Circadian control of mitochondrial dynamics and functions

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Physiology and behavior in mammals is predominantly under circadian clock control. Circadian clocks are present in nearly every cell of the body and oscillate with a frequency of about 24 h in a self-sustained and cell autonomous manner. These clocks not only play a principal role in metabolic control but concurrently respond to a wide variety of metabolic cues. While various aspects of circadian regulation of metabolic functions have been extensively studied, our knowledge regarding circadian mitochondrial biology is just emerging. We review herein the current literature addressing circadian mitochondrial biology: from diurnal changes in mitochondrial make-up, through dynamics and functions. We will discuss as well potential mechanisms that are implicated in circadian control of mitochondrial biology in mammals.

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Introduction

Circadian clocks are present in almost all light sensitive organisms, from cyanobacteria through plants, flies, mice and humans. These molecular oscillators cycle with periodicity of about a day and thus enable organisms to synchronize a wide variety of biological functions with the geophysical time. In mammals, the circadian timing system is structured in a hierarchical manner [1–3]. A master pacemaker is located in the suprachiasmatic nucleus (SCN) of the brain and synchronizes subsidiary oscillators in the rest of the body. While the former is primarily entrained by daily light–dark cycles, the latter are mainly synchronized by feeding time. Remarkably, circadian rhythmicity is preserved in cultured cells in a self-sustained and cell-autonomous manner [1–3]. At the molecular level, circadian clocks function is based on negative transcription–translation feedback loops that are generated through the action of several core clock genes. The transcription factors CLOCK and BMAL1 heterodimerize and activate the transcription of *Per* and

Cry genes, which in turn inhibit their own expression. In addition, the nuclear receptors family members of *Rev-Erb* and *Ror* regulate *Bmal1* expression. Nowadays, it is widely accepted that circadian clocks and metabolism are tightly intertwining. Circadian clocks not only play a central role in metabolic control but concurrently respond to a wide variety of metabolic cues. Several comprehensive reviews have covered in depth the molecular architecture of the core clock machinery [1–3], as well as their interplay with metabolism [4–8].

In eukaryotic cells mitochondria are major metabolic hubs that play key roles in many vital processes; among others energy production, lipid biosynthesis, and calcium homeostasis. However, relatively little is known regarding the circadian nature of mitochondrial biology. We will review herein the current literature related to circadian mitochondrial biology in mammals, and highlight the recent key findings related to daily changes in mitochondrial content, dynamics and functions (Figure 1), as well as potential underlying mechanisms and physiological implications.

Rhythms in mitochondrial content

In recent years, high throughput omics methodologies have provided an informative view of the daily changes in the mitochondrial make-up. Extensive daily changes in the mitochondrial proteome were uncovered by whole liver proteomics [9,10], as well as by proteomics analyses of isolated mitochondria [11••]. Over a third of the mitochondrial proteins accumulate in a daily manner [11••]. Remarkably, the vast majority of rhythmic proteins reach their zenith levels about the same time, during the early light phase. Functional annotation of the rhythmic mitochondrial proteome revealed the pervasive rhythmicity of key catabolic and oxidative functions of mitochondria [11••]. Notably, components of the pyruvate dehydrogenase complex (PDC) that catalyzes the rate-limiting step in mitochondrial carbohydrate metabolism accumulate early during the light phase. While carnitine palmitoyl-transferase 1 (CPT1), the rate-limiting enzyme in the transport of fatty acids into the mitochondrial matrix, cycles with peak levels at the transition between the dark and light phases. At the molecular level, the mechanisms accounting for these rhythmic changes are yet unknown. Although the transcript levels of several nuclear-encoded mitochondrial proteins are altered in clock mutant mice [12,13] and BMAL1 binds their promoter regions [14••,15], global analysis exhibits poor correlation between the phase of the mitochondrial proteome and its respective transcriptome [11••]. It is, therefore, conceivable that the observed daily changes in the

Figure 1
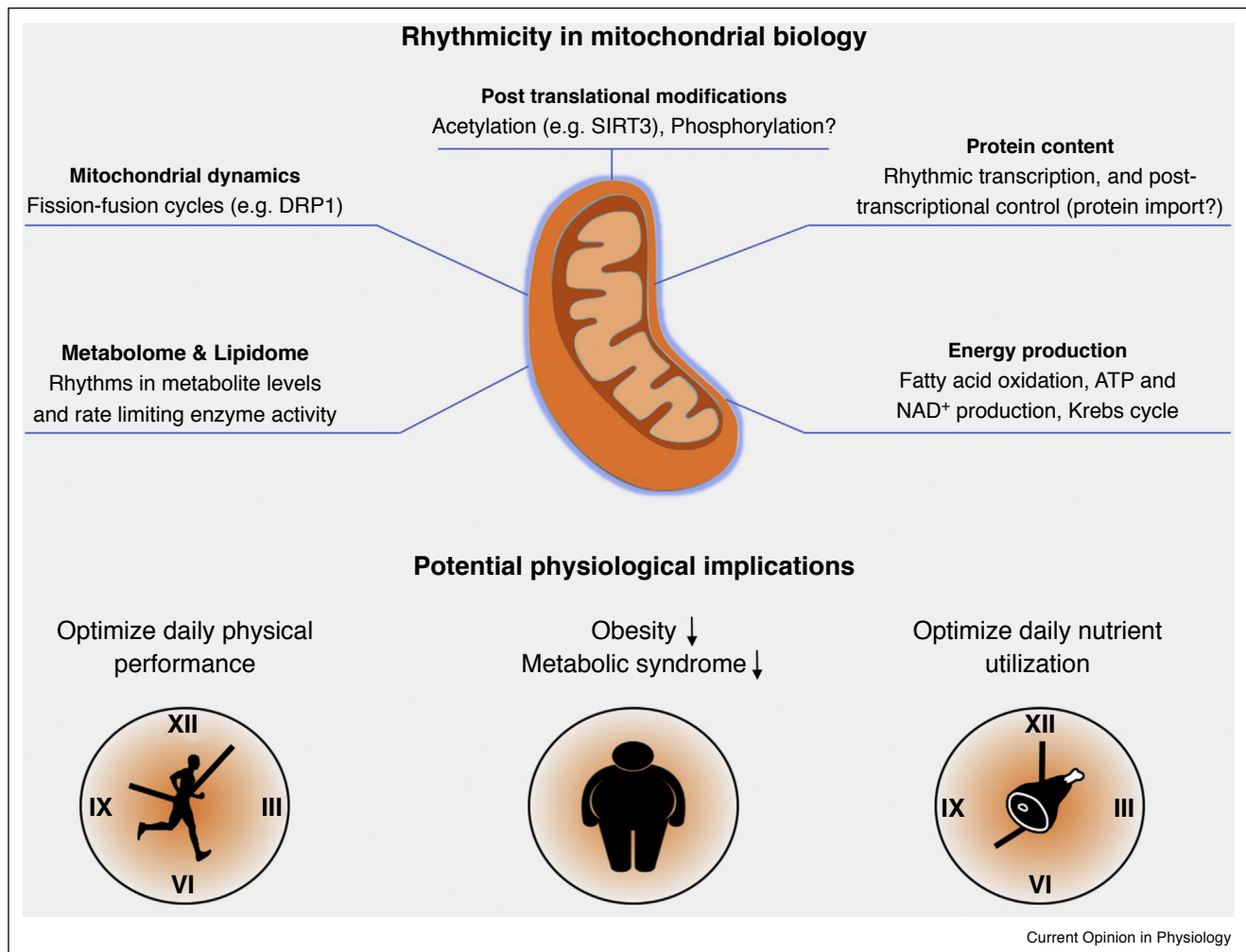
Schematic depiction of the circadian nature of mitochondrial biology; from content and functions; thorough potential molecular mechanisms to physiological implications.

mitochondrial proteome arise from post-transcriptional mechanisms; among others rhythmic translation, protein import and/or degradation. Future studies are expected to shed light on the identity and role of these different mechanisms.

Post-translational modifications have been implicated in control of mitochondrial functions and dynamics. Global acetylome analysis of mouse liver revealed daily changes in the acetylation status of many mitochondrial proteins with enrichment for CLOCK-dependent acetylation sites in proteins associated with Krebs cycle and glutathione metabolism [16]. Along this line, the acetylation levels of many mitochondrial proteins differ between wild type and BMAL1 deficient mice [17••]. For instance, acetylation of fatty-acid metabolism enzymes corresponds to their activity and are BMAL1-dependant. Likewise, the respiratory complex I is rhythmically acetylated, in accordance with changes in mitochondrial respiration [18]. Taken together, it appears that rhythmic mitochondrial protein acetylation is under circadian clock control and hence regulate daily changes in mitochondrial function. It raises the question whether other post-translation modifications of mitochondrial proteins, such as phosphorylation, play a similar role.

A wide range of metabolites associated with mitochondrial metabolism display circadian rhythmicity both in cultured cells and in animal models. Among others, rhythms in NAD^+ levels [19,20], ATP levels, reactive oxygen species and Krebs cycle [21•] were reported. Furthermore, high throughput lipidomics analyses on isolated mitochondria from mouse liver evinced that about one third of the lipids in mitochondria exhibit daily rhythms [22•]. Both the composition and phase of the rhythmic lipids are PER1/2 and feeding time dependent.

Notably, in *ad libitum* fed mice the majority of mitochondrial lipids reach their zenith levels at the transition between the light and the dark phase, while an opposite phase is observed in mice fed exclusively during the dark phase [22•]. Similarly, mitochondrial fatty acid composition and metabolism were found to depend on BMAL1 [17••]. Upcoming studies on these rhythmic lipids will likely clarify their relevance for the daily changes in mitochondrial functions and dynamics. Moreover, studies conducted with isolated mitochondria purified through biochemical [22•] or pull down approaches [23] will provide a more accurate and detailed depiction of the circadian mitochondrial landscape.

Rhythms in mitochondrial dynamics

Mitochondrial function is largely dependent on mitochondrial dynamics, namely changes in shape and size due to fission and fusion, with elevated respiration in fused compared to fragmented mitochondria [24]. Morphological changes (i.e. shape and volume) between the light and dark phases in mitochondria of rat hepatocytes were detected using electron microscopy imaging already decades ago [25]. More recently, daily rhythms in mitochondrial dynamics were reported in mouse liver as well, and revealed that many genes participating in mitochondrial dynamics are expressed in a daily manner and are BMAL1-dependent [14••]. Consequently, mitochondria isolated from BMAL1-liver specific deficient mice are bigger, more rounded, and maintain similar morphology throughout the day. Recently, circadian clock controlled changes in mitochondrial morphology were observed in cultured cells as well. These cycles of fission and fusion and consequently rhythms in ATP production are regulated by Dynamin-Related Protein 1 (DRP1). DRP1 is phosphorylated in a circadian manner, and suppression of its activity not only eliminates circadian ATP production but feeds back and influence the core circadian oscillator [21•].

The dependency of mitochondrial morphology on clock genes was corroborated in mouse skeletal muscle [26] and heart [12], and is linked to impaired mitochondrial function in these organs. Macrophages mitochondria exhibit daily morphological changes *in vitro* as well [27]. By contrast, the overall number of mitochondria, assessed by mitochondrial genome copy number, appears to be constant throughout the day and is independent of clock genes [11••,12,14••,28]. Overall, these studies point towards circadian control of mitochondrial dynamics, with implications on mitochondrial functions (see also below).

Rhythms in mitochondrial functions

The most prominent role of mitochondria is energy production from different nutrients. Pyruvate and fatty acids are catabolized into acetyl CoA through the action of the pyruvate dehydrogenase complex and Fatty Acid Oxidation (FAO), respectively. The acetyl groups are

then fed into the Krebs cycle, and the process culminates with the transfer of acetyl-derived high-energy electrons along the respiratory chain that is coupled to production of ATP by the ATP-synthase complex.

Several studies tested the circadian control of mitochondrial nutrient utilization and respiration, using assays that measure oxygen consumption rate (OCR) in cultured cells and isolated mitochondria. OCR measurements of synchronized C2C12 muscle cells in culture are rhythmic with ~24 h period [17••]. Similar results were obtained with HepG2 cells, albeit with a significantly shorter period (~15 h), [18]. Analysis of isolated hepatocytes from wild type mice harvested throughout the day revealed BMAL1-dependent elevated respiration during the dark phase compared to the light phase in the presence of pyruvate [14••]. Analyses of mitochondrial respiration were conducted as well with isolated mitochondria from mouse liver, muscle and rat brain [11••,17••,26,29]. Mitochondria isolated from livers of wild type mice exhibit higher OCR than those of *Bmal1* knockout mice [17••], *Bmal1* liver-specific knockout mice [14••] and *Per1/2* double knockout mice [11••]. Likewise, measurements of FAO by [14C] labeled fatty acid supplementation evinced that this property is also reduced in *Bmal1* deficient mice [17••].

Experiments performed with mitochondria isolated from mice around the clock uncovered the daily preference of mitochondrial nutrient utilization. In the presence of FAO substrates (palmitoyl-carnitine or palmitoyl-CoA + carnitine) mitochondrial respiration is rhythmic with zenith level early in the light phase, in accordance with CPT1 protein levels. Whereas carbohydrates (pyruvate) utilization is rhythmic as well, but peaks later during the light phase [11••]. The differences in peak time of mitochondrial respiration in experiments conducted with isolated mitochondria [11••] versus hepatocytes [14••] might reflect the role of mitochondrial extrinsic cellular mechanism in control of mitochondrial respiration. Remarkably, these daily rhythms in mitochondrial respiration are strongly influenced not only by the molecular circadian clock, but also by nutrition type (e.g. High Fat Diet), and eating pattern (i.e. nighttime restricted feeding). Each of these factors differentially affects the overall level, rhythm, and phase of oscillation for several mitochondrial enzymes and the catabolism of their respective substrates [11••]. Taken together these studies suggest that mitochondrial respiration exhibits daily rhythms that are dependent on the molecular clock, nutrients, feeding pattern and diet composition.

Concluding remarks and future directions

The literature summarized herein highlights the circadian nature of mitochondrial biology (Figure 1). At the same time, it emphasizes the need for future studies addressing the daily changes in mitochondrial content

and functions as well as deciphering the underlying molecular mechanisms.

Evidence emerging from different clock mutant models support the potential role of circadian clocks in control of mitochondrial rhythmicity. However, it cannot be excluded that some of these effects are attributed to specific clock genes regardless of their function within the core clock circuitry.

Furthermore, the dissection whether mitochondrial rhythmicity is achieved through systemic cues (such as feeding-fasting or rest-activity cycles), or via cell autonomous mechanisms remains unresolved. It is likely that both scenarios co-regulate mitochondrial homeostasis throughout the day. In this conjuncture, experiments addressing mitochondrial function in cultured cells support a cell autonomous effect on mitochondrial function. Whereas experiments with mice show that feeding-rhythms are sufficient to restore some mitochondrial functions even in the absence of a functional clock. Remarkably, the ability of mitochondria to preserve functional differences when isolated in different hours of the day indicates that these alterations are not simply because of daily changes in substrate availability, but rather due to inherent changes in mitochondrial composition.

The lack of phase correlation between the mitochondrial proteome and its respective transcriptome [11••] is another puzzling point. As aforementioned, this finding highlights the importance of post-transcriptional mechanisms in control of mitochondrial protein homeostasis throughout the day. Recently, translation efficiency was reported to exhibit daily rhythms, specifically in respect to genes implicated in mitochondrial function [30,31]. Bass and colleagues [17••] proposed another model wherein circadian clocks generate oscillations in NAD^+ levels, a cofactor for Sirtuin, a family of NAD^+-dependent deacetylases, among them the mitochondrial SIRT3. Thus, NAD^+ serves as a metabolic link between circadian clocks and mitochondrial function through NAD^+ and SIRT3-dependent deactylation. In this conjuncture, they showed that reduction in mitochondrial activity in the absence of BMAL1 could be rescued by restoring NAD^+ levels.

As abovementioned, daily oscillations in mitochondrial rate-limiting metabolic enzymes and co-factors correspond to daily changes in mitochondrial substrate utilization. Moreover, recent studies identified daily rhythms in tissue oxygenations as well as hypoxic response [32•,33•, 34•]. It is conceivable that these molecular events carry physiological implications, in particular in the case of physical activity. The importance of exercise as lifestyle treatment for metabolic diseases is well established. Major efforts are dedicated not only to uncover the

underlying mechanisms but to maximize these beneficial metabolic effects. A recent study point towards a functional interaction between circadian clocks, metabolism and athletic performance. CRY 1/2 null mice exhibit repressed fatty acid transport and oxidation, and perform better upon intense exercise challenge. This effect is achieved in part due to reduced PPARδ inhibition in these mice [35•], which improves endurance performance, and regulates the shift from glucose to fatty acid catabolism during prolonged exercise [36]. Given that nutrient consumption is critical for physical activity, and that nutrient utilization is subjected to circadian regulation, it is conceivable that the health benefits of physical performance exhibit daily variance. Hence, performing exercise during different times of the day might carry distinct metabolic outcomes; a conjuncture that was to date never tested.

Future studies on the circadian nature of mitochondrial biology are expected to shed light on many of these intriguing open questions.

Conflict of interest statement
Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as

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