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COMMENTARY

A context-specific circadian clock in adipocyte precursor cells modulates adipogenesis

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ABSTRACT

The circadian clock is an intricate molecular network that paces a variety of physiological process to ~ 24 hour day/night cycles. Whereas the central circadian clock in the brain is primarily entrained by light signals, peripheral circadian clocks, which are in most cells in the body, receive cues not only from the central pacemaker but also endocrine and other systemic and tissue-specific signals. Prior studies have connected peripheral circadian clocks to metabolism, primarily with studies focused on the robust clock in the liver that responds to feeding/fasting cycles. Adipose tissue is also critical for metabolism and adipocytes have circadian clocks. Yet, the role of the circadian clock in adipocytes is poorly understood. Here we describe our studies that revealed components of the circadian clock in primary adipocyte precursor cells (APCs) in mice. We made the surprising discovery of a particularly prominent role for the circadian gene Period 3 (*Per3*) in the APC clock. Furthermore, we elucidated that *Per3* directly regulates an output pathway of the APC clock to modulate the expression of the Kruppel-like factor 15 (*Klf15*) gene. Finally, we discovered that this clock-Klf15 pathway regulates adipogenesis in APCs. These finding have important implications for our understanding of adipose tissue biology and metabolism and, we speculate, will generate opportunities to develop novel therapeutic strategies based on the context-specific features of the circadian clock in APCs.

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Introduction

A circadian clock is an endogenous 24-hr-cycle timekeeping system, controlled by oscillations in molecular components.[1,2] The core circadian clock machinery uses positive feedback components (i.e. *Clock* and *Bmal1*) and negative feedback components (i.e. *Per* genes), to regulate the periodicity of circadian and clock-controlled genes.[1] Although, three *Period* paralogs (Per 1–3) are reported in mammals, *Per3* is the least understood and was not regarded as a core circadian clock gene because deleting *Per3* had minimal effect on circadian locomotion in mice.[3,4]

The central circadian clock is located in the suprachiasmatic nuclei of the brain and most cells in the body appear to contain peripheral circadian clocks.[2,5] Interestingly, there are context specific differences in the components used in these peripheral clocks[6] but the implications and mechanisms driving theses differences are incompletely understood. Signals from photoreceptors in the retina to the central clock are generated by diurnal light cycles and entrain the central clock.[2,5] The central clock serves as an important pacemaker for peripheral circadian clocks. A number of functions of peripheral tissues are modulated by central clock pacing including: insulin secretion from the pancreas and sensitivity in muscle.[7,8] However, peripheral clocks are also subject to distinct inputs and contextual regulation that may uncouple oscillations from the central clock.[1,9–12] For example, the timing of food intake can alter peripheral clock oscillations and rhythmicity of bile synthesis in liver.[13-16] These, and a body of other related studies, indicate that peripheral circadian clocks provide a mechanism that links peripheral tissues with systemic metabolism.[17-20]

Another example of a metabolically relevant biological process that is influenced by peripheral circadian clocks is adipogenesis- a differentiation pathway where adipocyte precursor cells develop into mature adipocytes.[21-23] Dysregulated adipogenesis contributes to expansion of adipocyte tissue and thus is often implicated in obesity.[21] Furthermore, disruption of circadian rhythms is a risk-factor for obesity.[1,24] To begin to exam the mechanisms by which the circadian clock is connected to adipogenesis, previous studies employed *in vitro* adipocyte differentiation protocols using mouse embryonic fibroblasts cells (MEFs) and immortalized 3T3-L1 cells, e.g..[25] Recently, we performed studies using primary adipocyte precursor

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cells (APCs) and approaches that monitor adipogenesis *in vivo* to establish the presence of peripheral clocks in bona fide APCs and mechanisms by which the circadian clock regulates the process of adipogenesis.[23]

Results

As defined by a Sca1 (stem cell marker) positive and lineage marker negative population,[26] we isolated primary APCs from subcutaneous adipose depots of circadian clock reporter mice (m*Per2*^{Luc}).[27] These studies demonstrated that primary APCs contain a circadian clock.[23] However, to our surprise, our further investigations elucidated that expression of the classic core clock component *Per1* does not significantly oscillate in APCs.[23] Strikingly, we discovered that *Per3*, has the most robust circadian expression profile of the Per paralogs in primary APCs.[23] These findings suggested to us that *Per3* might have a particularly prominent role in the context of APC biology. Indeed, deletion of the *Per3* gene altered the circadian cycling of core clock components, supporting our hypothesis that *Per3* activity impacts the clock machinery in APCs.[23]

Intrigued by the above results, we investigated the functional role of *Per3* in APC biology. To probe this question, we compared the level of adipogenesis occurring in *Per3* deficient mice (*Per3*$^{-/-}$) *in vivo* to that of wild type mice using a pulse-chase approach we had previously validated.[23,28,29] Using this protocol, we revealed that *Per3*$^{-/-}$ mice have increased levels of adipogenesis *in vivo*.[23] The increased adipogenesis upon *Per3* ablation was also captured in *ex vivo* culture assays by monitoring the levels of spontaneous adipogenesis of isolated primary APCs, indicating that disruption of *Per3* in APCs was sufficient to cell autonomously promote adipogenesis in APCs.[23]

Next, to elucidate the mechanisms by which *Per3* regulates adipogenesis in APCs, we performed unbiased genome-wide expression profiling studies comparing APCs isolated from wild type and Per3$^{-/-}$ mice. Among the genes with significant expression changes across the genotypes, *Klf15* was previously reported as having a potential role in modulating adipogenesis.[30] Therefore, we decided to examine whether *Klf15* is responsible for connecting circadian clock to *in vivo* adipogenesis. We confirmed that both the messenger and protein expression levels of *Klf15* were significantly increased in APCs from Per3$^{-/-}$ mice.[23] Conversely, *Per3* overexpression in wild type APCs decreased *Klf15* expression level, suggesting that PER3 inhibits *Klf15* expression.[23] Interestingly, we discovered *Klf15* expression has a circadian oscillation pattern in APCs from wild type mice and, furthermore, these oscillations were abrogated in the absence of *Per3* gene.[23] Together, these findings indicate that PER3 plays a prominent role in the regulation of *Klf15* expression in APCs. Indeed, our molecular and biochemical studies elucidated that PER3 forms a complex with BMAL1 at an E-box in the *Klf15* gene to regulate expression.[23] Our results suggest that when PER3 levels are low BMAL1 occupancy of this E-box promotes *Klf15* expression and, as PER3 levels increase, PER3 binds to BMAL1 and Klf15 expression is repressed.[23] This mechanism reveals a direct pathway by which the peripheral circadian clock controls *Klf15* expression, resulting in the circadian oscillations in *Klf15* expression observed in wild type APCs.[23] Finally, we tested if this clock-Klf15 pathway was relevant for the regulation of adipogenesis. To this end, we found that knocking-down *Klf15* was sufficient to rescue the Per3$^{-/-}$ adipogenesis phenotype.[23] These results indicate that PER3-KLF15 pathway is critical to regulating adipogenesis.

Discussion

In conclusion, we discovered that primary APCs contain a peripheral circadian clock. Further, we exposed what appear to be context-specific elements of the APC clock. In particular, the poorly understood *Per3* clock component has a significant role in the APC clock, directly regulating an output pathway that modulates adipogenesis. As the rate of adipogenesis has systemic implications, including for metabolism and body composition, these new insights have broad implications.

In addition, our study further delineates the intriguing relationship between circadian oscillations and metabolic processes. For example, recent studies by others have demonstrated the importance of cycling between feeding and fasting states for maintaining healthy systemic metabolism in humans.[31,32] This is particularly interesting to us because circadian cycles of feeding and fasting provide potent entrainment cues for peripheral clocks. This has most extensively been studied in the liver, where disruption in feeding cycles, as well as direct abrogation of the circadian clock, leads to metabolic derangement.[33-35] To date, the effect of circadian feeding/fasting cycles for the clock in APCs and adipocytes has yet to be extensively explored. We speculate that, like in the liver, disruption of feeding/fasting cycles will have detrimental functional implications in adipose tissue. Indeed, we hypothesize that the rate of adipogenesis might be modulated by altering these circadian cycles by mechanisms that impinge upon the same peripheral clock-Klf15 pathway exposed in our study (Figure 1).

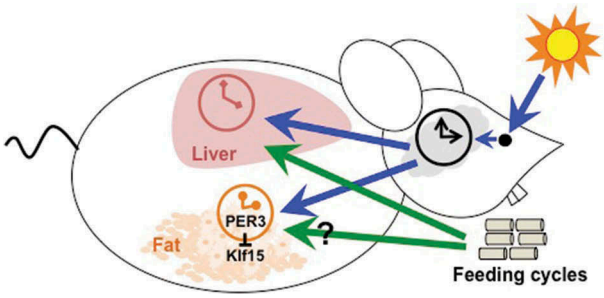

Figure 1. Schematic of the central circadian clock and peripheral clocks in the liver and APCs. The peripheral circadian clock in the liver receives multiple inputs including signals from the central clock and feeding. *Per3* has a context specific significant role in the circadian clock in APCs and regulates an output pathway to Klf15 that modulates adipogenesis.

However, we also note that, while liver and adipose clocks may share some commonality in receiving entrainment from feeding cycles, there are likely to be important distinctions as well. In particular, our study found that, consistent with a more canonical peripheral clock, hepatocytes maintain robust oscillations in the expression of the core clock component *Per1*, while in APCs, we found that oscillations in *Per1* expression is not significant.[23] This may signal a unique role for *Per3* in APCs and, by extension, adipose tissue biology in general. The logic behind this context difference still needs to be elucidated, but it is consistent with enabling tissue specific input and output responses to systemic changes and signals. If correct, this context-specificity of peripheral clocks would offer opportunities for the development of more targeted tissue-specific therapeutic interventions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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