Time-dependent sequestration of RVE8 by LNK proteins shapes the diurnal oscillation of anthocyanin biosynthesis

Pablo Pérez-García[a], Yuan Ma[a], Marcelo J. Yanovsky[b], and Paloma Mas[a,1]

[a]Center for Research in Agricultural Genomics, Consejo Superior de Investigaciones Científicas (CRAG, CSIC), 08193 Bellaterra, Barcelona, Spain; and [b]Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires–Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina, C1405BWE Buenos Aires, Argentina

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Circadian clocks sustain 24-h rhythms in physiology and metabolism that are synchronized with the day/night cycle. In plants, the regulatory network responsible for the generation of rhythms has been broadly investigated over the past years. However, little is known about the intersecting pathways that link the environmental signals with rhythms in cellular metabolism. Here, we examine the role of the circadian components REVEILLE8/LHY-CCA1-LIKE5 (RVE8/LCL5) and NIGHT LIGHT–INDUCIBLE AND CLOCK-REGULATED genes (LNK) shaping the diurnal oscillation of the anthocyanin metabolic pathway. Around dawn, RVE8 up-regulates anthocyanin gene expression by directly associating to the promoters of a subset of anthocyanin biosynthetic genes. The up-regulation is overcome at midday by the repressing activity of LNK proteins, as inferred by the increased anthocyanin gene expression in *lnk1/lnk2* double mutant plants. Chromatin immunoprecipitation assays using LNK and RVE8 misexpressing plants show that RVE8 binding to target promoters is precluded in LNK overexpressing plants and conversely, binding is enhanced in the absence of functional LNKs, which provides a mechanism by which LNKs antagonize RVE8 function in the regulation of anthocyanin accumulation. Based on their previously described transcriptional coactivating function, our study defines a switch in the regulatory activity of RVE8–LNK interaction, from a synergic coactivating role of evening-expressed clock genes to a repressive antagonistic function modulating anthocyanin biosynthesis around midday.

circadian clock | anthocyanin accumulation | transcriptional regulation | protein–protein interaction | *Arabidopsis thaliana*

Circadian clocks are broadly present in nature and allow organisms to anticipate and prepare for the predictable changes that occur during the day/night cycles (1). Synchronization by the environmental signals ensures proper coordination of metabolism and physiology in many organisms, including plants (2). In *Arabidopsis*, the molecular architecture depends on a complex regulatory network, in which the morning-expressed single Myb-like transcription factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) (3) and LATE ELONGATED HYPOCOTYL (LHY) (4) repress the expression (5) of the evening-phased pseudoresponse regulator, *TIMING OF CAB EXPRESSION 1* (*TOC1*/*PRR1*) (6, 7). TOC1 in turn represses *CCA1* and *LHY* (8, 9) as well as the other members of the PRR family (*PRR9*, *7*, and *5*) (10) that in turn act as repressors of *CCA1* and *LHY* expression (11). TOC1 also represses *LUX ARRHYTHMO* (*LUX*) and *EARLY FLOWERING 4* (*ELF4*) (9), whose protein products interact with EARLY FLOWERING 3 (ELF3) to form the so-called Evening Complex (EC) (12).

Chromatin changes at the promoters of the core oscillator genes also play an important role modulating clock gene expression and function (13, 14). The single Myb-like transcription factor REVEILLE8/LHY-CCA1-LIKE5 (RVE8/LCL5) (15) antagonizes CCA1 repressing function in the regulation of Histone3 acetylation at *TOC1* promoter (16). RVE8 overexpression and mutation affect not only circadian gene expression but other clock-regulated processes such as hypocotyl elongation and the photoperiodic regulation of flowering time (15, 17). Analysis of loss-of-function mutants of *rve8* and its close homologs (*rve4*/*rve6*/*rve8* triple mutants) showed a significant lengthening of the circadian period most likely through the decreased expression of evening-phased clock genes (18). These results assigned an important activating function for the RVE protein family at the core of the clock.

Precise timekeeping by the clock also relies on the coordinated synchronization by environmental cues. The circadian transcriptional machinery integrates diurnal signals to keep track of time and of seasonal changes (19). Recent studies have identified the *NIGHT LIGHT–INDUCIBLE AND CLOCK-REGULATED* genes (*LNK*) as key circadian components with an important role in seasonal adjustment (20). The LNKs integrate environmental signals to control the expression of afternoon genes, allowing plants to perceive and respond to seasonal changes in day length and temperature (20–22). Two members of the LNK family (LNK1 and LNK2) interact with RVE8 and RVE4 and form a protein complex that is important for the transcriptional activation of the evening-phased clock genes, *PRR5* and *TOC1* (21).

Less information is available on the regulatory networks connecting the synchronizing signals and the core oscillator with the rhythmic biological processes or clock outputs. Classical studies and more recent systems biology approaches have provided clues about key plant processes regulated by the clock including metabolism, plant development, as well as abiotic and biotic

Significance

The circadian clock plays a crucial role controlling a wide variety of physiologic and metabolic processes in plants. However, the components and mechanisms involved in such regulation remain to be fully elucidated. Here we show that a protein complex composed of the clock components REVEILLE8/LHY-CCA1-LIKE5 (RVE8/LCL5) and the NIGHT LIGHT–INDUCIBLE AND CLOCK-REGULATED (LNK) proteins has a dual regulatory function. The complex acts as a repressor of the clock-controlled anthocyanin biosynthetic pathway at midday but it switches to an activating function in the control of evening-expressed clock genes. The interaction with LNKs impedes RVE8 binding to the promoters of the anthocyanin genes but not to the clock genes, which provides a mechanism by which LNKs antagonize RVE8 function to repress anthocyanin accumulation.

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[1]To whom correspondence should be addressed. Email: paloma.mas@cragenomica.es.

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stresses (23). A downstream response to stress conditions is the induction of genes involved in the synthesis of secondary metabolites such as flavonoids (24). Anthocyanins, the water-soluble pigments that are only present in plants, are the most ubiquitous type of flavonoids with a broad variety of functions ranging from attraction of insects for pollination and seed dispersal, protection from UV irradiation, or defense against pathogens (25). The anthocyanin biosynthesis pathway is composed of early biosynthetic genes (e.g., *CHS*, *CHI*, and *F3H*), which are common in the flavonoid pathway, and late biosynthetic genes (e.g., *DFR*, *LDOX*, and *UF3GT*), which are induced following the expression of the early biosynthetic genes (26).

Deciphering how and when oscillations in gene and protein expression engaged to coordinately regulate clock outputs is essential to fully understand plant circadian structure and organization. In our study, we have identified that the direct interaction of LNK proteins with RVE8 shapes the oscillating waveform of anthocyanin-related gene expression under light/dark cycles. As opposed to the coactivating function in the regulation of circadian gene expression, LNKs act as repressors of the expression of anthocyanin structural genes, a repressive role that counteracts RVE8-activating function. Our study thus unravels a dual sign for the regulatory activity of RVE8–LNK interaction, with opposing functions in the control of evening-expressed clock genes and in anthocyanin biosynthesis around midday.

Results and Discussion

Structural Genes Involved in Anthocyanin Biosynthesis Are Up-Regulated in Plants Overexpressing RVE8. To identify the transcriptional network controlled by RVE8, we compared the transcriptomic profiles of wild-type (WT) and RVE8-overexpressing plants (RVE8-ox) using genome-wide RNA sequencing (RNA-seq). To reduce the effects due to changes in the circadian phase by RVE8 overexpression, sampling was performed with plants grown under constant light and temperature conditions (without light or temperature entrainment). We found 1,074 differentially expressed genes with *RVE8* at the top most significantly different (Dataset S1). Functional categorization of the proteins encoded by the misregulated target genes revealed a wide variety of biological processes, including among others, signal transduction, response to stress, and developmental processes (*SI Appendix*, Fig. S1).

Inspection of the data also revealed that a number of up-regulated genes were highly coexpressed (*SI Appendix*, Fig. S1) and could be ascribed to the flavonoid biosynthetic pathway (*SI Appendix*, Figs. S1 and S2). The major genes comprising the anthocyanin biosynthetic pathway were up-regulated in RVE8-ox plants (Fig. 1 *A*–*C* and *SI Appendix*, Fig. S2). The expression of most of these genes is controlled by the clock, with a rhythmic oscillatory pattern peaking around dawn under constant light conditions (LL) (*SI Appendix*, Fig. S3) in a similar trend to that of *RVE8* (15, 17). Intriguingly, the peak phase of expression for the anthocyanin-related genes appears to change under light/dark (LD) cycles, and in some instances, the waveforms displayed a double peak around zeitgeber time 4 and 12 (ZT4 and ZT12) with a clear decrease around midday (*SI Appendix*, Fig. S3).

To verify the RNA-seq data, we performed a time course analysis under LD conditions (*SI Appendix*, *SI Materials and Methods*) to analyze the expression of the anthocyanin-related genes in WT and RVE8-ox plants. Our results showed that transcript abundance was significantly increased in RVE8-ox plants, particularly during daytime (Fig. 1 *D*–G), whereas no significant differences in gene expression were observed in WT and RVE8-ox during the night period. The decreased expression around ZT7 (*SI Appendix*, Fig. S3) was quite evident in RVE8-ox plants, suggesting a complex mechanism of regulation that is able to overcome the activating function of RVE8 overexpression around midday. The fact that nearly all of the structural genes comprising the anthocyanin pathway were up-regulated in RVE8-ox

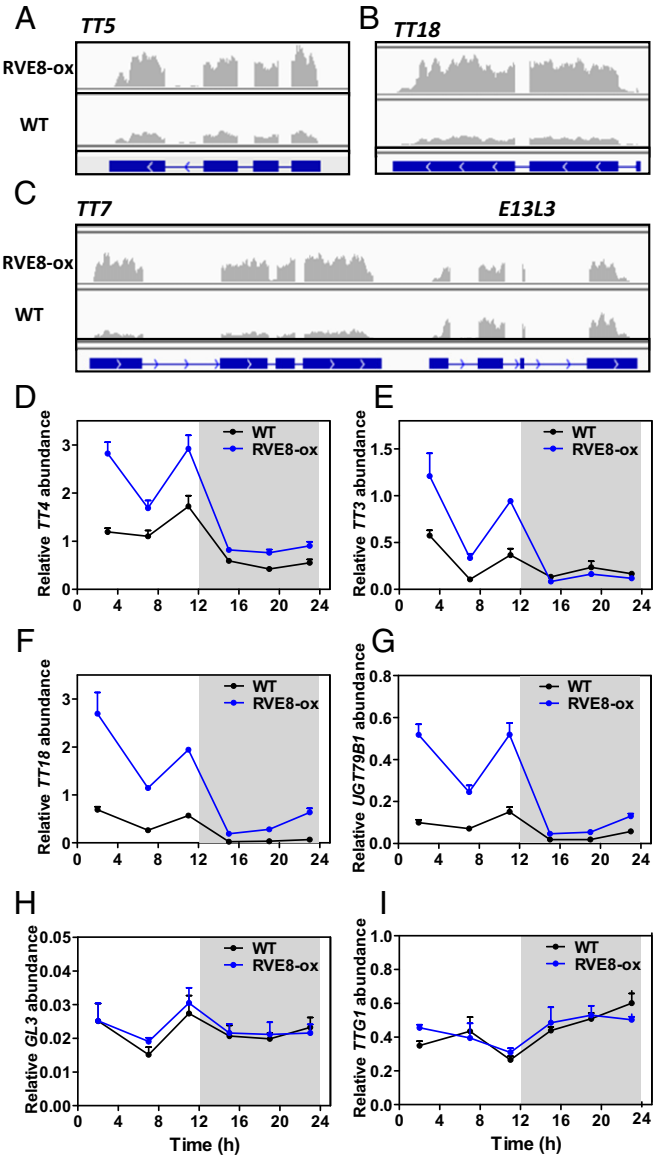

Fig. 1. Up-regulation of anthocyanin biosynthetic genes in RVE8-ox plants. (*A*–*C*) Visualization of RNA-seq reads by using the Integrative Genomics Viewer (IGV) browser for the indicated anthocyanin biosynthetic loci. As a control, RNA-seq data from the *E13L3* locus (*GLUCAN ENDO-1,3-BETA-GLUCOSIDASE-LIKE PROTEIN 3*) just downstream of *TT7* is shown in *C*. Time course analysis by RT-QPCR of *TT4* (*D*), *TT3* (*E*), *TT18* (*F*), *UGT79B1* (*G*), *GL3* (*H*), and *TTG1* (*I*) in WT and RVE8-ox plants grown under LD cycles. mRNA abundance was normalized to *IPP2* (*ISOPENTENYL PYROPHOSPHATE:DIMETHYL-ALLYL PYROPHOSPHATE ISOMERASE*) expression. Values represent means + SEM. White, day; gray, night.

plants (*SI Appendix*, Fig. S2) suggests a specific role for RVE8 in the control of the anthocyanin biosynthetic pathway. The expression of other regulatory nonbiosynthetic anthocyanin genes was not significantly affected (Fig. 1 *H* and *I*) with the exception of *PAP1* and *TT8* (*SI Appendix*, Fig. S3). The regulation appears to be gated mostly during the day but it is not constant, as at midday, other factors and/or mechanisms are partially able to overcome the RVE8-mediated activating function of the anthocyanin pathway.

LNK Proteins Directly Interact with RVE8. To further dissect the molecular mechanism underlying RVE8 function, we performed a yeast two-hybrid screening to identify RVE8 interacting proteins.

The full-length coding sequence of *RVE8* was used as a bait to screen a random-primed *Arabidopsis thaliana* seedling cDNA library. Using a high confidence score (predicted biological score, PBS) (27), we identified three RVE8 interacting factors belonging to the LNK protein family (20) (*SI Appendix*, Fig. S4). Similar to the *RVE8* oscillation, the expression of *LNKs* rhythmically oscillate under LD cycles and under LL conditions with a peak close to dawn (20–22). Analysis of the *RVE8* coexpressed gene network uncovered the members of the *LNK* family as highly significant genes coexpressed with *RVE8* (*SI Appendix*, Fig. S5). The yeast two-hybrid screening is thus consistent with a previous report showing the rhythmic interaction of LNK1 and LNK2 with RVE8 and with RVE4 (21).

To further support the interactions and expand the studies to LNK3 and LNK4, we performed coimmunoprecipitation experiments with plants overexpressing RVE8 and LNK3 or LNK4 proteins. The results of our coimmunoprecipitation experiments revealed a clear interaction at ZT7 and a weaker interaction at ZT11 (Fig. 2*A*). No evident immunoprecipitation was observed at other time points examined (ZT2, ZT15, ZT19, and ZT23), which suggests that despite the constitutive overexpression, the interaction is timely controlled. A similar pattern was observed for LNK1 (*SI Appendix*, Fig. S4). Competition with endogenous LNK proteins is not likely responsible for the observed pattern of interaction, as no evident immunoprecipitation was observed at time points when the endogenous expression is very low (ZT15, ZT19, and ZT23). RVE8 and LNK protein abundance did not manifestly change at the different time points examined, suggesting that changes in protein stability are not driving the interaction. No bands with mobility close to that of the LNK proteins were observed when similar procedures were performed with WT plants or with samples similarly processed but without antibody, which confirmed the specificity of the interactions. In vitro studies using the proteins expressed in *Escherichia coli* also showed that the LNKs proteins were effectively immunoprecipitated with RVE8 (*SI Appendix*, Fig. S4), confirming that the proteins are also able to interact in vitro. The results are consistent with the yeast two-hybrid screening and suggest a direct, specific interaction between RVE8 and the LNK proteins in *Arabidopsis*.

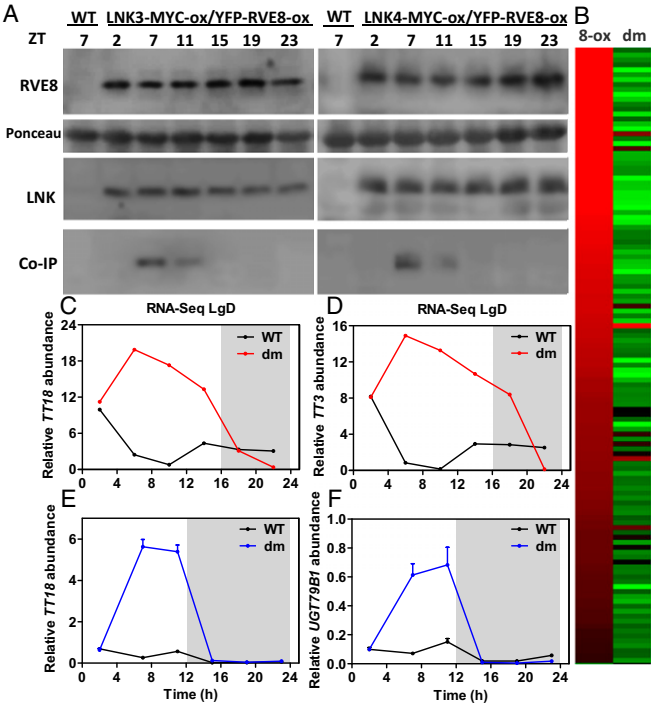

Fig. 2. LNK proteins interact with RVE8 and regulate anthocyanin gene expression. (*A*) Western blot analysis of LNK3-MYC-ox/YFP-RVE8-ox and LNK4-MYC-ox/YFP-RVE8-ox plants immunoprecipitated (Co-IP) with anti-GFP antibody following detection with anti-MYC antibody. Western blot analysis of RVE8 and LNK protein accumulation is shown above and below the Ponceau staining. Plants were grown under LD cycles and processed at the indicated ZTs. (*B*) Heatmap comparing the up-regulated transcripts in RVE8-ox (8-ox) and the corresponding expression in dm plants. Red indicates high expression and green, low expression. Time course analysis of *TT18* (*C*) and *TT3* (*D*) transcriptional profiles from RNA-seq data of dm plants under LgD (20). Time course analysis by RT-QPCR of *TT18* (*E*) and *UGT79B1* (*F*) in WT and dm plants grown under LD cycles. mRNA abundance was normalized to *IPP2* expression. Values represent means + SEM. White, day; gray, night.

The Anthocyanin-Related Target Genes of RVE8 Are Regulated by LNKs. We next interrogated previously published RNA-seq datasets of *lnk1/lnk2* double mutant (dm) plants (20). Comparisons of RVE8-ox and dm RNA-seq experiments under constant light and temperature conditions revealed that among the overlapping genes in both datasets (154), about 72% of the up-regulated genes in RVE8-ox plants were down-regulated in dm plants (Fig. 2*B* and *SI Appendix*, Fig. S6), whereas only about 9% of the overlapping genes up-regulated in RVE8-ox plants were also up-regulated in the dm. Similar low percentages were obtained when down-regulated genes in RVE8-ox RNA-seq dataset were compared with up- or down-regulated genes in dm plants (*SI Appendix*, Fig. S6). These results suggest that without light or temperature entrainment, RVE8 and LNKs might coactivate a subset of their target genes, as previously suggested (21).

When we focused on the up-regulated anthocyanin genes in the RVE8 RNA-seq dataset, we found that nearly all of them were significantly down-regulated in dm plants (*SI Appendix*, Fig. S7). Intriguingly, RNA-seq analysis with plants grown under long-day (LgD) conditions (20) showed that many of the anthocyanin-related genes were not down-regulated but highly up-regulated in the dm plants (Dataset S2 and Fig. 2 *C* and *D*). RT-QPCR analysis of dm plants grown under LD cycles (12-h light:12-h darkness) confirmed a clear up-regulation particularly during the day (Fig. 2 *E* and *F*). These intriguing results are consistent with the observed different waveforms of the anthocyanin genes under LD and LL cycles (*SI Appendix*, Fig. S3) and suggest that timing by the clock and/or the external environmental conditions are important for LNK function in the anthocyanin pathway.

Collectively, the data suggest that LNKs are responsible for the acute down-regulation of anthocyanin genes around midday, as judged by the dramatic up-regulation observed in dm plants (Fig. 2 *C*–*F*). The fact that under LD cycles, the anthocyanin biosynthetic genes were up-regulated in both RVE8-ox and dm plants also suggests that RVE8 might act as an activator, whereas LNK1 and LNK2 might be repressors of the anthocyanin biosynthetic pathway. A previous report has demonstrated that LNK1 and LNK2 together with RVE4 and RVE8 act as transcriptional coactivators in the regulation of circadian gene expression (21). Our results open the interesting possibility that under LD cycles, the role of LNK–RVE8 interaction in the control of anthocyanin regulation might be opposed to that exerted on circadian core gene expression.

The Phase-Specific Binding of RVE8 to the Promoters of Anthocyanin Biosynthetic Genes Is Antagonized by LNKs. RVE8 is able to directly bind to the promoters of its target circadian genes (15, 17, 21). Therefore, we next investigated whether RVE8 binds in vivo to the promoters of the anthocyanin biosynthetic genes. First, we performed chromatin immunoprecipitation (ChIP) assays with RVE8-ox plants and examined by QPCR the amplification of promoters. We found a significant enrichment of the *TT18*, *UGT79B1*, and *TT4* promoters and a lower amplification of other anthocyanin-related gene promoters (Fig. 3*A*). The binding appeared to be specific as we obtained lower amplification

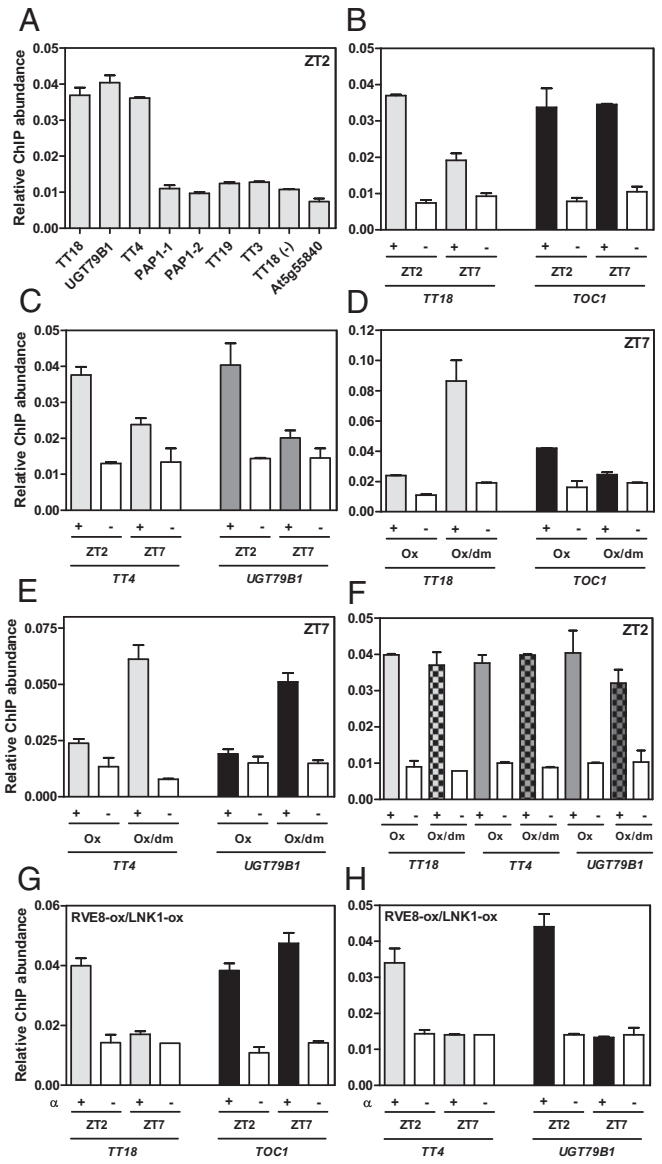

Fig. 3. RVE8 binding to the promoters of anthocyanin genes is antagonized by LNKs. (*A*) ChIP assays using RVE8-ox plants grown under LD cycles and sampled at ZT2. The promoters of the indicated genes were amplified by QPCR. Samples similarly processed but in the absence of antibody [*TT18(–)*] were used as control. The promoter of an unrelated gene (At5g55840) was used as a negative control. (*B* and *C*) ChIP-QPCR comparing RVE8 binding at ZT2 and ZT7 to the promoters of (*B*) *TT18* and *TOC1* and (*C*) *TT4* and *UGT79B1*. (*D* and *E*) Comparison of RVE8 binding by ChIP-QPCR using RVE8-ox (Ox) and RVE8-ox/*dm* (Ox/dm) plants sampled at ZT7. The promoters of (*D*) *TT18* and *TOC1* and (*E*) *TT4* and *UGT79B1* were amplified. (*F*) ChIP-QPCR using RVE8-ox (Ox) and RVE8-ox/*dm* (Ox/dm) plants sampled at ZT2. (*G* and *H*) Comparison of RVE8 binding by ChIP-QPCR assays using RVE8-ox/LNK1-ox plants sampled at ZT2 and ZT7. The promoters of (*G*) *TT18* and *TOC1*, and (*H*) *TT4* and *UGT79B1* were amplified. Samples were also similarly processed but in the absence of antibody (–). Values are represented as means + SEM.

when samples were similarly processed but without antibody or when a promoter of an unrelated gene was used as a negative control (Fig. 3*A*). Our results also revealed that the declining mRNA accumulation from ZT2 to ZT7 (Fig. 1) was accompanied by a concomitant decrease in RVE8 binding to the promoters of the *TT18*, *UGT79B1*, and *TT4* genes (Fig. 3 *B* and *C*). Remarkably, the decreased binding at ZT7 was specific for the anthocyanin-related genes and not for other previously described RVE8 circadian targets such as *TOC1* (Fig. 3*B*) or *PRR5* (*SI Appendix*, Fig. S8). ChIP analysis at ZT11 also revealed the absence or very reduced RVE8 binding to the anthocyanin-related gene promoters but not to the *TOC1* promoter (*SI Appendix*, Fig. S8). These results suggest a different mechanism in the regulation of anthocyanin-related genes and the evening-expressed clock genes.

We next examined whether RVE8 binding was altered in plants misexpressing LNKs. First, we compared binding in RVE8-ox and in RVE8-ox/dm plants using sets of lines that expressed comparable amounts of RVE8 (*SI Appendix*, Fig. S8). We found that RVE8 binding to the anthocyanin gene promoters was significantly enriched in the absence of functional LNK1 and LNK2 (Fig. 3 *D* and *E*), whereas the opposite effect was observed for binding to the *TOC1* promoter (Fig. 3*D*). These results are in agreement with data showing that RVE8, LNK1, and LNK2 act together as cotranscriptional activators of *PRR5* and *TOC1* expression. The results are also in line with the notion that anthocyanin and circadian gene expression are oppositely modulated by the RVE8–LNK interaction. Notably, the increased RVE8 binding in RVE8-ox/dm plants was phase specific, as no significant differences in binding were observed when the ChIP assays were performed at ZT2 (Fig. 3*F*). Therefore, the phase-specific interference of LNKs on RVE8 binding might be responsible for the decreased anthocyanin gene expression around midday. If our conclusions are correct, then RVE8 binding should be affected by LNK overexpression. Indeed, ChIP analysis with RVE8-ox and double RVE8-ox/LNK1-ox plants showed that RVE8 binding was abolished in the double overexpressing lines, specifically at ZT7 but not at ZT2 (Fig. 3 *G* and *H*). However, again, the effect was not observed at the *TOC1* and *PRR5* promoters (Fig. 3*G* and *SI Appendix*, Fig. S8). ChIP analysis of RVE8-ox/LNK3-ox plants rendered similar results (*SI Appendix*, Fig. S8).

RVE8–LNK Regulation of Anthocyanin Accumulation. To dissect the physiological relevance of RVE8–LNK interaction, we measured anthocyanin content in the different genetic backgrounds. As shown in Fig. 4*A*, increased anthocyanin accumulation was observed in RVE8-ox plants, whereas the anthocyanin content was even higher

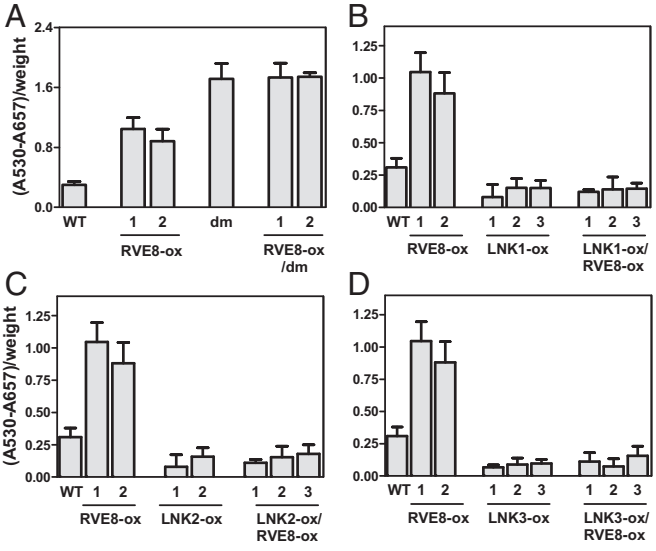

Fig. 4. RVE8 and LNKs antagonistically regulate anthocyanin accumulation. (*A*) Anthocyanin content in WT, dm, in two different lines of RVE8-ox and RVE8-ox/dm. Anthocyanin content in (*B*) single LNK1-ox and double overexpressing (RVE8-ox/LNK1-ox) plants, (*C*) single LNK2-ox and double overexpressing (RVE8-ox/LNK2-ox) plants, and (*D*) single LNK3-ox and double overexpressing (RVE8-ox/LNK3-ox) plants.

in dm plants. The RVE8-ox phenotypes were not due to decreased *LNK* gene expression in RVE8-ox plants (*SI Appendix*, Fig. S9). These results are consistent with the transcriptional changes observed in these plants and with the positive role for RVE8 and the negative function of LNK1 and LNK2 in the control of the anthocyanin pathway. Our studies also showed an increased accumulation of anthocyanin in RVE8-ox/dm compared with RVE8-ox (Fig. 4*A*). The anthocyanin content correlated with the up-regulation of the anthocyanin-related genes, particularly around the mid-to-late day (*SI Appendix*, Fig. S10). We next reasoned that anthocyanin content in double overexpressing plants should revert the RVE8-ox phenotype. Indeed, single LNK and double RVE8–LNK overexpression led to a significant reduction in anthocyanin content (Fig. 4 *B*–*D* and *SI Appendix*, Fig. S8). Consistently, analysis of LNK-ox/RVE8-ox plants revealed a down-regulation of anthocyanin gene expression, particularly evident around ZT7 (*SI Appendix*, Fig. S10). Comparisons of anthocyanin gene expression in LNK-ox plants in the presence or absence of RVE8-ox showed that overexpression of RVE8 in LNK-ox plants led to increased expression particularly around ZT2, although the overall expression was still lower than in WT plants (*SI Appendix*, Fig. S10). Together, the results are consistent with an activating function of RVE8 by direct binding to the promoters on anthocyanin genes that is antagonized by the repressing activity of LNKs on anthocyanin accumulation. Different factors and mechanisms might be involved in the regulation of a clock output such as anthocyanin accumulation versus regulation of core clock gene expression. The different factors might influence the regulatory activity of RVE8 and LNKs. The fact that activation of *TOC1* by RVE8 occurs later during the day compared with the earlier activation of anthocyanin gene expression by RVE8 might be responsible for a timely regulated set of different activities. The results showing that overexpression of LNK1 and LNK2 does not render a circadian phenotype (21) but leads to anthocyanin phenotypes also highlight fundamental differences in the regulatory functions.

Gene expression analysis under different photoperiodic conditions also provided some clues about the physiological relevance of RVE8–LNK interaction (*SI Appendix*, Fig. S11). Indeed, a recurrent pattern was observed consisting of (*i*) a clear up-regulation and a peak of expression about 4 h after dawn that is facilitated by RVE8 activating function; (*ii*) a down-regulation around midday, favored by LNK repressing activity that is followed by a second peak of expression under longer photoperiods; and (*iii*) a subsequent declining phase that coincides in all cases with the dark period. Notably, the down-regulation was completely abolished under LL conditions, which demonstrates the inductive role of light during the night period. Based on our results, we envision a complex scenario in which anthocyanin content is modulated by the phase-dependent interaction of RVE8 (and most likely other RVEs) with LNKs. The interaction defines the timing of RVE8 binding to the promoters of the anthocyanin structural genes; and thus in consonance with the photoperiodic conditions, plants might precisely control anthocyanin accumulation.

Materials and Methods

A. thaliana seeds (Columbia ecotype) were stratified at 4 °C in the dark for 3 d on Murashige and Skoog (MS) agar medium supplemented with 3% (wt/vol) sucrose. Unless otherwise indicated, seedlings were grown under LD conditions (12-h light:12-h dark) with 60–100 μmol $m^{-2}{\cdot}s^{-1}$ of cool white fluorescent light at 22 °C. Further detailed information is presented in *SI Appendix*, *SI Materials and Methods*.

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