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Journal
Nucleus, 6(2)

ISSN
1949-1034

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Publication Date
2015-03-04

DOI
10.1080/19491034.2015.1010949

Peer reviewed

Nucleus 6:2, 114–117; March/April 2015; © 2015 Taylor & Francis Group, LLC

Nuclear envelope regulates the circadian clock

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Daily 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.

Keywords: BMAL1, circadian clock, lamin B receptor, lamin B1, MAN1, nuclear envelope

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Submitted: 12/15/14
Revised: 01/05/2015
Accepted: 01/09/2015

http://dx.doi.org/10.1080/19491034.2015.1010949

Original Article: Lin ST, Zhang L, Lin X, Zhang LC, Garcia VE, Tsai CW, Ptacek L, Fu YH. Nuclear envelope protein MAN1 regulates clock through BMAL1. eLife 2014; 3:e02981.

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-erb*α and *Ror*α. 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 1*ε (*Pdp1*ε) 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

period, while over-expression shortens the period. Reducing or increasing LMNB1 in mouse via genetic manipulations delays or advances the phase of molecular oscillation, respectively, which is likely a result of lengthening or shortening of the endogenous period. Similarly, reducing or increasing d*Lamin* (d*Lam*, also known as *Dm0*) in flies lengthens or shortens the behavioral period, respectively. This implicates an evolutionarily conserved role of *LMNB1*/d*Lam* in modulating the molecular clock. Given that our *in vitro* assays were conducted in human osteosarcoma cell lines, whereas mouse clock was analyzed using liver tissues, and fly behavioral period is a reflection of the clock in circadian neurons,[9] we can draw the conclusion that the effect of *LMNB1*/d*Lam* on the clock is likely ubiquitous. This is also consistent with the ubiquitous expression of B-type lamin in all somatic cells.[10] The effects of LBR and MAN1 on the clock are more complex. In contrast to cell culture, over-expressing *LBR* and *MAN1* lengthens behavioral period in flies, while knocking down *MAN1* also lengthens period,[8] suggestive of differential underlying mechanisms which will be discussed in greater details in subsequent sections.

NE may be important in the transcriptional regulation of not only the core clock genes, but also other genes that are under circadian control. In prokaryotes, which lack the NE, a specific clock gene promoter is not essential for mediating the transcriptional feedback loop, unlike in eukaryotes.[1,11] In these organisms, the circadian rhythm of the transcriptome is believed to be achieved by circadian changes in DNA topology.[11,12] It has also been shown that approximately 30 ~ 64% of the transcriptome accumulate rhythmically in these organisms,[13,14] which is considerably larger than that of the eukaryotic system.[4] 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.[15] 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 [16] and interaction with protein partners, such as chromosome modifying enzymes and transcription factors with different spatial and temporal expression.[7]

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.[8] Consistently, over-expressing *MAN1* in flies increases the mRNA levels of *cyc*. Promoter-fused reporter assays revealed that the enhancement of *BMAL1* mRNA levels is a result of 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.[7] MAN1 has been identified to tether Smads and antagonize BMP/TGFβ signaling, resulting in less activation of gene expression.[17-21] We found that both TGFβ1 and TGFβ-responsive Smad2 increase *BMAL1* transcription, but likely via a pathway independent from that of MAN1.[8] 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-erb*α and *Ror*α, 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),[22] which binds dsDNA, chromatin, histones and various transcription factors.[23] 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.[8] 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.[24] Therefore, misregulation of *TIM* could contribute to the

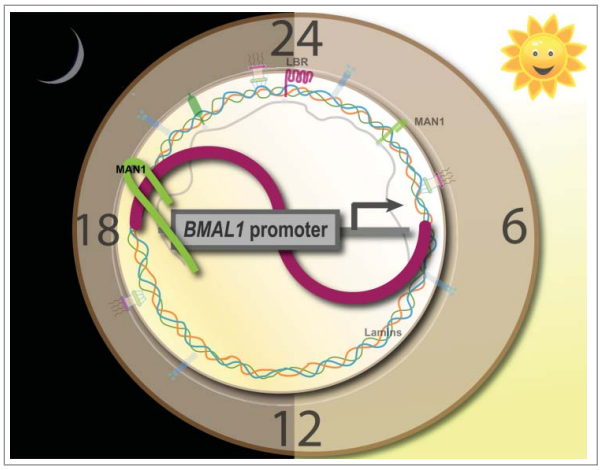

Figure 1. MAN1 regulates the circadian clock by promoting *BMAL1* transcription. The schematic demonstrates a model for the action of MAN1 on the molecular clock. MAN1 binds to *BMAL1* promoter and promotes its transcription, thus exerting effects on the clock. Besides MAN1, LMNB1 and LBR also participate in modulating the clock, but the molecular mechanisms remain unclear.

pathology underlying human diseases caused by *MAN1* deficiency.[25] 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.[8] 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,[10] 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 deficient for these genes.[8]

As previously mentioned, MAN1 physically associates with BAF,[22] while both LMNB1 and LBR can directly bind DNA and chromatin.[10] In addition, B-type lamin and LBR interacts with histones H2A/H2B and H3/H4, respectively.[26,27] LBR also interacts with heterochromatin protein HP1,[28] which regulates gene expression by binding to methylated H3 tail.[29] Interestingly, LBR/HP1 binding to H3/H4 is strongly inhibited by CREB-binding protein (CBP)-mediated acetylation.[27] Moreover, the promoter regions of *Per1*, *Per2* and *Cry1* exhibit circadian rhythms in H3 acetylation,[30] and CBP has been shown to regulate the clock by impinging on transcriptional activity of CLOCK/BMAL1.[31,32] Although LBR levels do not exhibit circadian oscillation, LBR could exert effects on the transcription of clock genes through cooperative actions with CBP.

Concluding Remarks

Each NE protein interacts with a distinct set of proteins (including chromatin modifying enzymes and transcription factors) 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 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 different organisms at different life stages in adaptation to the 24-hour solar cycle.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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