Circadian Rhythms Require Proper RNA Splicing

Circadian rhythms have evolved in a wide variety of organisms, allowing them to anticipate the 24-h diurnal cycle of daylight and darkness and adjust endogenous biological processes to this predictable cycle. This rhythm, which is regularly entrained by the actual onset of daylight, involves a set of circadian clock-regulated genes that operate in a feedback-controlled loop to ensure proper expression of target genes (Nakamichi, 2011). Although most research has focused on transcriptional regulation of clock-associated genes, there is considerable involvement of posttranscriptional processes, such as mRNA stability, translatability, and post-translational regulation (Cibois et al., 2010; Kojima et al., 2011).

Jones et al. (2012) mutagenized Arabidopsis thaliana plants containing a clock-regulated bioluminescent CCR2:LUC reporter to identify genes whose products are involved in maintaining circadian regulation. This reporter construct encodes firefly luciferase expressed under the control of the COLD CIRCADIAN RHYTHM–RNA BINDING2 promoter, resulting in a circadian pattern of bioluminescence in transgenic plants expressing this construct. They isolated a mutant defective in circadian regulation that showed enhanced bioluminescence and a long-period phenotype. This mutation, mapped to the At1g17070 locus, caused a G-to-A transition in the 3′ untranslated region (UTR) of the STIPL1 gene, resulting in a premature stop codon. The gene mutated has significant homology with genes encoding the STIP (for Septin and TufTalin Interacting Protein) family of RNA binding proteins. Because of the observed altered circadian phenotype and the identification of the human homolog, TIP11, as a component of the spliceosome, the Arabidopsis protein has been named SPLICEOSOMAL TIMEKEEPER LOCUS1 (STIPL1).

In the CCR2:LUC reporter plants, the stipl1 mutation caused enhanced bioluminescence and lengthened the circadian period by ∼1.5 h (see figure). Expression of the wild-type STIPL1 protein in transgenic mutant plants complemented this circadian defect. The authors examined the circadian patterns of mRNA accumulation for six clock-regulated genes in stipl1 mutant plants and discovered a similar lengthening of the circadian period.

Expression of a STIPL1:GFP fusion construct showed that STIPL1, which contains two putative nuclear localization signals, was localized to the nucleus (see figure, right panel), consistent with a role in pre-mRNA splicing. They then examined splicing patterns using a set of eight clock-regulated genes. Splicing defects, such as intron retention, exon skipping, and alternative 5′ and 3′ splice site selection, were noted in ∼33% of the transcripts tested. The most common defect was retention of introns, resulting in some unspliced transcripts. The authors also examined 70 non-clock-regulated genes and found similar splicing defects in a similar proportion of transcripts tested, suggesting a global impact on pre-mRNA splicing due to the stipl1 mutation. To better define the effect of the stipl1 lesion, the authors continued with a detailed look at the types of alternative splicing events in the clock-regulated genes.

Whereas the STIP proteins in animals are encoded by single-copy genes, the Arabidopsis genome encodes two related STIP proteins. Interestingly, mutation of STIPL2 does not cause splicing defects or changes in circadian rhythms in Arabidopsis plants, demonstrating that the functions of these genes have diverged. However, the high level of STIPL2 mRNA in seed and the observation that no stipl1 stipl2 double mutants could be isolated suggest a potential role of STIPL2 in splicing events during embryogenesis.

Gregory Bertoni
Science Editor
gbertoni@aspb.org

REFERENCES


Circadian Rhythms Require Proper RNA Splicing
Gregory Bertoni

*Plant Cell*; originally published online October 30, 2012;
DOI 10.1105/tpc.112.241012

This information is current as of October 20, 2017