Plant Signaling & Behavior 6:4, 541-543; April 2011; © 2011 Landes Bioscience

Histone acetylation and the circadian clock

A role for the MYB transcription factor RVE8/LCL5

Benoit Farinas and Paloma Mas*

Centre for Research in Agricultural Genomics (CRAG, Consortium CSIC-IRTA-UAB); Barcelona, Spain

Most organisms have developed an internal timing mechanism or circadian clock that is able to generate 24-hour biological rhythms in synchronization with the diurnal environmental changes. Despite our increasing understanding of the molecular machinery underlying circadian clock function, a complete picture of the components and regulatory mechanisms governing the circadian system in *Arabidopsis thaliana* is still lacking. In a recent study, we have characterized the role of the MYB-like transcription factor REVEILLE8/LHY-CCA1-LIKE5 (RVE8/LCL5) within the Arabidopsis circadian clock. We have generated *RVE8/LCL5* mutant and overexpressing plants and showed that similar to the MYB-like transcription factor CIRCADIAN CLOCK-ASSOCIATED1 (CCA1), RVE8/LCL5 binds to the promoter of key clock component *TOC1* (Timing of CAB expression 1) and regulates its circadian expression. However, the mechanisms of RVE8/LCL5 and CCA1 circadian function seem to differ: while CCA1 represses *TOC1* expression by facilitating a hypo-acetylated state of Histone H3, RVE8/LCL5 contributes to *TOC1* expression by favouring H3 acetylation at the *TOC1* locus. Although CCA1 has a more predominant role on this regulation, our results showing the opposing function of RVE8/LCL5 open interesting questions about the complex networks of transcriptional regulators and chromatin remodelling activities that need to be integrated in synergistic and antagonistic ways to generate the circadian periodicity.

Key words: *Arabidopsis thaliana*, circadian clock, transcriptional regulation, single MYB, histone acetylation

Submitted: 01/14/11

Accepted: 01/15/11

DOI: 10.4161/psb.6.4.14837

*Correspondence to: Paloma Mas;
Email: paloma.mas@cid.csic.es

Addendum to: Farinas B, Mas P. Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. Plant J 2011; 2:318-29;
PMID: 21205033;
DOI: 10.1111/j.1365-313X.2011.04484.x.

The circadian clock is able to generate 24-hour biological oscillations in resonance with the diurnal environmental changes.[1] The mechanisms underlying circadian clock function are complex and seem to be conserved throughout evolution. In broad sense, the 24-hour period oscillations rely at its basis on negative feedback loops at the core of the circadian oscillator.[2] In addition to a very precise transcriptional/translational regulation of clock gene and protein expression, recent evidence is also pointing towards changes in chromatin structure as a key mechanism for circadian clock progression.[3,4] In Arabidopsis, the transcriptional oscillation of the clock component *TOC1* (*Timing of CAB expression 1*) is preceded by rhythmic changes in the pattern of histone H3 acetylation.[5] The histone acetylation state seems to be regulated, at least in part, by the clock component CCA1 (CIRCADIAN CLOCK-ASSOCIATED1) as plants misexpressing this MYB-like transcription factor exhibit an altered pattern of histone acetylation at the *TOC1* locus.[5] CCA1 might thus facilitate repressive chromatin structures at the *TOC1* locus to regulate *TOC1* expression at dawn. In a recent study, we have characterized the implication on the Arabidopsis circadian system of RVE8/LCL5, a MYB-like transcription factor with a high degree of sequence homology to CCA1.[6] By using RVE8/LCL5 overexpressing and mutant plants, we found that RVE8/LCL5 binds to the *TOC1* promoter and contributes to proper circadian expression of *TOC1*. Our studies also show that in contrast to CCA1, RVE8/LCL5 seems to favor H3 acetylation, most likely by antagonizing CCA1

function during *TOC1* raising phase. Therefore, the counteracting functions of CCA1-RVE8/LCL5 (and possibly other members of the LCL subfamily) might regulate chromatin compaction to precisely shape the waveform of *TOC1* circadian expression.

The Expression of the MYB-Like Transcription Factor RVE8/LCL5 is Regulated by the Circadian Clock

Two essential regulators of the Arabidopsis circadian system, CCA1 and LHY (ELONGATED HYPOCOTYL) belong to the REVEILLE (RVE) family composed of eleven proteins. These proteins show a high amino acid sequence similarity within the MYB-like domain (SHAQKYF class).[7,8] Five of the eleven proteins can also be grouped into a subfamily, previously identified as LHY/CCA1-LIKE (LCL) proteins.[9] The LCL protein subfamily shows a domain at the C-terminal end of the proteins (LCL domain) that is not present in CCA1, LHY or in the rest of the RVE protein family. Based on the sequence similarities and divergences, we explored the possible role of one of the RVE/LCL proteins, RVE8/LCL5, within the Arabidopsis circadian system. To that end, we first analyzed *RVE8/LCL5* expression in wild-type (WT) plants grown for two days under constant light (LL) conditions after synchronization under light:dark cycles. Northern-blot and RT-Q-PCR analysis showed that the expression of *RVE8/LCL5* was controlled by the circadian clock, with a rhythmic oscillation under constant light (LL) conditions. *RVE8/LCL5* circadian waveform showed a morning acrophase similar to that reported for *CCA1*[10,11] and almost antiphasic to the one described for *TOC1*.[12,13]

TOC1 Rhythmic Expression is Affected by Overexpression and Mutation of *RVE8/LCL5*

To analyze the role of RVE8/LCL5 in the Arabidopsis circadian system, we characterized two independent T-DNA insertion lines, *RVE8/LCL5-E5* and *RVE8/LCL5-I2*. We also generated different lines overexpressing *RVE8/LCL5* and examined the effects of *RVE8/LCL5* mis-expression on circadian gene expression. Northern-blot and RT-Q-PCR analysis revealed that overexpression of *RVE8/LCL5* advanced the phase of the evening-expressed gene *TOC1*, leading to higher abundance than in WT plants, particularly during the subjective day. Luminescence assays of *TOC1:LUC* plants transformed with the RVE8/LCL5-ox construct were consistent with the northern-blot and RT-Q-PCR assays and revealed that *TOC1* promoter activity rose earlier in RVE8/LCL5-ox than in WT plants, leading to an advanced phase and a short-period phenotype. RT-Q-PCR and luminescence analysis of *RVE8/LCL5* mutant plants revealed the opposite phenotypes, i.e., a delayed phase and a long-period phenotype with decreased *TOC1* accumulation mostly during the subjective day. Altogether, these results suggest that directly or indirectly RVE8/LCL5 contributes to proper regulation of *TOC1* circadian oscillation.

In vivo Binding of RVE8/LCL5 to the *TOC1* Promoter

We next examined whether regulation of *TOC1* expression by RVE8/LCL5 might be achieved by binding of RVE8/LCL5 to the *TOC1* promoter. To that end, we performed chromatin immunoprecipitation (ChIP) experiments with RVE8/LCL5-ox plants. Our results showed an evident amplification of the Evening Element (EE)-containing region of the *TOC1* promoter, with a peak during the subjective day, at times when *TOC1* expression is clearly affected by overexpression of RVE8/LCL5. No evident amplification was observed when other clock-unrelated genes were analyzed or when samples were equally processed but in the absence of GFP antibody. Together, the results suggest that in a similar way to CCA1,[5] RVE8/LCL5 is able to bind to the *TOC1* promoter to regulate *TOC1* expression.

RVE8/LCL5 Regulates the Pattern of H3 Acetylation at the *TOC1* Promoter

Based on previous results showing that circadian rhythms of H3 acetylation at the *TOC1* locus are important for *TOC1* circadian expression,[5] we next examined whether mis-expression of *RVE8/LCL5* affected the pattern of H3 acetylation at the *TOC1* promoter. Our ChIP analysis with an anti-acetylated H3 antibody showed that the pattern of H3 acetylation was significantly increased in RVE8/LCL5-ox plants compared to that of WT, particularly during the subjective day. As H3 acetylation favors transcription, these results were consistent with the early raising phase of *TOC1:LUC* expression observed in RVE8/LCL5-ox plants. We also performed ChIP experiments using the *rve8/lcl5* T-DNA insertion lines. Our studies showed that H3 acetylation was reduced during the subjective day with a delayed raising phase. These results suggest that *RVE8/LCL5* overexpression and mutation alter the normal pattern of H3 acetylation at the *TOC1* promoter. This regulation might be particularly relevant during the subjective day, at times when *TOC1* expression is most affected in RVE8/LCL5-ox and in *rve8/lcl5* T-DNA insertion lines.

Effects on *TOC1* Circadian Waveform after Pharmacological Inhibition of Histone Deacetylation

To further confirm our hypotheses, we examined *TOC1:LUC* expression in the absence or in the presence of trichostatin A (TSA), a potent HDAC inhibitor (Chang and Pikaard, 2005). As previously reported in reference 5, TSA treatment of WT plants delayed *TOC1:LUC* expression but the phase-delays were more evident when TSA was applied at the beginning or at the middle of *TOC1* raising phase, which suggested that treatments closer to the time of HDAC action (just after *TOC1* peak of expression) had more severe effects on *TOC1:LUC* waveform. In RVE8/LCL5-ox plants, TSA treatment led to a high and close to arrhythmic *TOC1:LUC* expression regardless the time of inhibitor administration. It is therefore possible that the HDAC activities that contribute to *TOC1* declining phase[5] might interfere with RVE8/LCL5 regulatory function at the *TOC1* promoter. When TSA effects on *TOC1:LUC* expression were examined

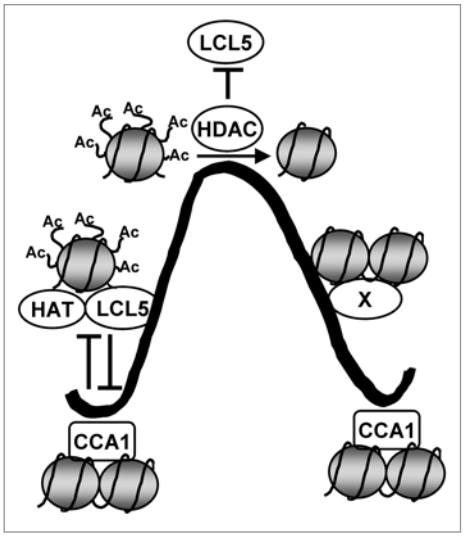

Figure 1. Schematic representation depicting the rhythmic regulation of *TOC1* expression. CCA1 contributes to *TOC1* repression at dawn most likely by antagonizing HAT activities and favoring a hypo-acetylated state of H3. Decreased CCA1 binding throughout the day together with the counteracting function of RVE8/LCL5 and HAT activities allow transcriptional activation by facilitating histone acetylation. After *TOC1* peak of expression, HDAC activities interfere with HAT/LCL5 activating function, promoting the switch to repressive chromatin structures and contributing to the declining phase of *TOC1* waveform. Additional unknown factors and mechanisms of circadian gene regulation (X) can account for *TOC1* declining phase until a rhythmic cycle starts again with CCA1 binding. The thick black line represents *TOC1* circadian waveform; nucleosomes are shown as gray circles with the H3 N-terminal tails as black, curved lines; lines ending in perpendicular dashes indicate antagonistic function.

in *rve8/lcl5* T-DNA insertion lines, we observed that adding the inhibitor at the mid of *TOC1:LUC* raising phase led to a very evident upregulation of *TOC1:LUC* expression suggesting that adding TSA is enough to partially restore the *TOC1:LUC* amplitude phenotypes in *rve8/lcl5* T-DNA insertion lines. Together, our results suggest that at the beginning of *TOC1* declining phase, HDAC activities might antagonize RVE8/LCL5 function while *TOC1* raising phase would be regulated by antagonistic functions in which CCA1 repression would be counterbalanced by RVE8/LCL5 activating function.

Conclusions

Our results suggest that RVE8/LCL5 (and most likely other members of the RVE/LCL subfamily) might function as a molecular rheostat of the CCA1-mediated H3 deacetylation at the *TOC1* promoter. We propose that CCA1 repressive function at dawn might act by antagonizing RVE/LCL and HAT activities (**Fig. 1**). Decreased CCA1 protein abundance throughout the day together with RVE8/LCL5 association to the *TOC1* promoter may lead to transcriptional activation by the recruitment of HAT activities (**Fig. 1**). In turn, HDAC activities contributing to *TOC1* declining phase might interfere with the RVE8/LCL5 function. Additional factors and different mechanisms of circadian gene regulation (X in **Fig. 1**) might account for *TOC1* declining phase until a rhythmic cycle starts again with CCA1 repressive binding. We suggest that regulated patterns of H3 acetylation by RVE/LCL-CCA1 and by HAT-HDAC activities might provide a fine-tune mechanism for accurate clock-controlled regulation of *TOC1* gene expression.

References

1. Wijnen H, Young MW. Interplay of circadian clocks and metabolic rhythms. Annu Rev Genet 2006; 40:409-48.
2. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, et al. Circadian rhythms from multiple oscillators: Lessons from diverse organisms. Nat Rev Genet 2005; 6:544-56.
3. Nakahata Y, Grimaldi B, Sahar S, Hirayama J, Sassone-Corsi P. Signaling to the circadian clock: plasticity by chromatin remodeling. Curr Opin Cell Biol 2007; 19:230-7.
4. Stratmann T, Mas P. Chromatin, photoperiod and the Arabidopsis circadian clock: a question of time. Semin Cell Dev Biol 2008; 19:554-9.
5. Perales M, Mas P. A functional link between rhythmic changes in chromatin structure and the Arabidopsis biological clock. Plant Cell 2007; 19:2111-23.
6. Farinas B, Mas P. Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. Plant J 2011; 2:318-29. DOI: 10.1111/j.1365-313X.2011.04484.x; PMID: 21205033.
7. Carré IA, Kim JY. MYB transcription factors in the Arabidopsis circadian clock. J Exp Bot 2002; 53:1551-7.
8. Andersson CR, Harmer SL, Schultz TF, Kay SA. The Reveille (REV) family of DNA binding proteins and the circadian clock. In: Abstracts of the 10th international conference on Arabidopsis research, Melbourne, 4–8 July 1999. Available at: http://arabidopsis.org/abstract_australia.pdf.
9. Schmied KC, Merkle T. A small family of LHY-CCA1-like (LCL) MYB1R transcription factors: potential co-regulators of the circadian oscillator. EMBL/GenBank/DDBJ databases 2005.
10. Wang ZY, Tobin EM. Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) gene disrupts circadian rhythms and suppresses its own expression. Cell 1998; 93:1207-17.
11. Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carré IA, et al. The late elongated hypocotyl mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. Cell 1998; 93:1219-29.
12. Matsushika A, Makino S, Kojima M, Mizuno T. Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: insight into the plant circadian clock. Plant Cell Physiol 2000; 41:1002-12.
13. Strayer C. Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. Science 2000; 289:768-71.