Nuclear envelope regulates the circadian clock

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Dailу rhythms of behavior and physiology arise from endogenous circadian clocks. At the molecular level, the circadian clock consists of intricate transcriptional and post-transcriptional feedback loops that drive 24h rhythms in a vast repertoire of basic cellular processes. The nuclear envelope, as a fundamental component of the cell, has been shown to function as a global transcriptional regulatory machinery. Recently we found that nuclear envelope proteins regulate the circadian clock both in the mammalian system and in fruit flies. One of these proteins, MAN1, impinges on the clock by binding to the promoter region of the core clock gene BMAL1 and enhances its transcription. Here we discuss about other potential mechanisms employed by nuclear envelope proteins to regulate the circadian clock and possible biological relevance of these modulations.

Introduction

Many organisms exhibit 24-hour or circadian rhythms in various cellular, physiological and behavioral processes. In mammals, circadian rhythms are driven by a molecular clock consisting of a series of transcriptional/post-transcriptional feedback loops with Clock and Bmal1 at the center of the loops.¹,² CLOCK/BMAL1 dimers activate the transcription of 3 Period genes (Per1, 2 and 3) and 2 Cryptochrome genes (Cry1 and Cry2). PER and CRY dimerize and translocate into the nucleus, inhibiting the transcriptional activity of CLOCK/BMAL1. In a second loop, CLOCK/BMAL1 activates the transcription of retinoic acid-related orphan receptors, Rev-erba and Rora. The former inhibits whereas the latter activates the transcription of Bmal1. A highly similar molecular clockwork exists in fruit flies, with CLOCK (CLK) and CYCLE (CYC, the fly homolog of BMAL1) driving the transcription of per and timeless (tim) in one loop, and vrille (vri) and PAR-domain protein 1s (Pdp1s) in a second loop.³ This molecular circadian machinery is believed to contribute to rhythmicity in up to 10% of the transcriptome.⁴ Indeed, several core clock genes have been shown to interact with chromatin modifying enzymes, serving as part of the epigenetic mechanism underlying rhythmic transcription of clock-controlled genes.⁵ A recent study demonstrated circadian rhythm in temporal and spatial organization of the chromosomes, which is driven by the molecular clock.⁶

The nuclear envelope (NE), aside from being a barrier that separates the nucleus from the cytoplasm, also regulates spatial genome organization and gene expression by interacting with chromatin modifying enzymes and transcription factors.⁷ Therefore, it is tempting to speculate that the circadian changes in spatial organization of the chromosomes are mediated by the NE, and this contributes to circadian-controlled transcription.

A Role for Nuclear Envelope in Modulating Circadian Oscillations

To test the hypothesis that the NE modulates circadian regulation of transcription, we conducted a screen in human osteosarcoma U2OS cells and identified 3 nuclear lamina proteins, Lamin B1 (LMNB1), Lamin B receptor (LBR) and MAN1 that participate in determining the circadian period of molecular oscillation.⁸ Knocking down any one of these genes lengthens the

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circadian neurons, we can draw the conclusion that the molecular clock is a reflection of the clock in behavioral period, respectively. This implicates an evolutionarily conserved role of circadian control. In prokaryotes, which lack the NE, a specific clock gene promoter is not essential for mediating the trancriptional feedback loop, unlike in eukaryotes. In these organisms, the circadian rhythm of the transcriptome is believed to be achieved by circadian changes in DNA topology. It has also been shown that approximately 30 ~ 64% of the transcriptome accumulate rhythmically in these organisms, which is considerably larger than that of the eukaryotic system. A recent study in mouse found that 43% of all protein coding genes showed circadian rhythmicity in transcription somewhere in the body, but largely in an organ-specific manner. It is possible that NE engages in the regulation of specific chromosomal regions (and thus genes) which facilitates the spatial (different tissues/cells) and temporal (different life stages) specificity of circadian gene expression. This could be accomplished by tissue/cell type-specific expression of NE components and interaction with protein partners, such as chromosome modifying enzymes and transcription factors with different spatial and temporal expression.

**Mechanistic Actions of MAN1 on the Clock**

Knocking down MAN1 in cell culture leads to significantly reduced BMAL1 mRNA and protein levels, with less prominent effects on the other clock genes. Consistently, over-expressing MAN1 in flies increases the mRNA levels of cyc expression. This is not due to increased transcription, while chromatin immunoprecipitation demonstrated direct physical association between MAN1 and BMAL1 promoter. Taken together, these results implicate a role for MAN1 as a transcriptional activator that promotes BMAL1 transcription. This is particularly interesting since the NE is generally believed to repress transcription. MAN1 has been identified to tether Smads and antagonize BMP/TGFβ signaling, resulting in less activation of gene expression. We found that both TGFβ1 and TGFβ-responsive Smad2 increase BMAL1 transcription, but likely via a pathway independent from that of MAN1. Because MAN1 expression levels do not exhibit prominent circadian oscillation, it may function as a permissive signal to the clock, maintaining a basal level of BMAL1. The effects of MAN1 can be overridden by Rev-erba and Rorγ, which provides instructive signals to the clock and drives the oscillation of BMAL1. Alternatively, chromosomes may be rhythmically tethered to the NE, rendering rhythmic interaction between MAN1 and BMAL1 promoter. MAN1 is known to interact with barrier-to-autointegration factor (BAF), which binds dsDNA, chromatin, histones and various transcription factors. It is possible that BAF exhibits circadian oscillation in protein levels, sub-nuclear localization, and/or interaction with MAN1.

In flies over-expressing MAN1, tim mRNA levels are also significantly increased, suggesting that cyc is not the only target of MAN1 in the clock. This is further supported by the observation that knocking down MAN1 in flies lengthens the circadian period but the mRNA level of cyc is not affected. Besides targeting cyc, MAN1 may also modulate the clock by regulating tim levels. Although Drosophila TIM is believed to function mainly in the circadian clock, TIM in other eukaryotes plays essential roles in vital functions including DNA replication and maintaining genome stability. Therefore, mis-regulation of TIM could contribute to the

*Figure 1. MAN1 regulates the circadian clock by promoting BMAL1 transcription. The schematic demonstrates a model for the action of MAN1 on the molecular clock. MAN1 binds to BMAL1 promoter and promotes its transcription, thus exerting effects on the clock. Besides MAN1, LMNB1 and LBR also participate in modulating the clock, but the molecular mechanisms remain unclear.*
pathology underlying human diseases caused by MANI
deficiency. It would be interesting to test whether TIM is
affected by MAN1 manipulations in the mammalian
system.

Potential Mechanistic Actions
of LMNB1 and LBR on the Clock

We found that knocking down LMNB1 or LBR in cell culture reduces
mRNA and protein levels of MAN1, while knocking down MAN1 does not alter
LMNB1 or LBR levels. Therefore, the effects of LMNB1 and LBR on the clock
could be at least partially through MAN1. However, MAN1, LMNB1 and LBR all
interact with a distinct set of chromatin binding partners, implying that these 3
proteins may also influence the clock via different mechanisms. This could explain
the different circadian phenotypes observed in flies over-expressing or defic-
ient for these genes.

As previously mentioned, MAN1 physically associates with BAF, while both
LMNB1 and LBR can directly bind DNA and chromatin. In addition, B-type
lamin and LBR interacts with histones H2A/H2B and H3/H4, respectively. LBR also
interacts with heterochromatin protein HP1, which regulates gene
expression by binding to methylated H3 tail. Interestingly, LBR/HP1 binding to
H3/H4 is strongly inhibited by CREB-binding protein (CBP)-mediated acetyl-
ation. Moreover, the promoter regions of Per1, Per2 and Cry1 exhibit circadian
rhythms in H3 acetylation, and CBP-Per1 binding protein (CBP)-mediated acetyla-
tion is strongly inhibited by CREB-1, which regulates gene
expression.116 Volume 6 Issue 2

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Concluding Remarks
Each NE protein interacts with a distinct set of proteins (including chromatin
modifying enzymes and transcription fac-
tors) to exert its unique effects and some of these effects are on the core molecular

Clockwork and clock-controlled genes (Fig. 1). The regulation of NE on circadian
day clock is evolutionarily conserved, and it may help confer spatial and temporal
specificity to meet the divergent demands of varying cell types and tissues in differ-
ent organisms at different life stages in adaptation to the 24-hour solar cycle.

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