PERSPECTIVE

The ticking tail: daily oscillations in mRNA poly(A) tail length drive circadian cycles in protein synthesis

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In this issue of *Genes & Development*, Kojima and colleagues (pp. 2724–2736) examined the impact of mRNA poly(A) tail length on circadian gene expression. Their study demonstrates how dynamic changes in transcript poly(A) tail length can lead to rhythmic protein expression, irrespective of whether mRNA accumulation is circadian or constitutive.

Circadian clocks are timing devices that enable organisms to adjust their physiology in a proactive manner to daily oscillations in environmental conditions caused by the rotation of the Earth around its own axis. In higher organisms, virtually every cell in the body possesses a self-sustained, cell-autonomous clockwork circuitry that is thought to be based on multiple interconnected gene expression loops (for review, see Dibner et al. 2010). Over the past decade, the transcriptional regulation underlying rhythmic mRNA and protein expression has been the main focus of circadian gene expression studies in various organisms (for review, see Lowrey and Takahashi 2004; Pegoraro and Tauber 2011). However, recent studies employing genome-wide analyses of transcription and mRNA accumulation could attribute only a minor fraction of daily mRNA expression cycles to rhythmic transcription (Koike et al. 2012; Menet et al. 2012). Moreover, only about half of the proteins with circadian accumulation cycles in mouse livers were found to be generated by cyclically expressed mRNAs (Reddy et al. 2006). Indeed, several groups have already initiated work on diurnal post-transcriptional mechanisms, and, based on their studies, it appears that many steps in the lifetime of an mRNA can be subject to circadian regulation. These steps, schematically illustrated in Figure 1, include regulation of pre-mRNA splicing efficiency (Low et al. 2008), alternative splicing (McGlinchey et al. 2012; Wang et al.

2012), poly(A) site selection (Sumiyoshi et al. 2011), mRNA editing (Hughes et al. 2012), nuclear export (Morf et al. 2012), mRNA stability, and translation efficiency (Gatfield et al. 2009; Guo et al. 2009; Kojima et al. 2010; Woo et al. 2011; Mehta and Cheng 2012; Vodala et al. 2012; for review, see Kojima et al. 2011; Zhang et al. 2011).

The study by Kojima et al. (2012) in this issue of *Genes & Development* adds yet another important mRNA processing step—namely, poly(A) tail length regulation—to the growing list of circadian post-transcriptional mechanisms (Fig. 1). Polyadenylation is an important determinant of eukaryotic mRNA stability and translation efficiency (for review, see Zhang et al. 2010). In the nucleus, poly(A) tails of ~250–300 adenosine residues are added to the nascent transcripts by poly(A) polymerase after the endonucleolytic cleavage at polyadenylation sites. These poly(A) stretches are then bound by poly(A)-binding proteins (PABPs), which supposedly protect the transcripts from precocious degradation by the exosome but also fulfill other functions associated with mRNA maturation (Lemay et al. 2010). In general, the length of the poly(A) tail is still large in freshly exported mature mRNA but gets subsequently shortened in most mRNAs in a transcript- and cell type-specific manner. According to current models, the PABP-decorated poly(A) tail not only protects transcripts from exonucleolytic degradation, but also facilitates translation initiation through the formation of a closed loop between PABPs and the 5' cap-bound translation initiation factor 4F complex (eIF4F) (for review, see Millevoi and Vagner 2010; Weill et al. 2012). Aging transcripts progressively lose their poly(A) tails through the action of poly(A) nucleases (PANs, PARN, and the CCR4–NOT complex). The consecutive loss of protective PABPs exposes their 5' ends to the activity of decapping enzymes, such as the Dcp1p–Dcp2p complex and DcpS (for review, see Parker and Song 2004). Deadenylation and decapped mRNAs are then targeted for degradation via the exosome or Xrn pathways (3'–5' or 5'–3' nucleolytic activity, respectively) (for review, see Sloan et al. 2012). However, shortening of poly(A) tails does not inevitably lead to transcript degradation. Under

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Figure 1. Mechanisms underlying the circadian activity of PAR transcripts. In mouse livers, PAR mRNAs are rhythmically (class I and class II) or constitutively (class III) transcribed by RNA polymerase II. The m7Gppp-capped nascent chains are released from the chromatin by endonucleolytic cleavage at the poly(A) site. Subsequently, the nuclear polyadenylation machinery adds ~250 A residues to the 3' end of the transcripts. The polyadenylated precursors are then spliced into mature mRNAs, which are exported into the cytoplasm (note that some transcripts may undergo cotranscriptional splicing, not shown in the cartoon). As indicated in the scheme, some transcripts are degraded before being spliced or exported into the cytoplasm. In the cytoplasm, the long-tailed transcripts are translated more efficiently than the short-tailed transcripts. The latter are either degraded (class I and class II) or readenylated in a circadian fashion (class III).

some circumstances (for example, in oocytes, neurons, and lymphocytes), mRNAs can be deadenylated and stored for extended time periods before they are readenylated and actively translated. This process is mediated through the interaction of transcript-specific cytoplasmic polyadenylation elements (CPEs), mainly present in the 3' untranslated regions of mRNAs and their cognate binding proteins (CPEBs). Reactivation of stored silent transcripts includes the readenylation, initiated through the interaction of CPEBs with cytoplasmic polyadenylation complexes, as well as adenylation-independent mechanisms (e.g., DAZL-PABP and SLBP-eIF4G-mediated transcript stabilization) (for review, see Weill et al. 2012). In addition to the above-mentioned scenarios of cytoplasmic polyadenylation, the circadian expression pattern of a deadenylase named Nocturnin (Wang et al. 2001; Kojima et al. 2010) and daily poly(A) tail length variations in two mRNAs observed in previous studies (Robinson et al. 1988; Gerstner et al. 2012) suggested an implication of cytoplasmic poly(A) length regulation in circadian gene expression. In this study, Kojima et al. (2012) examined the connection between circadian transcript oscillations and poly(A) tail length dynamics in a genome-wide manner. Most interestingly, their work

shows that rhythmic changes in poly(A) tail length of mRNAs accumulating at nearly constant levels throughout the day can drive circadian protein translation cycles.

To examine whether the length of poly(A) tails oscillates in a circadian fashion in mouse liver transcripts, Kojima et al. (2012) fractionated mRNAs harvested at 4-h intervals around the clock according to their elution properties on oligo(dT) columns. mRNAs harboring short (<60-nt) and long (60- to 250-nt) poly(A) tails can be eluted from such columns with moderate and low-salt concentrations, respectively. The fractionated mRNAs were then subjected to an oligonucleotide microarray hybridization analysis in order to estimate the prevalence of long-tailed or short-tailed transcripts in a daytime-dependent manner. Their analysis identified 237 transcripts (2.3% of expressed mRNAs) with rhythmic L/S (long/short) poly(A) tail length ratios, which were termed "poly(A) rhythmic" (PAR) mRNAs. The PAR transcripts were divided into three classes based on the temporal accumulation patterns of the corresponding pre-mRNAs (reflecting transcription) and mature mRNAs.

As anticipated, the most abundant PAR mRNAs (class I) showed circadian rhythmicity at all levels. High L/S poly(A) tail ratios of these transcripts at specific time

points likely reflect circadian synthesis, as pre-mRNAs and newly synthesized mRNAs carry longer poly(A) tails than aged ones. An intriguing observation in this group of PAR transcripts is the ~5-h delay between maximal pre-mRNA and mRNA accumulation. Since this delay appears to be much shorter for the majority of rhythmic transcripts, additional experiments will be required to examine the mechanisms specifically retarding the accumulation of PAR mRNAs after their synthesis.

Class II PAR transcripts exhibited circadian rhythmicity at the pre-mRNA and poly(A) tail length levels but, supposedly owing to their long half-lives, accumulated at virtually constant amounts throughout the day. The daily rise and fall in the L/S tail ratio of class II transcripts most probably reflect their circadian transcription, as has been argued for class I transcripts. Since long poly(A) tails may be beneficial for translation, the oscillations in poly(A) tail length could manifest themselves in protein synthesis rhythms and hence in circadian protein accumulation. Immunoblot experiments for MIF and GSTT2, two polypeptides encoded by class II PAR mRNA, are consistent with this scenario. Interestingly, some class II PAR mRNAs display maximum poly(A) tail lengths at the opposite time of the day, probably reflecting a regulation of their transcription by anti-phasic circadian feedback loops.

Clearly, class III PAR mRNAs are the least expected and thus the most interesting PAR transcripts. These mRNAs are characterized by daily oscillations in their poly(A) tail length in the absence of significant temporal changes in pre-mRNA and mRNA levels. Hence, for these transcripts, cytoplasmic polyadenylation must participate in the generation of circadian poly(A) tail length fluctuations and, as a consequence, protein synthesis rhythms. As shown by this study, circadian cytoplasmic polyadenylation of a subset of class III transcripts is mediated by the known CPEB factors (CPEB1 and CPEB2). Interestingly, CPEB2 as well as other factors of the polyadenylation machinery (e.g., CPEB4, PARN, and GLD2) exhibit rhythmic changes in their mRNA expression. However, the investigators' data also suggest the existence of additional, yet-unknown mechanisms of cytoplasmic polyadenylation that do not exclusively rely on CPEB-mediated activity. The notion that class III PAR transcripts comprise a special group of mRNAs is further emphasized by their relatively long half-lives and the phase of their poly(A) length oscillation. While most class I and class II PAR transcripts exhibit maximal poly(A) tail lengths during the night, class III PAR mRNAs reach longest poly(A) tails during the day. In future studies, it will be exciting to identify all of the *cis*-acting RNA sequences and their cognate *trans*-acting factors and dissect the mechanisms by which they control the cytoplasmic oscillations in poly(A) length of class III PAR transcripts.

In conclusion, the study by Kojima et al. (2012) opens new avenues in studying circadian gene expression by demonstrating that cyclic protein expression can be generated by rhythmic changes in poly(A) tail length of both rhythmically and constitutively expressed mRNAs

(see Fig. 1). Probably due to the limitations of the microarray technology used (for review, see Sun et al. 2012), no PAR transcript-specific sequence motifs could yet be identified. However, the use of ultradeep RNA sequencing procedures might help address these issues in the near future.

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Gotic and Schibler

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