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## Metabolism control by the circadian clock and vice versa

Kristin Eckel-Mahan and Paolo Sassone-Corsi

Department of Pharmacology, University of California, Irvine, California USA

Paolo Sassone-Corsi: psc@uci.edu

### Abstract

Circadian rhythms govern a wide variety of physiological and metabolic functions in most organisms. At the heart of these regulatory pathways in mammals is the clock machinery, a remarkably coordinated transcription-translation system that relies on dynamic changes in chromatin states. Recent findings indicate that regulation also goes the other way, as specific elements of the clock can sense changes in the cellular metabolism. Understanding in full detail the intimate links between cellular metabolism and the circadian clock machinery will provide not only crucial insights into system physiology but also new avenues toward pharmacological intervention of metabolic disorders.

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A staggering number of our biological functions are controlled by the circadian clock. Whether we feed, rest, sleep or exercise, the 24 h–based cycle that governs our life has intimate links with our body’s metabolism. Is the endogenous clock present in most organisms, regulating their metabolism, or do variations in their metabolic cycles feed back on the clock? To what extent do changes in our rhythmic life patterns—for example, restricted feeding or altered sleep-wake cycles—coordinately impinge on unique molecular gears common to the body’s clock and metabolism?

The anatomical center of the circadian clock consists of a small region of the brain (approximately 20,000 neurons) known as the suprachiasmatic nucleus (SCN, see Table 1). As circadian rhythms control activities as disparate as the sleep-wake cycle, hormone secretion, glucose metabolism, memory formation and changes in body temperature, the finding that there are autonomous oscillations in many tissues of the body constituted a crucial discovery<sup>1-3</sup>. Indeed, the picture that emerges from numerous studies indicates that a network of interconnected peripheral oscillators operates in the body, with the SCN playing the part of conductor<sup>4,5</sup>. Central to the core clock mechanism are the *Clock* and *Bmal1* (also known as *Arntl*) genes, which encode basic-helix–loop–helix (bHLH)-PAS transcription activators that heterodimerize and induce the expression of the genes for two main oscillators, period (*Per*) and cryptochrome (*Cry*), which contain E-box elements (CACGTG) in their promoters<sup>6,7</sup>. Additional genes with promoters containing E-box, D-box and retinoic acid responsive element (RRE) consensus sequences are also clock regulated and make up a large body of circadian clock–controlled genes (CCGs). The *Per* and *Cry* genes encode other elements of the core clock machinery that in the classical view function to form heterodimeric complexes that translocate to the nucleus and inhibit Clock–Bmal1-mediated transcription through direct protein-protein interactions<sup>8-12</sup>. Thus, at its core, circadian rhythmicity is supported by intracellular transcriptional and translational feedback loops that

perpetuate oscillations in gene expression. Such an explanation is deceptively simple; auxiliary proteins necessarily impinge on circadian core components by such mechanisms as methylation, acetylation, phosphorylation and ubiquitination, keeping the core clock strictly timed and yet remarkably plastic, able to adapt to changes in its environment<sup>13-18</sup>. MicroRNAs and RNA binding proteins that affect RNA stability also participate in timekeeping, providing further complexity to the clock machinery<sup>19,20</sup>. Moreover, cells cope with the spectacular task of rhythmically synthesizing 10–15% of their transcripts<sup>21-23</sup>, an undertaking that can be achieved only by a powerful and cyclic mechanism of chromatin remodeling. This is likely to be obtained, at least in part, through the histone acetyltransferase (HAT) activity of the master regulator Clock<sup>24</sup>.

There is increasing evidence that a smoothly running endogenous clock is crucial for energy balance in organisms from cyanobacteria to mammals<sup>25-28</sup>. Internal and external clock resonance seems to be energetically favorable for an organism. For example, *Arabidopsis thaliana* with mismatching internal and environmental periods experience a reduction in leaf chlorophyll content, reduced growth and an increase in mortality<sup>29</sup>. The alignment of endogenous rhythms with the environment allows coordination of physiology with predictable zeitgebers (Table 1). There may be several reasons for this advantage in mammals, including the prevention of aging and dementia pathologies by the efficient removal of DNA damage–induced by oxidative stress<sup>29-34</sup>. The recent discovery that the activity of sirtuin-1 (Sirt1), a longevity-associated protein belonging to a family of NAD<sup>+</sup>-activated histone deacetylases<sup>14</sup>, oscillates in a circadian fashion broadens our knowledge about the communication between the circadian clock and metabolism, but it also reveals a void in our understanding about the molecular support for such interplay<sup>14,15</sup>. Sirt1 (the mammalian ortholog of yeast Sir2) shows an oscillation in activity, impinging back on the circadian clock by altering Bmal1 acetylation and CLOCK–BMAL1-induced gene transcription<sup>14,35</sup>. The discovery of metabolite oscillations during the yeast metabolic cycle<sup>26,36,37</sup> combined with evidence of circadian sirtuin activity allows speculation as to whether metabolites such as NAD<sup>+</sup> themselves serve a preponderant role in the cellular link between metabolism and the circadian clock (Fig. 1).

## Physiological evidence linking circadian rhythms and metabolism

Accumulating physiological evidence indicates that circadian rhythms and energy state are tightly linked. First of all, rodents that lack expression of circadian clock genes or that express mutant variants of clock genes have abnormal metabolic phenotypes. For example, the *Clock* mutant mouse (*Clock*<sup>19</sup>) on a BALB/c and C57BL/6J background is obese and shows signs of abnormal lipid and glucose metabolism, revealing the importance of a transcriptionally active Clock protein for both normal body weight regulation and glucose and lipid homeostasis<sup>27,38</sup>. Interestingly, and possibly revealing the epistatic effect of yet unidentified factors, *Clock*<sup>19</sup> mice on an ICR genetic background also show abnormal lipid distribution, although the effect seems to be protection from weight gain on a high fat diet due to decreased fat absorption<sup>39</sup>. What is clear is that mutations in the *Clock* gene affect metabolism in each genetic background tested, but in different ways. *Bmal1*<sup>-/-</sup> mice also show metabolic abnormalities with impaired insulin responsiveness and reduced gluconeogenesis<sup>40</sup>. Similarly, mice that lack *Bmal1* expression in the liver show fasting-

phase hypoglycemia and excessive glucose clearance in response to insulin, probably resulting from the loss of the circadian glucose transporter-2 (Glut2) protein<sup>28</sup>. These altered metabolic states are somewhat expected, as nuclear hormone receptors, many of which participate directly in fatty acid and glucose metabolism, undergo circadian oscillations<sup>38</sup>.

The levels of the metabolic hormones glucagon, insulin, ghrelin, leptin and corticosterone are also reported to oscillate in a circadian fashion, as is that of peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) an essential activator of gluconeogenesis in the nutrient-deprived state<sup>41-43</sup>. PGC-1 $\alpha$  seems to be dually regulated by circadian processes: it is both rhythmically expressed and it is deacetylated by Sirt1 in an NAD<sup>+</sup>-dependent fashion<sup>44,45</sup>. As is the case with Sirt1, PGC-1 $\alpha$  feeds back on the circadian clock, affecting metabolic rate, body temperature and daily locomotion patterns. These PGC-1 $\alpha$ -mediated changes in physiology may be due to alterations in *Bmal1* transcription, as PGC-1 $\alpha$  and RAR-related orphan receptor- $\alpha$  (ROR $\alpha$ ) coactivate the *Bmal1* promoter<sup>43</sup>.

Finally, restricted feeding is a powerful zeitgeber, one of a limited number of cues that can entrain circadian rhythms in non-SCN tissues but which probably depends on signaling to the dorsomedial hypothalamus<sup>46</sup>. Some evidence suggests that sterol regulatory element-binding protein-1 (SREBP-1) activation and HMG-CoA reductase expression in the liver, both of which participate in sterol metabolism, may serve a central role in restricted feeding-induced phase shifting<sup>47</sup>. Because the endogenous circadian clock has an intrinsic ability to anticipate changes in its environment, restricted feeding-induced phase shifting provides convincing evidence for the codependence of circadian and metabolic processes.

Chromatin remodeling by histone acetylation seems to be a key molecular pathway in the translation of the metabolic message to the circadian clock<sup>24</sup>. The recent findings that another histone deacetylase, HDAC3, interacts with the co-repressor NCOR1 to regulate circadian behavior, insulin signaling and energy expenditure<sup>48</sup> underscore the critical role that epigenetic regulation has in circadian and metabolic physiology.

Connections between circadian rhythmicity and energy balance are poignantly apparent in aging studies. Although neurodegenerative diseases and aging frequently coincide with disrupted circadian rhythms and fragmented sleep patterns<sup>49</sup>, perhaps the *Bmal1*<sup>-/-</sup> mice present the most convincing evidence linking an aging phenotype to disrupted circadian rhythmicity<sup>50</sup>. These mutant mice show numerous signs of early aging and have a reduced lifespan. Tissues from *Bmal1*<sup>-/-</sup> mice demonstrating an age-dependent reduction in size also show high accumulation of reactive oxygen species (ROS), consistent with the idea that Bmal1 participates in the oxidative stress response<sup>51</sup>.

Mitochondria are subject to high levels of ROS production, an inexorable byproduct of respiration. As a result, mitochondrial proteins are central targets for oxidative damage and, when damaged, may contribute to aging disorders such as neurodegeneration. ROS-induced damage of mitochondrial DNA, which inevitably leads to reduced respiration efficiency and a further increase in the level of intracellular ROS production, is thought to be central to the aging process, an idea known as the 'mitochondrial theory of aging'<sup>52</sup>. Accumulating ROS

in the brain probably lead to the age-related neurodegenerative pathologies of Alzheimer's and Parkinson's disease. Although still debated<sup>53</sup>, there is much evidence to back up this theory, particularly in pathologies associated with neurodegeneration<sup>54</sup>. Interestingly, nicotinamide mononucleotide adenylyl-transferase-1 (Nmnat1), an enzyme involved in the biosynthesis of NAD<sup>+</sup>, protects against axonal degeneration in Wallerian degeneration slow mice (Wld<sup>s</sup>). These mice have a spontaneous mutation that increases activation of the Nmnat1 protein, resulting in elevated NAD<sup>+</sup> levels and consequent Sirt1-dependent protection against axonal injury<sup>55,56</sup>. The discovery that Sirt1 activity oscillates suggests that there may be a link between neuroprotection, redox state and circadian rhythmicity<sup>14</sup>. This association is also supported in *Drosophila melanogaster*, where the administration of superoxide radical-producing compounds, such as paraquat, results in reduced clock gene cycling in peripheral tissues and a forkhead box O protein (FOXO)-dependent sensitivity to oxidative stress in the central pacemaker<sup>32</sup>. These studies help bring into focus the importance of circadian control in metabolism and aging, but raise questions as to what fundamentally fuels their interaction at the cellular level.

### NAD<sup>+</sup>, central to both metabolism and circadian rhythmicity?

During the conversion of energy from food to ATP, cells harness energy from the oxidation of food molecules to drive reactions that are required for normal cell function but that may be energetically unfavorable. At the crossroad of cellular metabolism is the mitochondrion, host to the ATP-generating process of oxidative phosphorylation. In the mitochondria, the carrier molecules such as ATP and NAD<sup>+</sup> are used in oxidation-reduction reactions that are crucial for the maintenance of energy balance. In the case of NAD<sup>+</sup>, the acquisition of energy-rich electrons and a hydrogen atom from substrate molecules to form reduced NADH promotes subsequent biosynthesis by the release of hydride ions from reduced NAD to donor molecules. NAD<sup>+</sup> is derived from niacin and operates as a coenzyme for multiple cellular dehydrogenases (such as those necessary for the  $\beta$ -oxidation of fatty acids or for the Krebs cycle), at which time its reduction to NADH precedes its subsequent oxidation by the respiratory chain. Therefore, the role of NAD<sup>+</sup> as a hydrogen carrier is paramount for the production and maintenance of energy stores.

In addition to its redox roles, NAD<sup>+</sup> also serves as a substrate for ADP ribosylation. When NAD<sup>+</sup> is depleted by sustained increases in the activity of the NAD<sup>+</sup>-dependent ribosylating enzyme poly(ADP-ribose) polymerase (PARP)-1, cell death can ensue<sup>57-59</sup>. Mitochondrially localized PARP activity is induced by DNA damage and is thought to contribute to cell death during periods of intense oxidative stress<sup>60</sup>. Vitamin B3 (consisting of the NAD<sup>+</sup> precursors nicotinamide and nicotinic acid) can be particularly useful in preventing NAD<sup>+</sup> turnover, as nicotinamide inhibits PARP activity<sup>61</sup>. The nicotinamide phosphoribosyltransferase enzyme NAMPT (which mediates the biosynthesis of NAD<sup>+</sup> from nicotinamide in mammals) is expressed in the mitochondria, among other cellular loci, and as a sirtuin activator can also protect cells from PARP-mediated cell death. Restoration of mitochondrial NAD<sup>+</sup>, specifically, seems to rescue cells from the PARP-mediated apoptosis that normally occurs when PARP-1 depletes cytosolic pools of NAD<sup>+</sup>, a rescue that probably depends on one of the sirtuin proteins (for example, Sirt3) that localizes to the mitochondria<sup>62</sup>.

Not all NAD<sup>+</sup> functions are restricted to the mitochondria. On the contrary, its role in both PARP and sirtuin regulation can be cytosolic or nuclear<sup>63</sup>. The fact that the NAMPT protein, for example, can localize to the cytoplasm, nucleus and mitochondria suggests that microdomains of NAD<sup>+</sup> are probably important for stimuli-specific responses<sup>64</sup>. In fact, it has been proposed that increasing the nuclear availability of NAD<sup>+</sup> specifically promotes longevity in yeast in a Sir2-dependent manner<sup>65</sup>. The Sir2 protein seems to control aging in yeast by mediating transcriptional silencing at telomeres<sup>66</sup>. The biosynthesis of NAD<sup>+</sup> in mammals relies on the activity of the rate-limiting enzyme NAMPT, whose activation seems to be sufficient to drive Sir2-mediated changes in gene expression and activity<sup>67</sup>. Importantly, recent data reveal that NAD<sup>+</sup> levels oscillate with a 24-h cycle, a rhythm that is driven by the circadian clock<sup>68,69</sup>. The Clock–Bmal1 activator complex regulates *Nampt* expression, in conjunction with Sirt1, which thereby contributes to the cyclic synthesis of its own coenzyme. Thus, the circadian clock is directly implicated in controlling the intracellular levels of critical metabolites, generating an interlocking of the transcriptional feedback clock loop with the enzymatic feedback loop of the NAD<sup>+</sup>-salvage pathway<sup>69</sup> (Fig. 2).

Like mitochondrial PARPs, nuclear PARP proteins are also activated following DNA damage, where they recruit factors necessary for subsequent DNA repair, thereby preserving viability and normal cellular function<sup>70</sup>. Cytosolic and nuclear NAD<sup>+</sup> also carries out an important role via its activation of the deacetylase Sirt1, whose expression has been determined to be both cytosolic and nuclear but not mitochondrial. The recent evidence that Sirt1 activity oscillates over a 24-h cycle<sup>14</sup> provides a novel mechanism for how the cell might rely on circadian-controlled cycling in energy systems to maintain energy balance. Because Sirt1 is a histone deacetylase, its primary function may be to translate cellular energy states to chromatin remodeling and then into changes in gene expression. Although Sirt1 activity is not confined exclusively by NAD<sup>+</sup> levels, Sirt1 has been described to function in part as a redox sensor, using the coenzyme NAD<sup>+</sup> to catalyze the acetyl transfers from substrate proteins<sup>71</sup>. Therefore, central to the redox state of the cell, as a substrate for PARP proteins, and as an activator of the sirtuin family of longevity-associated histone deacetylases, NAD<sup>+</sup> maintains a privileged position in the cell, able to monitor both its metabolic state and, possibly, its fate. Intriguingly, a molecular and functional link has been described between Sirt1 and PARP-1 in the control of cell death<sup>72</sup>.

## Cycling proteins or metabolites?

The interplay of circadian rhythmicity and energy balance may result from the fluctuations of proteins required for normal metabolic function, fluctuations in metabolites themselves or both. Some evidence suggests that neither takes center stage, but rather that both may work together to maintain energy balance. Respiration may itself be subject to circadian control; mRNA levels for several complex I proteins seem to oscillate in expression (reviewed in ref. 73). In rodents, a circadian oscillation in the expression of approximately 20 respiratory-chain protein subunits in the SCN peaks just before dawn, preceding the circadian peak of neuronal firing when metabolic demands are at their highest<sup>23,74</sup>. As respiration produces its own metabolites, the cells must find ways to avoid the damaging effects of this necessary activity. In mice, the oscillation of hexokinase and malate dehydrogenase, proteins that

utilize glucose or ketone bodies to provide the energy for oxidative phosphorylation, demonstrates the necessity for such compensation<sup>23</sup>.

Microarray analysis of *D. melanogaster* fly head RNA also reveals oscillations in numerous detox, stress response and metabolic proteins, some of which are NAD<sup>+</sup> or NADP<sup>+</sup> activated. These include aldehyde dehydrogenase, oxido reductase and short-branched chain acyl CoA dehydrogenase<sup>75</sup>. Detoxification enzymes constitute a large proportion of cycling genes in the fly head, accounting for at least six cytochrome P450 family members as well as glutathione *S*-transferase. Cycling genes are also manifest in the fly body, where many redox-associated genes show circadian oscillations. In keeping with the *D. melanogaster* microarray data, melatonin-driven glutathione peroxidase production peaks in both the brain and the liver in rodents. This event may be synchronized specifically for scavenging ROS as ROS levels peak just before maximal glutathione peroxidase production<sup>73</sup>. The expression profile of numerous other metabolism-associated mRNAs, such as those encoding the glucagon receptor, glucokinase, glucagon, Glut2, glucose-6-phosphate transport protein, pyruvate kinase and pyruvate dehydrogenase, show circadian oscillation<sup>23,76,77</sup>. These observations lend strength to the hypothesis that circadian oscillations of mitochondrial proteins influence the cell's metabolic state and allow the cell to respond efficiently to DNA damage induced by accumulating oxidative stress. As DNA damage is thought to contribute to neurodegenerative disorders such as Parkinson's and Alzheimer's disease, it is reasonable to speculate that the circadian fluctuations of proteins responsible for reducing oxidative stress may help prevent development of these disease states.

Although the circadian activity or expression of metabolic proteins is likely to contribute to energy balance, a recent study of the yeast metabolic cycle in nutrient-limiting conditions reveals that yeast metabolites themselves are actually cyclically produced and that they drive cell division, resulting in a peak of cell division while respiration is at its nadir<sup>37</sup>. Metabolite cycling seems to be finely tuned. For example, amino acid and nucleotide precursors peak during the oxidative phase, whereas metabolites associated with glycogen metabolism (such as pyruvate and glucose-6-phosphate) reach a maximum during reductive and building phases. Although these cycles are ultradian (with periodicities of less than 24 h), the synchronization required for normal cell cycling suggests that there are similar oscillations in mammalian cells, although they are temporally disparate. Whether cycles such as these occur on an ultradian or a circadian cycle in mammalian tissues has yet to be demonstrated.

The small molecule cyclic ADP-ribose (cADPR), which is produced from NAD<sup>+</sup> by ADP-ribosyl cyclases, modulates circadian oscillations in cytosolic calcium release in plant and animal cells in a ryanodine receptor-dependent manner<sup>78</sup>. It was recently reported to be synthesized in a circadian fashion in some organisms, where it contributes to circadian clock gene expression<sup>79</sup>. The NAD<sup>+</sup> dependency of cADPR production lends further support for the idea that oscillations in metabolites may serve as a prerequisite for circadian modulation of cellular metabolism. Notably, heme oxygenase-2, an enzyme involved in heme degradation, is light inducible. This is relevant considering that both PAS domains of Npas2 (a clock protein that dimerizes with Bmal1 and controls circadian gene expression) were recently reported to bind heme and respond to changes in the cell's redox state in the form of altered Bmal1 binding<sup>80,81</sup>. Heme also serves as a ligand for the REV-ERB proteins, an

interaction that results in transcriptional repression of *Bmal1* and negative regulation of hepatic gluconeogenic genes<sup>82,83</sup>. The contribution of heme to circadian rhythms is further supported by the fact that the gene controlling the rate-limiting step for heme biosynthesis, aminolevulinic synthase-1 (*Alas1*), is itself transcriptionally regulated by Bmal1–Npas2 heterodimers<sup>84</sup>.

Whereas circadian oscillations in the activity of the NAD<sup>+</sup>-activated Sir2 protein homologs may affect DNA repair or silencing, cell-cycle progression and glucose homeostasis in a manner reflective of the cell's metabolic state, oscillations in NAD<sup>+</sup> or NADP<sup>+</sup> levels may provide the explanation for why food remains such a potent zeitgeber. Specifically, high concentrations of the oxidized forms of NAD, NAD<sup>+</sup> and NADP<sup>+</sup>, have been described to inhibit the binding of Clock or Npas2 to Bmal1, preventing E-box binding and subsequent circadian target gene transcription. Conversely, NADH or NADPH have been described to promote dimerization of Bmal1 with Npas2 or Clock, thus contributing to Clock-driven gene transcription<sup>85</sup>. The profile of circadian gene expression, therefore, is likely to depend directly on the metabolic state of the cell.

### Implications for metabolite oscillations on circadian physiology

Whether there are mammalian metabolite oscillations analogous to those of the yeast metabolic cycle is still unclear, but it remains a tantalizing possibility. The fact that cellular demands are met temporally as a function of the cell's metabolic cycle is likely to be true for all cells, regardless of the organism. In the context of mammalian Sirt1 circadian activity, it seems likely that metabolite oscillations in the coenzyme NAD<sup>+</sup> must also occur in a cyclical, circadian manner. If metabolite fluctuations are organized temporally in a circadian manner, what might this mean physiologically? The central functions of NAD<sup>+</sup> in DNA repair, gene silencing, the cell cycle and circadian control (Fig. 1) indicate that the consequences of its aberrant regulation could be numerous and physiologically severe. It is conceivable that food restriction impinges on circadian rhythms because it disrupts NAD<sup>+</sup>-NADH cycling, essentially allowing the redox state of individual cells and tissues to alter rhythmicity. The absence of Clock–Bmal1 dimerization in the presence of increased levels of oxidized NAD is one piece of evidence supporting this idea<sup>85</sup>. As such, it is easy to imagine sophisticated schemes coordinating SCN-driven rhythms with those of a phase-shifted periphery for drug administration and efficacy. Already there are numerous drugs, perhaps most commonly known within cancer chemotherapeutic strategies, administered following a circadian protocol so that the maximal benefit might be achieved from their use<sup>86,87</sup>.

Sirtuins are also a powerful example of the extent to which metabolite oscillations might affect physiology as its protein substrates include not only histones but transcription factors and cofactors such as p53, FOXO, PGC-1 $\alpha$ , NF- $\kappa$ B and PPAR $\gamma$ <sup>88</sup>. These proteins have been implicated in disease states ranging from the metabolic syndromes and obesity to cancer. Furthermore, as it is the coenzyme for Sirt1, timed production of NAD<sup>+</sup> may be important in neuronal and axonal integrity, promoting neuronal survival under conditions of metabolic stress<sup>56,89</sup>.

As observed throughout circadian biology, peripheral loops and feedback mechanisms are crucial for proper timing. In this sense, NAD<sup>+</sup> fits stereotypically what is required in an oscillating system. For example, oxidative phosphorylation requires NADH for ATP production, but then NAD<sup>+</sup> provides itself as substrate for PARP-mediated repair to deal with the resulting oxidative stress. Likewise, as coenzyme for Sirt1, NAD<sup>+</sup> puts the brakes *per se* on the system, by slowing the metabolic cycle—or rather by restoring it in preparation for the next cycle of energy production. Deciphering the molecular interplay between these regulators is likely to provide a new and much awaited glimpse of how the circadian clock ‘senses’ cellular metabolism, and vice versa.

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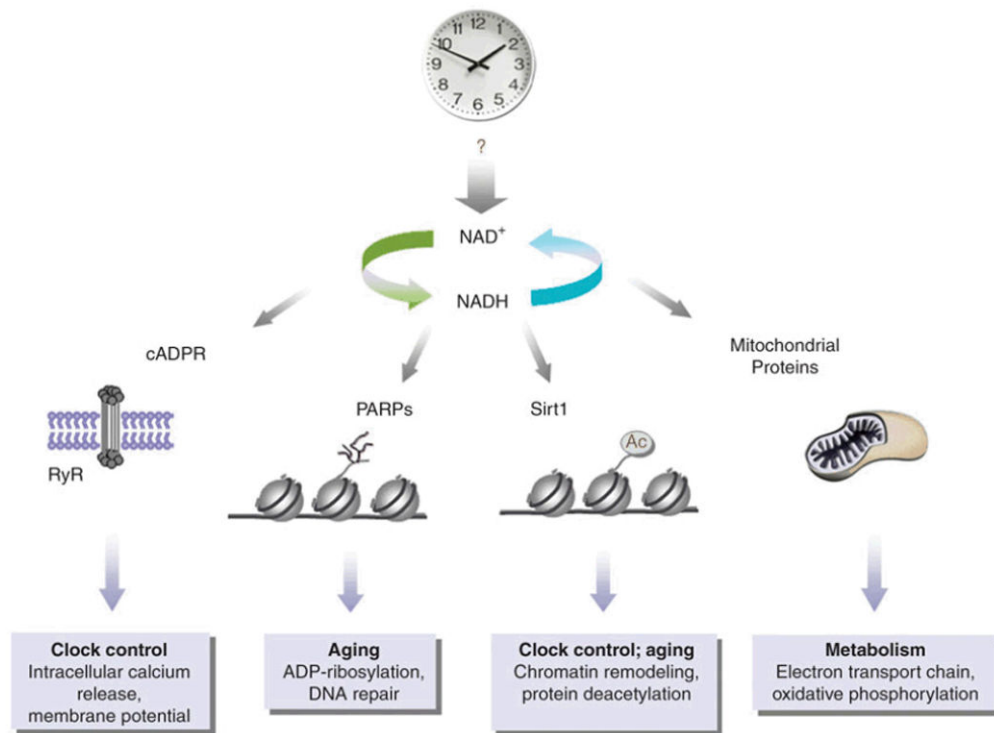
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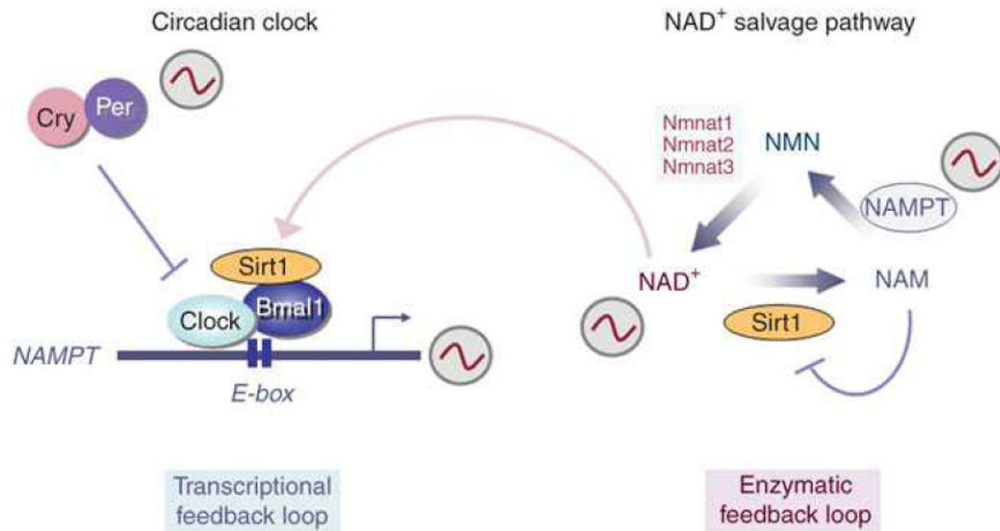
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**Figure 1.**

The interplay between the circadian clock and cellular metabolism occurs at various levels. Central to cellular metabolism is the synthesis of  $\text{NAD}^+$ , a process influenced by the circadian clock. Variable levels of  $\text{NAD}^+$  may influence numerous intracellular pathways. The small molecule cyclic ADP-ribose (cADPR) is produced from  $\text{NAD}^+$  by ADP-ribosyl cyclases and contributes to circadian oscillations in a ryanodine receptor (RyR)-dependent manner<sup>79</sup>. Metabolites also have an attractive role in epigenetic control<sup>84</sup>.  $\text{NAD}^+$  modulates the activity of chromatin-associated enzymes, such as PARP-1 and Sirt1. The activity of the  $\text{NAD}^+$ -dependent histone deacetylase Sirt1 oscillates and participates in chromatin remodeling at circadian genes. There is no evidence to date for a circadian function of PARP-1, but its role in DNA damage repair<sup>64</sup> and the reported interplay with Sirt1 (ref. 66) may be predictive of a circadian role. Finally, the central role of  $\text{NAD}^+$ -activated mitochondrial proteins in energy metabolism begs the question of how they might contribute to circadian control.

EdSumm: The mammalian circadian clock controls many biological functions, including metabolic activity. In this Perspective, the authors present recent literature and discuss the two-way relationship between the clock and metabolism, with  $\text{NAD}^+$  playing a central part in their integration.



**Figure 2.**

By activating Sirt1, NAD<sup>+</sup> conjoins two feedback loops necessary for cross-talk between the circadian clock and metabolite production. The NAD<sup>+</sup>-salvage pathway is important for regulating intracellular NAD<sup>+</sup> levels. After the conversion of nicotinamide (NAM) into nicotinamide mononucleotide (NMN) by NAM phosphoribosyl transferase (NAMPT), NMN is further modified into NAD<sup>+</sup> by the nicotinamide mononucleotide adenylyl transferases (Nmnat1,-2 and -3). Whereas NAM inhibits Sirt1 activity, NAD<sup>+</sup>-activated Sirt1 feeds back into the NAD<sup>+</sup>-salvage pathway by directly regulating *Nampt* gene expression in a Clock–Bmal1-dependent manner<sup>69</sup>. By this mechanism, NAD<sup>+</sup> conjoins the two feedback loops, contributing to the fine tuning necessary for achieving energy balance. EdSumm: The mammalian circadian clock controls many biological functions, including metabolic activity. In this Perspective, the authors present recent literature and discuss the two-way relationship between the clock and metabolism, with NAD<sup>+</sup> playing a central part in their integration.

**Table 1**

List of common circadian terms and their definitions

<b>Term</b>	<b>Definition</b>
Circadian	A modifier referring to the approximately 24-h nature of an event. The word 'circadian' is derived from the Latin roots <i>circa</i> (about) and <i>diem</i> (day).
Entrainment	The process of clock synchronization. For example, light 'entrains' the biological clock.
Zeitgeber	German word meaning 'time giver'. An external cue (such as light or food) that entrains the circadian clock.
Suprachiasmatic nucleus (SCN)	A small region of the brain consisting of bilateral nuclei that coordinately function to synchronize circadian rhythms in other tissues.
Circadian clock-controlled gene (CCG)	A gene expressed in a circadian-dependent manner, usually under the control of a promoter that contains E-box, D-box or RRE elements.
Brain and muscle Arnt-like protein-1 (Bmal1)	The mammalian bHLH-PAS transcription factor that dimerizes with Clock to activate gene transcription.
CLOCK	A bHLH-PAS transcription factor that dimerizes with BMAL1 to activate promoters that contain E boxes (CACGTG).
Cryptochrome proteins (Cry)	Transcriptional repressors that dimerize with Per to inhibit Clock-Bmal1-mediated gene transcription. In plants and invertebrates, these function as light-responsive flavoproteins.
Neuronal PAS domain protein 2 (Npas2)	A transcription factor similar to Clock and highly expressed in the forebrain. Npas2 dimerizes with Bmal1 to activate gene transcription.
Period homolog proteins (Per)	PAS domain-containing proteins that dimerize with TIM (in <i>Drosophila</i> ) or Cry (in mammals) to inhibit Clock-Bmal1-induced gene transcription.