Expression and Alternative Splicing in Circadian Clock Genes Respond to Temperature Changes

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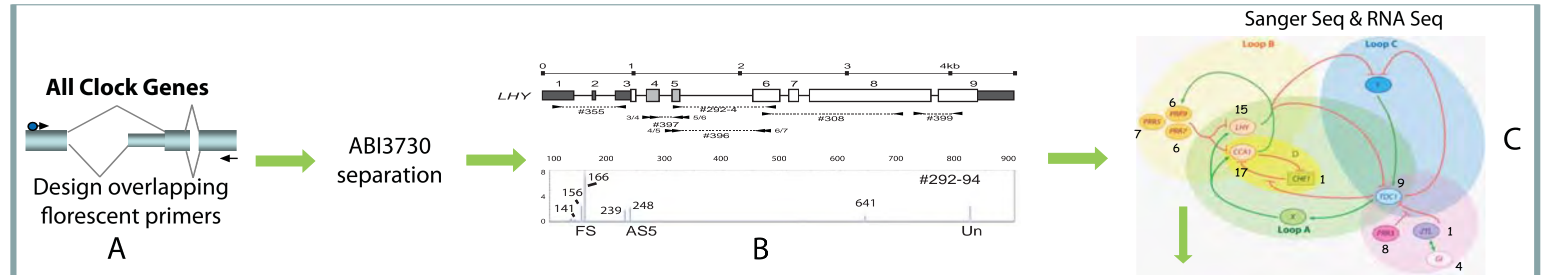
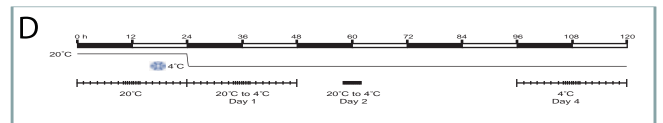
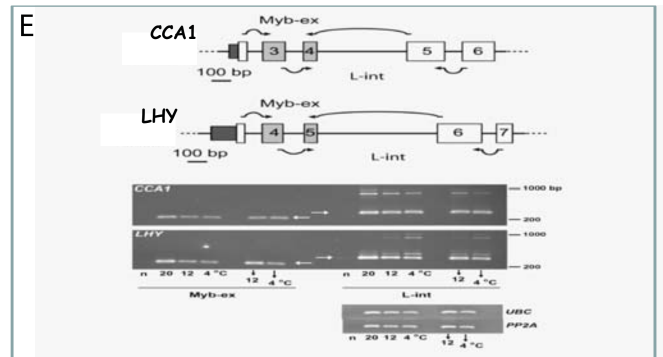
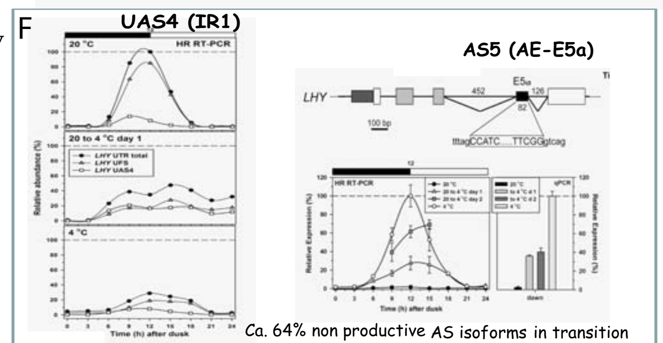
Introduction

Alternative splicing (AS) is a well-established mechanism in eukaryotic cell function which controls both transcriptome and proteome diversity and regulates protein structure/function and gene expression. In plants, alternative splicing is widespread with a current estimate of 42% of intron-containing genes in Arabidopsis being alternatively spliced. Alternative splicing has also been reported in the Arabidopsis circadian clock genes (CCA1 and PRR9) and output genes (GRP7 and GRP8). Recent work has provided further evidence of links between the circadian clock and AS. In Arabidopsis, PROTEIN ARGININE METHYL TRANSFERASE 5 (PRMT5) is required for correct pre-mRNA splicing. PRMT5 methylates a wide variety of substrates including histones and spliceosomal proteins, and *prmt5* mutants show a long circadian period and dramatic changes in alternative splicing of PRR9 transcripts.
By employing a high resolution RT-PCR system we have systematically investigated the expression of Arabidopsis circadian clock genes in response to temperature changes at the level of both transcription and AS. We show the occurrence of numerous AS events in the core clock genes, many of which are temperature sensitive and some of which have a major role in the effects of temperature on gene expression, thus implicating AS as a further mechanism involved in operation and control of the circadian clock.

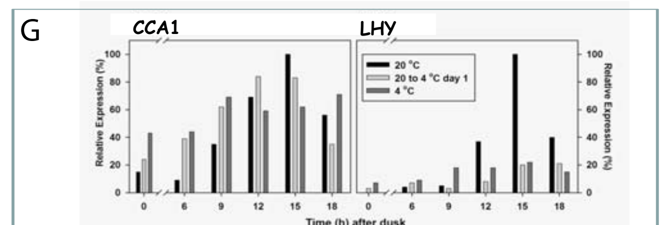


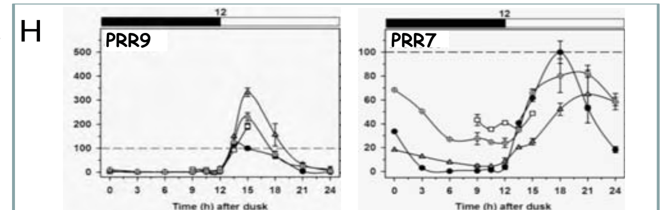






Ca. 64% non productive AS isoforms in transition





Results

A, B. Overlapping florescent primers were designed across the length of all core clock genes and used in RT-PCR reactions using pooled RNA samples harvested at different temperatures and light conditions during the day-night cycle. RT-PCRs were separated on an ABI3730 sequencer.

C. Extensive alternative splicing for most clock genes was discovered as shown in the clock model (adapted from Harmer S, 2010). Different AS transcripts were identified using Sanger or RNA Seq.

D. Experimental scheme where plants grown at 20°C were transferred to 4°C. AS variants and canonically spliced products were measured during transition as well as acclimation to 4°C.

E. AS variants in CCA1 and LHY decrease and increase respectively at low temperatures. Primers spanning the Myb domain show no variation for both LHY and CCA1. Partially redundant CCA1 and LHY show opposite effects of AS with low temperature.

F. Expression patterns of two AS events (UAS4 retains intron 1 in the 5'UTR and AS5 adds an alternative exon of 82 bp in the transcript) in LHY according to D. AS5 is not seen at 20°C but steadily increase at 4°C to ~20% of the total LHY transcript. UAS4 increases to 50% during the first day of transfer to 4°C and becomes about one third of the total transcript after acclimation. A bar chart in (F) shows qRT-PCR results of AS5 for dawn samples showing almost identical rersults. UAS4 and AS5 do not code for full length protein owing to the presence of premature termination codons (PTCs) and together represent about 64% of the total transcript of LHY during the transition.

G. Western analysis: Protein levels of LHY are dramatically reduced 3 h after dawn at 4°C wheras CCA1 is much less affected. LHY transcription does not change so the presence of non-functional LHY transcripts through AS may cause the reduction in LHY protein.

H. qRT-PCR of the two redundant genes PRR9 and PRR7 show opposite effects in response to cool temperature. PRR9 expression goes up with decrease in temperature while PRR7 shows an opposite response. Splice variants of PRR9 and PRR7 also show dramtic effects during transition as well as acclimation to 4°C. Similarly, PRR3 and PRR5 also respond to temperature changes in opposite directions. HR RT-PCR also gave identical results.

Conclusions

- Alternative splicing is widespread among the plant cicadian genes and many of these AS events result in non-functional transcripts and/or induction of nonsense-mediated decay.
- Several clock genes show marked changes in alternative splicing with temperature and these temperature transitions distinguish functionally between partially redundant clock genes (LHY/CCA1; PRR7/PRR9 etc.).
- Temperature associated alternative splicing is an additional mechanism involved in the control and operation of the plant circadian clock.