levels are increased upon starvation in the liver. SIRT1 interacts with PGC-1 $\alpha$  to enhance the gluconeogenic pathway (66). In addition, SIRT1 is important in adipocyte physiology, and, on food withdrawal, it antagonizes the action of PPAR- $\gamma$ , one of the master regulators of adipogenesis, on genes mediating fat storage, such as the *aP2* promoter, thereby promoting fat mobilization in starved mice (59). SIRT1 also interferes with the adipogenic process and triggers lipolysis in differentiated adipose cells (59). To catalyze the removal of an acetyl group, SIRT1 uses NAD<sup>+</sup>, which directly reflects the metabolic status of the cell. Caloric restriction, by increasing the content of NAD<sup>+</sup>, stimulates SIRT1 activity (8). Moreover, SIRT1 activity and NAD<sup>+</sup> metabolism are modulated by AMPK, a "fuel gauge" that senses the intracellular

Table 1. *Metabolic consequences of genetically induced or spontaneous mutations in different clock genes in murine models*

Genotype	Metabolic Phenotype	Reference No.
clock $\Delta$ 19 mutant	C57Bl/6 genetic background Fed a regular chow diet: increased body weight gain and energy intake; altered circadian pattern in food intake and energy expenditure; hypercholesterolemia, hypertriglyceridemia, hyperglycemia, and hypoinsulinemia; increased leptin levels during the light phase; altered circadian pattern of hypothalamic expression of neuropeptides involved in food intake regulation (ghrelin, orexin, CART) Fed a high-fat diet: further increase in body weight, energy intake, triglycerides, cholesterol, and glucose levels; adipocyte hypertrophy and hepatic steatosis	81
<i>Bmal1</i> <sup>-/-</sup>	Jcl-ICR genetic background Decreased gluconeogenesis (when fed a regular chow diet)	67
	Whole body deficiency Moderate weight gain on a high-fat diet due to impaired dietary lipid absorption	57
	Decreased body weight (when fed a regular chow diet)	51
	Blunted gluconeogenesis, altered circadian variations of blood glucose (decreased at CT4, increased at CT16, vs. weight) when fed a regular chow diet	67
Liver-specific deficiency	Increased fat mass, glucose intolerance, decreased insulin levels when fed a regular chow diet	35
	Normal feeding behavior, normal fat mass (as compared to weight); fasting hypoglycemia accompanied by altered circadian pattern of expression of GLUT-2, LPK, PEPCK and G6P-T (regular chow diet)	35
Total knockout + muscle rescue	Normalization of body weight (regular chow diet)	51
Rev-erb $\alpha$ <sup>-/-</sup>	Increased plasma triglycerides levels, abnormal bile acid metabolism, altered circadian expression pattern of bile acid metabolism-related genes in liver (when fed a regular rodent chow diet)	19; 65
Staggerer (sg/sg)	Increased liver apolipoprotein C-III expression and triglycerides levels, decreased HDL-Cho (when fed a regular chow diet) and aggravated atherosclerosis development Reduced fat depots when fed a regular chow diet, and resistance to high-fat diet-induced obesity likely due to compromised feeding capacity	49; 64 38

HDL-Cho, high-density lipoprotein-cholesterol; CART, cocaine and amphetamine-regulated transcript; CT, circadian time (CT0 = light on, CT12, light off); GLUT-2, hepatic glucose transporter; PEPCK, phosphoenolpyruvate carboxy kinase; G6P-T, glucose-6-phosphatase-translocase; LPK, L-pyruvate kinase.

AMP-to-ATP ratio (11). This implies not only that the clock modulates metabolism, but also the other way around: that the metabolic status of the cell influences the clock machinery. Indeed, it has been demonstrated that murine models of genetically (obese *ob/ob* and diabetic *db/db* mice) or high-fat feeding-induced obesity and diabetes have disrupted circadian expression of clock and clock-regulated genes and altered locomotor activity, feeding pattern, and sleep regulation (31, 33, 36, 37).

### Nuclear Receptors

Besides Rev-erb- $\alpha$ , which will be discussed below, several nuclear receptors (such as PPAR- $\alpha$  and PPAR- $\gamma$ ) and the nuclear receptor coregulator PGC-1 $\alpha$  show strong circadian pattern in numerous tissues, suggesting that they may link nutrient sensing and circadian control of metabolism. PGC-1 $\alpha$  has been shown to enhance *Bmal1* and *Rev-erb- $\alpha$*  transcription through ROR- $\alpha$  transcriptional activity potentiation, and liver-specific PGC-1 $\alpha$ -deficiency leads to arrhythmicity (46). Interestingly, PGC-1 $\alpha$  has been shown to play a pivotal role in the response to fasting, a condition under which its expression increases in the liver. In this organ, PGC-1 $\alpha$  triggers the fasting-induced activation of the gluconeogenic pathway and fatty acid oxidation, and mice deficient for PGC-1 $\alpha$  display hypoglycemia and hepatic steatosis (43, 61). The fasting/feeding transition is one of the most predictable daily changes. It implies coordinated metabolic adjustments in several metabolic tissues to maintain fuel supply and energy homeostasis at all times. Because PGC-1 $\alpha$  interacts with different nuclear receptors and transcription factors, it may be part of the coordinated response necessary to face these metabolic challenges.

Feeding time is a dominant zeitgeber of the peripheral clock (15), and food access restriction inverses the phase of circadian expression in mouse liver. Remarkably, glucocorticoids have been suggested to interfere with that process, since mice harboring a hepato-specific deletion of the glucocorticoid receptor invert their circadian liver gene expression more rapidly after the shift of food availability to daytime (39). With a different protocol, other data have been reported indicating restricted feeding, but not corticosterone injection, may entrain the liver clock in rat (74).

Nutrients derived from food, in particular fatty acids, may activate PPAR- $\alpha$ , a key regulator of fatty acid metabolism whose expression oscillates diurnally in liver (42). Interestingly, PPAR- $\alpha$  binds to the *Bmal1* promoter and regulates its expression (10). The CLOCK/*Bmal1* heterodimer reciprocally regulates PPAR- $\alpha$  (30). Finally, PPAR- $\alpha$  increases *Rev-erb- $\alpha$*  expression in human liver cells (25).

Thus nuclear receptors may entrain circadian variations in their target genes and thus participate in the circadian control of metabolic pathways.

### REV-ERB- $\alpha$ : A CLOCK GENE AND NUCLEAR RECEPTOR TRANSDUCING CLOCK SIGNALS INTO METABOLIC REGULATIONS

#### *Rev-erb- $\alpha$ : A New Target in Circadian Disorders?*

Mice deficient for Rev-erb- $\alpha$  display a 0.5-h shorter period and more heterogeneously distributed period length. They are

also more sensitive to light pulse-induced phase shift compared with wild-type controls (60). Heme has been identified as a natural Rev-erb- $\alpha$  ligand, which potentiates the recruitment of the NCoR, thereby enhancing Rev-erb- $\alpha$ -mediated transcriptional repression of its target genes (62, 89). The *Drosophila* homolog of Rev-erb- $\alpha$ , called E75, had already been reported to bind heme, which regulates E75 stability and transcriptional activity. Interestingly, intracellular heme levels oscillate and may, therefore, affect the transcriptional repressive activity of Rev-erb- $\alpha$ . A synthetic ligand for Rev-erb- $\alpha$  has been recently developed, which increases the recruitment of NCoR and Rev-erb- $\alpha$  transcriptional activity (52). Interestingly, this compound induces circadian phase shift in vitro. This indicates that one can modulate Rev-erb- $\alpha$  activity, and that this modulation impacts on circadian rhythms, identifying Rev-erb- $\alpha$  as an interesting pharmacological target for phase-resetting drugs. One might also speculate that other "orphans", such as the ROR isotypes, might be adopted in the future. This would offer the possibility to affect the ROR/Rev-erb regulatory loop pharmacologically, supposedly in a tissue-specific manner. It is also noteworthy that the synthetic Rev-erb- $\alpha$  ligand has been shown to induce phase advance as well as phase delay in vitro, depending on the abundance of Rev-erb- $\alpha$ . This observation indicates that treatment response to Rev-erb- $\alpha$  ligands will ultimately depend on the time of administration. Thus the discovery that Rev-erb- $\alpha$  acts as a liganded nuclear receptor opens a new area of research of more selective Rev-erb- $\alpha$  modulators.

#### *Rev-erb- $\alpha$ Links Circadian Clock to Energy Homeostasis*

Rev-erb- $\alpha$  is also an important metabolic regulator expressed in a circadian manner in tissues such as liver, adipose tissue, muscle, and pancreas. Indeed, Rev-erb- $\alpha$  modulates lipid, glucose, and bile acid metabolism, adipogenesis, and the inflammatory reaction. Intuitively then, it is likely that Rev-erb- $\alpha$  may relay circadian signals into metabolic and inflammatory regulatory responses and vice versa (Fig. 3).

*Rev-erb- $\alpha$  participates in lipid and glucose metabolism.* Rev-erb- $\alpha$  downregulates the expression of liver apolipoprotein C-III, a constituent of very-low-density lipoproteins, and Rev-erb- $\alpha$ -deficient mice exhibit a dyslipidemia characterized by increased triglyceride levels (19, 65). Rev-erb- $\alpha$  has also been suggested to repress the expression of *elovl3*, a gene encoding a very long chain fatty acid elongase (4). These data indicate that Rev-erb- $\alpha$  modulates both systemic and hepatic lipid metabolism. It also influences de novo glucose synthesis. Indeed, Yin and colleagues (89) have demonstrated that heme binding to Rev-erb- $\alpha$ , resulting in enhanced transcriptional activity, leads to increased repression of the gluconeogenic *Pepck* gene in vitro in human hepatoma HepG2 cells. It remains unclear whether Rev-erb- $\alpha$  affects circadian variations in glucose levels. Nonetheless, these data indicate that modulation of Rev-erb- $\alpha$  activity is likely to affect lipid and glucose metabolism in a circadian fashion.

*Rev-erb- $\alpha$  connects the adipose tissue clock and adipogenesis.* Altered circadian patterns due to work shift or sleep disorders are associated with increased body mass index. It is tempting to speculate that chronic alteration in circadian variations of various adipogenic regulators, such as PPAR- $\gamma$ , or other genes involved in lipid uptake might result in abnormal

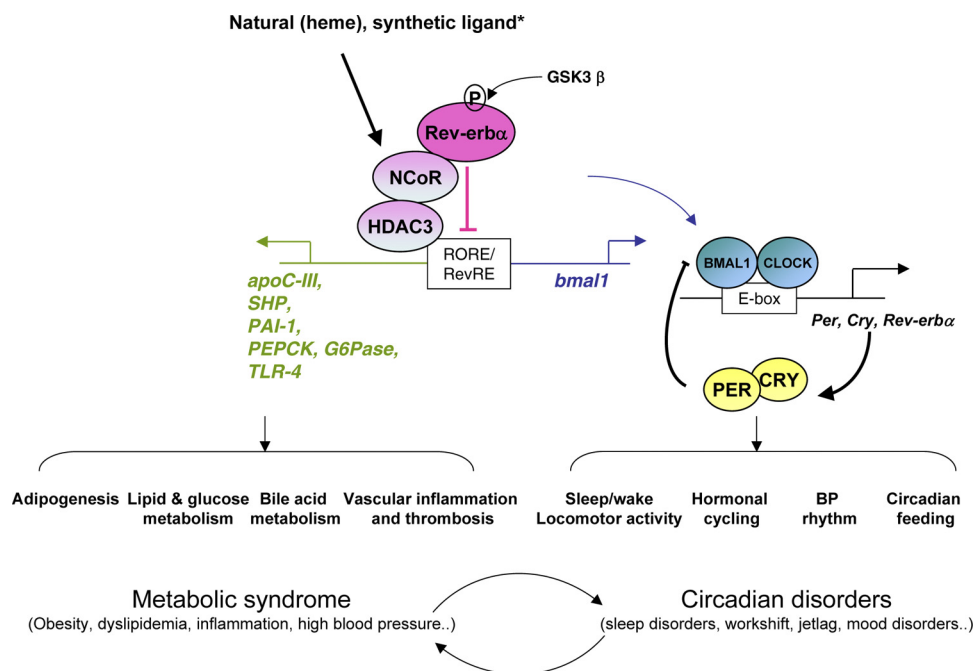


Fig. 3. Rev-erb- $\alpha$  binds to RORE/RevRE sites located in the promoter of its target genes. Rev-erb- $\alpha$  is critical for the proper timing of the core clock machinery. Rev-erb- $\alpha$  also regulates metabolic functions, including lipid and bile acid metabolism, adipogenesis, gluconeogenesis, the inflammatory response, and thrombosis. Rev-erb- $\alpha$  ligands modify its transcriptional activity and suggest that Rev-erb- $\alpha$  may constitute a promising target for the treatment of circadian alterations of metabolic pathways. \*Synthetic ligand: see Meng et al. (52). BP, blood pressure; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; apoC-III, apolipoprotein C-III. See text for definition of all other acronyms.

circadian pattern of adipocyte physiology with subsequent changes in differentiation, lipid storage, and adipokine secretion (9). *Rev-erb- $\alpha$*  expression cycles in adipose tissue (90) and is induced during the adipogenic process and by PPAR- $\gamma$  activation by rosiglitazone in rat adipose tissue (13, 22). Ectopic overexpression of *Rev-erb- $\alpha$*  in 3T3L1 preadipocytes promotes their differentiation into mature adipocytes and enhances lipid storage (22, 83). In a recent paper, Alenghat and colleagues (2) demonstrated that impaired recruitment of the NCoR/HDAC3 complex to the Rev-erb- $\alpha$  response element present in the *Bmal1* promoter results in aberrant circadian pattern of the canonical clock genes, leading, in turn, to altered diurnal expression of genes involved in metabolism. Strikingly, this was associated in vivo with a lean phenotype (NCoR mutant mice having less adipose tissue) and ameliorated metabolic phenotype. This does not exclude the fact that Rev-erb- $\alpha$  may exert both circadian-independent functions in the adipose tissue, e.g., on adipogenesis, and a circadian role through the control of the rhythmic alternation between fat storage and disposal.

*Rev-erb- $\alpha$  participates in the circadian control of bile acid metabolism.* Bile acids are biologically active molecules that serve for lipid solubilization in the intestinal lumen and modulate lipid and glucose metabolism (41). The cholesterol 7 $\alpha$ -hydroxylase (*Cyp7A1*) gene, which encodes the first and rate-controlling enzyme of the major bile acid biosynthetic pathway, exhibits a strong diurnal pattern of expression (14). We have recently shown that Rev-erb- $\alpha$  cross talks with the bile acid receptor FXR for the regulation of the small heterodimer partner (*Shp*) and modulates *Cyp7A1* expression through altered circadian expression of the two CYP7A1 regulators *Shp* and *E4BP4* (also known as Nfil3) (19). The albumin site D-binding protein is a clock output protein that, together with the hepatocyte leukemia factor and the thyrotroph embryonic factor, belongs to the PARBZip family of transcription factors. These well-conserved mammalian pro-

teins display strong circadian variations in numerous tissues and play important metabolic roles. In particular, they are important for xenobiotic detoxification. Since D-binding protein and E4BP4 bind to and regulate common sets of target genes (82), one might speculate that Rev-erb- $\alpha$  may play a more broad role in regulating clock output signals, as well as specific catabolic pathways.

#### *Rev-erb- $\alpha$ and Cardiovascular Diseases*

The cardiovascular system displays circadian variations in numerous outputs, such as blood pressure, heart rate, thrombolysis cascade, etc., and myocardial infarction and stroke are known to peak early in the morning. Rev-erb- $\alpha$  has been shown to modulate metabolism of triglyceride-rich lipoproteins, which are associated with increased risk of cardiovascular disease, thereby possibly affecting the development of atherosclerosis (65). On the other hand, atherosclerosis is also characterized by an inflammatory status of the vascular wall and the presence of macrophages within the lesion. Rev-erb- $\alpha$  is present in vascular wall cells, including macrophages (7, 23, 53). In human macrophages, Rev-erb- $\alpha$  represses the induction of toll-like receptor-4, the receptor of lipopolysaccharide, thereby diminishing the production of cytokines in response to lipopolysaccharide. These data demonstrate an anti-inflammatory role of Rev-erb- $\alpha$  (23) and suggest a positive impact of Rev-erb- $\alpha$  on atherosclerotic lesions.

Along the same line, Rev-erb- $\alpha$  has been identified as a potent negative regulator of plasminogen activator inhibitor (PAI)-1, an important inhibitor of the fibrinolysis cascade that may promote the development of atherothrombosis (84). In humans, PAI-1 oscillates diurnally with a zenith in the early morning, which coincides with acute thrombotic and cardiovascular events, such as myocardial infarction. GSK-3 $\beta$  enhances PAI-1 repression via phosphorylation and stabilization of the Rev-erb- $\alpha$  protein, and a phosphorylated form of Rev-



erb- $\alpha$  dampens PAI-1 oscillations. This suggests that Rev-erb- $\alpha$  may affect the circadian expression levels and rhythmicity of PAI-1 and affect the fibrinolysis cascade in a circadian manner.

#### FUTURE PERSPECTIVES

Although the biological functions of Rev-erb- $\alpha$  are still not completely understood, it plays a crucial role in the core clock regulation on the one hand and, on the other hand, in lipid and adipogenic control, the inflammatory response, and has been suggested to influence the gluconeogenic pathway. It is, therefore, likely that Rev-erb- $\alpha$  mediates metabolic circadian rhythmicity. Yet, except for the shorter circadian period displayed by the Rev-erb- $\alpha$ -deficient mice, we still have little knowledge on how Rev-erb- $\alpha$  connects circadian disorders to metabolic pathologies. A better characterization of the biological functions of Rev-erb- $\alpha$  in metabolism *in vivo* will contribute to an improved understanding of the integration between circadian and metabolic pathways.

Recently, several studies have identified novel natural (heme) and synthetic ligands for Rev-erb- $\alpha$  and revealed the possibility to increase Rev-erb- $\alpha$  activity through enhanced recruitment of its corepressor NCoR. In addition, Rev-erb- $\alpha$  modulation was proven to be efficient in phase-advance or delay circadian rhythms, at least *in vitro*. Therefore, Rev-erb- $\alpha$  ligands may be useful in resetting the clock after jetlag or work shift and reentrain and put in resonance our body clocks. One can also hypothesize that the time of administration of a drug affecting Rev-erb- $\alpha$  activity will be an important factor to consider for its therapeutic efficacy. Given the broadened role of Rev-erb- $\alpha$  in lipid metabolism, adipogenesis, and vascular wall pathophysiology, Rev-erb- $\alpha$  represents a promising target for the treatment of metabolic abnormalities resulting from chronic aberrant circadian rhythms. The next step will be to determine whether these ligands may affect its capacity to regulate target genes *in vivo* and normalize circadian disorder-related metabolic abnormalities.

#### GRANTS

The authors acknowledge funding supports from Institut National de la Santé et de la Recherche Médicale, Contrat Plan Etat Région, the Région Nord Pas-de-Calais/FEDER, the Sixth European Union Research Framework Program Diabesity (contract LSHM-CT-2003-503041), and COST-action BM0602.

#### DISCLOSURES

No conflicts of interest are declared by the author(s).

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