OUTLOOK

‘Cold cuts’ added to the circadian smorgasbord of regulatory mechanisms

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In mammals, rhythms in body temperature help to entrain and synchronize circadian rhythms throughout the organism, and the cold-inducible RNA-binding protein (CIRBP) is one of the mediators of these daily temperature changes. *Cirbp* mRNA expression is regulated by the daily subtle rhythms in body temperature, and a new study by Gotic and colleagues (pp. 2005–2017) reveals a surprising and novel mechanism that involves temperature-dependent enhancement of splicing efficiency.

Circadian clocks throughout the body drive rhythms in biochemistry, physiology, and behavior through broad control of gene expression in a tissue-specific manner. Steady-state levels of thousands of mRNAs in each tissue exhibit daily rhythms, many from rhythmic transcriptional control. However, a number of recent studies have revealed that transcription is only one of many mechanisms in the circadian clocks’ repertoire, with more than half of the rhythmic mRNAs the result of various post-transcriptional regulatory mechanisms. The clocks driving these rhythms in gene expression are entrained and synchronized by environmental stimuli such as light, food intake, and temperature. In homeothermic animals such as mammals, the central clock in the brain drives daily rhythms in core body temperature, varying just a few degrees from peak to trough, and these rhythms help to synchronize the clocks in cells throughout the body (Brown et al. 2002; Buhr et al. 2010; Saini et al. 2012).

The circadian interest in cold-inducible RNA-binding protein (CIRBP) originally came from its identification as one of a small subset of mRNAs that continued to exhibit rhythmicity in the livers of mice following a liver-specific genetic ablation of clock function (Kornmann et al. 2007), indicating that it was controlled by some systemic rhythmic signal. Because this mRNA was known to be induced by a relatively modest lowering of temperature in cell culture, Schibler and colleagues (Morf et al. 2012) tested whether temperature cycles mimicking physiological core body temperature rhythms in mice (34°C–38°C) would be sufficient to generate *Cirbp* mRNA rhythms in

fibroblasts. Indeed, temperature cycles generated rhythms in *Cirbp* expression, suggesting that the core body temperature rhythm is the systemic cue that confers diurnal rhythmicity to this mRNA. Furthermore, knockdown of *Cirbp* in these cells resulted in a broad loss of high-amplitude rhythms of circadian gene expression and a less robust circadian clock that was more prone to resetting (Morf et al. 2012). Among the identified CIRBP target mRNAs were several involved in clock function, including *Clock*, which encodes one member of the heterodimeric CLOCK/BMAL1 transcription factor that is at the core of the circadian negative feedback loop. Loss of *Cirbp* caused significantly reduced levels of *Clock* mRNA accumulation, leading to a model in which core body temperature rhythms drive rhythms in CIRBP levels, which then rhythmically regulates *Clock* mRNA accumulation, thereby enhancing the robustness of the circadian clock mechanism post-transcriptionally.

Although this elegant work demonstrated that CIRBP plays an important role in circadian clock function and is likely regulated by rhythmic core body temperature, little was known about how such small variations in temperature could generate these rhythms in *Cirbp* mRNA. Earlier work on temperature control of rhythmicity by this laboratory and others has focused on the regulation of heat-inducible genes, such as the core clock gene *Per2* (Reinke et al. 2008; Buhr et al. 2010; Tamaru et al. 2011; Saini et al. 2012). This mechanism is transcriptional and involves temperature-induced release of the transcription factor HSF1 from inert cytosolic complexes followed by nuclear translocation and transcriptional activation of genes containing heat-shock response elements.

In a new study in this issue of *Genes & Development*, Schibler and colleagues (Gotic et al. 2016) examine how low temperatures contribute to rhythmicity of *Cirbp* mRNA and, in doing so, uncover a novel regulatory mechanism that likely exerts temperature-dependent control over many mRNAs. The cold induction of *Cirbp* expression has been reported recently to be transcriptional (Sumitomo et al. 2012); however, Gotic et al. (2016)

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demonstrated that although the steady-state levels of the mature *Cirbp* mRNA increased significantly in response to mild cold exposure (32°C) in NIH3T3 cells, the levels of *Cirbp* pre-mRNA did not change. Chromatin immunoprecipitation (ChIP) assays showed that RNA polymerase II occupancy on the *Cirbp* promoter or gene body also does not change in response to lowered temperatures, further arguing against a transcriptional response. Additional evidence that a post-transcriptional regulatory mechanism is responsible for the cold induction came from a *Cirbp*-luciferase fusion construct under the control of a temperature-independent, constitutively active CMV promoter. This construct, which contained the entire genomic region containing the *Cirbp* gene (including introns) downstream from the CMV promoter, resulted in rhythmic luciferase activity in cells exposed to simulated core body temperature rhythms.

To discern which post-transcriptional processes might be regulating *Cirbp* induction by cold, Gotic et al. (2016) used a method called “approach to steady state” (ATSS) to estimate the *Cirbp* mRNA half-life in a noninvasive manner following abrupt shifts in temperature from 33°C to 38°C and vice versa. Mathematical modeling of expression levels following these transitions revealed that the half-life of *Cirbp* mRNA increased moderately upon transition to the lower temperature, but the change in half-life could not explain the very large induction in steady-state *Cirbp* mRNA levels that they observed. Only when splicing “proneness” was factored into the model did it fit the data well. Supporting this, inhibition of splicing through pharmacological perturbation or by antisense morpholino oligos prevented the increase in *Cirbp* mRNA levels at low temperatures. Furthermore, removal of the introns from the *Cirbp*-luciferase reporter gene abolished the cold-induced increase in luciferase activity. Through the generation of various deletion constructs, a 337-base-pair (bp) region within intron 1 was identified that was sufficient for conferring temperature sensitivity to the luciferase construct even when inserted into temperature-insensitive genes. In the cold, this 337-bp region somehow confers increased splicing efficiency, resulting in significant increases in the mature mRNA, while, in warmer temperatures, the presence of this region prevents efficient splicing, and the unspliced preRNAs are targeted for degradation, resulting in overall lower *Cirbp* mRNA levels.

RNA sequencing analysis of their ATSS samples following heat or cold transitions revealed dozens of mRNAs that changed in abundance. Application of the ATSS models to the expression data for these genes revealed that while some of these mRNAs are regulated at the level of mRNA half-life, many other mRNAs are regulated by temperature-dependent splicing efficiency.

This finding is remarkable for several reasons. First, the temperature changes causing this change in splicing efficiency are extremely modest; just a few degrees generates these large changes in steady-state mRNA levels. Second, although there are previous examples of temperature regulating alternative splicing, gene-specific regulation of temperature-dependent splicing efficiency has not been previously demonstrated. Finally, these data suggest that *Cirbp* is not the only mRNA regulated by this mechanism and that subtle changes in temperature likely regulate many other mRNAs through gene-specific changes in splicing efficiency. Therefore, cold-induced splicing efficiency is yet another item on the “smorgasbord” of regulatory strategies that the mammalian circadian clock uses to generate the complex and extensive rhythms of gene expression that drive the rhythms in metabolism, physiology, and behavior.

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