ISSN : 1225-9918
Journal of Life Science **2012** Vol. 22. No. 8. 999~1008 DOI : http://dx.doi.org/10.5352/JLS.2012.22.8.999

Regulation of *Arabidopsis* Circadian Clock by De-Etiolated 1 (DET1) Possibly via Histone 3 Acetylation (H3Ac)

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Received June 7, 2012 / Revised July 4, 2012 / Accepted July 11, 2012

The circadian clock is a self-sustaining 24-hour timekeeper that allows organisms to anticipate daily-changing environmental time cues. Circadian clock genes are regulated by a transcriptional-translational feedback loop. In *Arabidopsis*, *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) transcripts are highly expressed in the morning. Translated LHY and CCA1 proteins repress the expression of the *TIMING OF CAB EXPRESSION 1* (*TOC1*) transcripts, which peaks in the evening. The TOC1 protein elevates the expression of the *LHY* and *CCA1* transcripts, forming a negative feedback loop that is believed to constitute the oscillatory mechanism of the clock. In mammals, the transcription factor protein CLOCK, which is a central component of the circadian clock, was reported to have an intrinsic histone acetyltransferase (HAT) activity, suggesting that histone acetylation is important for core clock mechanisms. However, little is known about the components necessary for the histone acetylation of the *Arabidopsis* clock-related genes. Here, I report that DET1 (De-Etiolated1) functions as a negative regulator of a key component of the *Arabidopsis* circadian clock gene *LHY* in constant dark phases (DD) and is required for the down-regulation of *LHY* expression through the acetylation of histone 3 (H3Ac). However, the HATs directly responsible for the acetylation of H3 within *LHY* chromatin need to be identified, and a link connecting the HATs and DET1 protein is still absent.

Key words : *Arabidopsis*, circadian clock, *De-Etiolated1* (*DET1*), histone acetylations, *Late Elongated Hypocotyl* (*LHY*)

Introduction

Circadian rhythm, which is controlled by biological circadian clocks, is a rhythmic variation within 24 hours. It is found in diverse organisms ranging from prokaryotes to humans, and allows the organisms to coordinate with daily light and temperature changes. In animals and plants, circadian clocks regulate diverse physiological processes such as human sleep/wake cycles, *Neurospora* sporulation, plant flowering, and robust rhythmic gene expression [10,13, 14,21,27]. Functional circadian clocks are known to confer enhanced fitness and, therefore, allow organisms to be more adaptive [11,43]. According to experimental observations and mathematical modeling, circadian clocks are based on interlocking negative feedback loops of transcriptional activators and repressors [23,38,44]. In *Arabidopsis*, the central loop operates through the reciprocal regulation between the two morning-phased, partially redundant MYB transcription factors, LHY and CCA1, and the evening-phased pseudo-response regulator TOC1 [18,24]. LHY and CCA1 are the transcriptional repressors in the loop that bind to the *TOC1* promoter and negatively regulate its transcription [1,36,39]. On the other hand, TOC1 indirectly promotes the expression of *LHY* and *CCA1* partly through the CCA1-binding transcription factor, CCA1 HIKING EXPEDITION (CHE) [1,33]. Such daily interactions between transcriptional activators and inhibitors of the clockwork and their corresponding cis regulatory elements on target genes can achieve phase control over gene expression rhythms. More than 10% of the *Arabidopsis* transcriptome showed rhythms of 24 hr period [10,18], suggesting that a large portion of the genome is either directly or indirectly controlled by the circadian clock.

The *det1* mutant was first identified as a mutant that developed as a light-grown plant in the dark [9]. The *det1* mutant showed constitutive expression of light-regulated genes, suggesting that the wild-type *DET1* gene may function as a negative regulator of light-induced gene expression [30]. The *det1-1* mutant exhibited short periods of *Cab:luc* expression in both LL and DD [26]. Therefore, the *DET1* gene was proposed to mediate the transduction of light signals to the circadian clock. The *DET1* gene encodes a 541 amino

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acid protein containing two regions that are similar to known bipartite nuclear localization signals [30]. Analysis of localization of a GUS-DET1 fusion protein in *Arabidopsis* leaf protoplasts showed that DET1 was targeted to the nucleus. DET1 is known to be part of a nuclear localized complex of approximately 350 kDa, suggesting that DET1 may not act alone [37]. The *Arabidopsis* CDD complex consists of COP10 (Constitutive Photomorphogenic10), DET1, and DDB1 (Damaged DNA Binging1), and a similar complex is also present in humans [32,37,42]. CDD can associate with CULLIN4 (CUL4) to form a CUL4-based E3 ligase [7,32]. Further, DET1 may be involved in the function of some CUL4-based ligases [6,32]. Besides the role of DET1 in the ubiquitin system, DET1 is known to be involved in chromatin regulation. DET1 associates with the histone gene cluster in *Drosophila* embryos and has been shown to interact with the nonacetylated tail of histone H2B in plants [3,4]. However, the significance of DET1's role in transcriptional regulation remains to be explored. In this study, DET1 is required for proper regulation of circadian period rhythms, and for repression of the core clock component gene expression, *LHY*, in DD possibly through modification of H3Ac.

Materials and Methods

Plant materials and growth conditions

The *det1-1* mutant (ecotype Columbia, Col) was obtained from Dr. Joanne Chory. Transgenic lines carrying *LHY:luc* reporter constructs have been described elsewhere [35]. The *LHY:luc* transgene was introduced into the *det1-1* mutant background by genetic crossing. Homozygous F3 plants showing both dwarf morphology and luciferase activity were selected for further analyses. Seeds were sown on Murashige and Skoog (MS)-agar medium supplemented with 1% sucrose, and refrigerated for at least 3-4 days before growing at 22°C under 70-100 μE m^{-2} s^{-1} cool white fluorescent light. Photoperiod durations (12L12D, LL or DD) were programmed according to the purpose of the experiments.

ChIP assays

ChIP was performed as described by [17] using 14- to 16-day-old seedlings. Seedlings were vacuum infiltrated with 1% formaldehyde for cross-linking and ground in liquid nitrogen after quenching the cross-linking process. Chromatin was isolated and sonicated into ~0.5 to 1 kb fragments. H3Ac and H3K9Ac-specific antibodies (MILLIPORE) were added to the chromatin solution, which had been precleared with salmon sperm DNA/ProteinA agarose beads (MILLIPORE). After subsequent incubation with salmon sperm DNA/Protein A agarose beads, immunocomplexes were precipitated and eluted from the beads. The cross-links were reversed, and residual proteins in the immunocomplexes were removed by incubation with proteinase K, followed by phenol/chloroform extraction. DNA was recovered by ethanol precipitation. The amount of immunoprecipitated chromatin was determined by PCR. The sequences of the primer pairs used for each PCR reaction were listed in Table 1.

RT-PCR and qPCR analyses

Total RNA was isolated from seedlings using TRI Reagent (Molecular Research Center, INC.), according to the manufacturer's instructions. Reverse transcription was performed with M-MuLV Reverse Transcriptase (Fermentas) and an oligo-dT primer according to the manufacturer's instructions using 3μg of total RNA isolated as described above. Semi-quantitative PCR was performed using first strand DNA with i-Taq DNA polymerase (iNtRON Biotechnology Inc., Seoul, Korea) and the primer pairs listed in Table 2. qPCR was performed in 96-well blocks with an Applied Biosystems (Foster City, CA) 7300 real-time PCR system using the SYBR Green I master mix (Bio-Rad, Hercules, CA) in a volume of 20μl. The reactions were performed in triplicate for each run. The comparative $_{\Delta\Delta}$CT method was used to evaluate the relative quantities of each amplified product in the samples. The threshold cycle (Ct) was automatically determined for each reaction by the system set with default parameters. The specificity of the PCR was determined by melt curve analysis of the amplified products using the standard method installed in the system.

Luminescence assay by photon-counting cameras

At least 2 weeks before imaging, seeds were sown on MS medium containing 1% sucrose. Plates were sealed with foil and stored at 4°C for 4-5 days then plants were grown in 12L12D conditions for at least 7 days. The day before starting imaging, plants were pre-sprayed with the luciferase substrate, luciferin (Promega, USA) at a 5mM concentration in 0.01% Triton X-100. Pre-sprays were repeated every 8 hours over a 24-hour period to remove luciferase activity accumu-

Table 1. The primers used in ChIP analyses

Genes	Primer names	Sequences
LHY	LP1_F	5′-GTGGCTGAGATTGCTTCTGG-3′
	LP1_R	5′-CTTGAGAGTAGCCATGGAGG-3′
	LX1_F	5′-CCGGTCCTGTTATGGATAC-3′
	LX1_R	5′-TCAGTCCATCGCTCTCGC-3′
	LX4_F	5′-TAGC AGGTAAGTGGCGAC-3′
	LX4_R	5′-GAGATACCATACCTGAGGG-3′
	L3U_F	5′-CTACATGACAGACTTGGAGG-3′
	L3U_R	5′-GTACAGAACCTGACATGACC-3′
CCA1	CP1_F	5′-CAGGTAGTCCCGGAACTCGTGG-3′
	CP1_R	5′-CGGAAATGGAGAAATCTCAGCC-3′
	CX1_F	5′-GGGGTCATGGTCTTTCTTATTG-3′
	CX1_R	5′-GTCGCAAATATGATGGACGC-3′
	CX6_F	5′-GGGAAGTCAGAATAACAGGG-3′
	CX6_R	5′-GGAAACGACTGATAATCTCCTGC-3′
	C3U_F	5′-CGGATGCGGTTGGAAACTC-3′
	C3U_R	5′-CAAG AGCCCCTTGAGTGAAG-3′
TOC1	TP2_F	5′-AGGGGATAAATTAGGCGAC-3′
	TP2_R	5′-C CATCTCCTCCTTTACACTC-3′
	TX1_F	5′-GAACGGT GAGTGTAAAGG-3′
	TX1_R	5′-AAGCCAGACGACAAGAACC-3′
	TX5_F	5′-GCTGGTAGTGGTCTGTTGC-3′
	TX5_R	5′-CAAAGAGAAACAATCACCTGG-3′
	T3U_F	5′-TACACCAAGAACTGAAAACCG-3′
	T3U-R	5′-CCAATGAGAGCTTTTCAATGC-3′
Actin1	Actin1_F	5′-CGTTTCGCTTTCCT TAGTGTTAGCT-3′
	Actin1_R	5′-AGCGAACGGATCTAGAGACTCACCTTG-3′

Table 2. The primers used in RT-PCR and qPCR analyses

Genes	Primer names	Sequences
LHY	LHY_F	5′-CAAAGCAGCGAGAGCGATGG-3′
	LHY_R	5′-GCGTGCCCGTGAGTTTCTTC-3′
TOC1	TOC1_F	5′-GGATTTGAACGGTGAGTG-3′
	TOC1_R	5′-CACTTGAAACTTCTCCGCC-3′
UBQ	UBQ_F	5′-GATCTTTGCCGGAAAACAATTGGAGGATGGT-3′
	UBQ_R	5′-CGACTTGTCATTAGAAAGAAAGAGATAACAGG-3′

lated prior to the first luciferin treatment [25]. Luminescence was imaged for 30 minutes every 2 hours for 3 days using photon-counting cameras (Hamamatsu). The luminescence levels were quantified from the images using MetamorphTM software (Universal Imaging Corp.).

Results

Expression of *LHY*, *CCA1* and *TOC1* in WT and *det1-1* seedlings under 12L12D

To test the effect of *det1* mutation on *LHY* expression, the *LHY:luc* reporter gene was introduced into the *det1-1* mutant by genetic crossing. Expression of homozygous *LHY:luc* was assayed in wild-type (WT) and *det1-1* plants under 12L12D (12 hours of light/12 hours of dark phases). In the *det1-1* mutant, the peaks of *LHY:luc* expression were identical to those of WT plants (Fig. 1A). However, luminescence began to increase four hours early (Fig. 1A). Luminescence in WT seedlings began to increase at ZT 16 (zeitgeber; 16hours after lights-on) while luminescence in *det1-1* mutant began to increase at ZT12, suggesting that the *det1* mutation may alter transcription or translation of the luciferase transgene. Expression of *LHY* transcripts was also observed by RT-PCR to test if *LHY:luc* reflects well the level of *LHY* mRNAs. WT

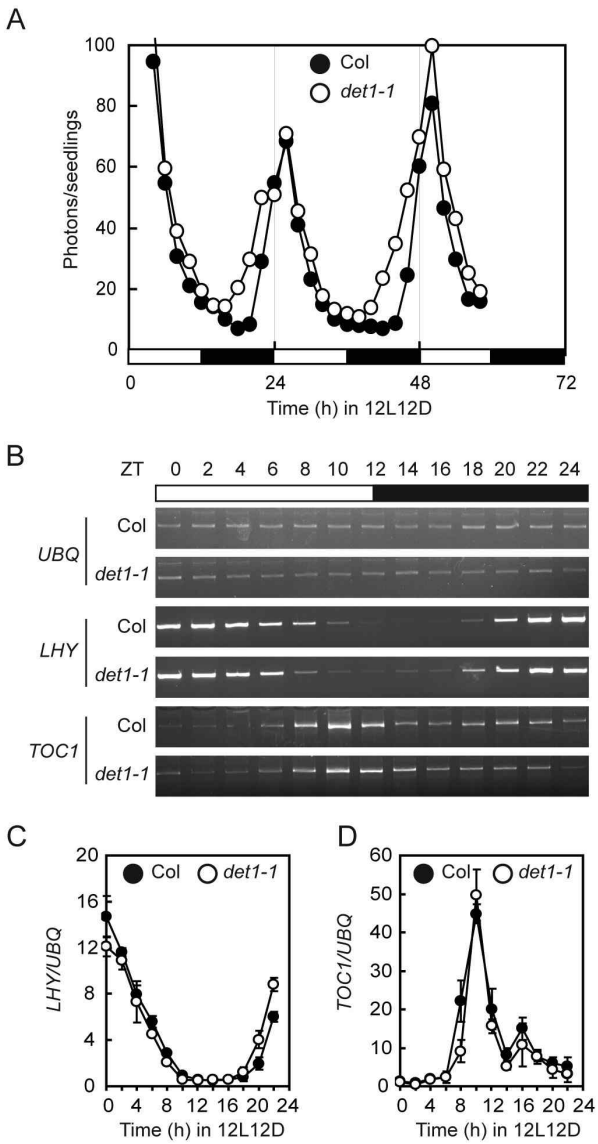

Fig. 1. Expression of *LHY* and *TOC1* transcripts in wild-type Col and *det1-1* seedlings under 12L12D. (A) Expression of *LHY:luc* in wild-type Col and *det1-1* seedlings in 12L12D. Col and *det1-1* plants carrying *LHY:luc* reporter construct were grown under 12L12D cycles for 7 days prior to imaging luminescence. White and black bars indicate light and dark phases, respectively. Data points represent average luminescence levels from 24 to 30 individual seedlings. (B) Expression of *CCA1* and *TOC1* transcripts in Col and *det1-1* seedlings grown in 12L12D. Col and *det1-1* seedlings were grown under 12L12D for 7 days, harvested at every 2 hours for one day and used for RT-PCR analyses. *UBQ* was included as an expression control. (C, D) qPCR analysis of *LHY* (C) and *TOC1* (D) transcripts. The same RNAs as used in Figure 1B were evaluated. *UBQ* was used as an internal control. Expression of *LHY* and *TOC1* transcripts was normalized by *UBQ*. Error bars represent SD (Standard Deviation) of three technical replicates.

and *det1-1* seedlings were grown and harvested at every 2 hours in 12L12D for RT-PCR. The level of *LHY* in both Wt and *det1-1* plants oscillated peaking at ZT0 and 24 (Fig. 1B and 1C). However, such early increase found in luminescence assays was not detected by RT-PCR and q-PCR analyses (Fig. 1B and 1C). Level of the evening expressing gene, *TOC1*, showed a peak at late afternoon, ZT10-12, showing no changes in both WT and *det1-1* transcript levels (Fig. 1B and 1D).

As one of the key factors in the photomorphogenesis pathway, DET1 has been shown to negatively regulate gene expression in response to light signals [22]. Furthermore, other studies suggested that DET1 might recruit HATs through binding to DDB1 and cause chromatin remodeling [3,37]. To test if the rhythmic oscillation of *LHY*, *CCA1*, and *TOC1* correlates with changes in chromatin structure, ChIP assays were performed. WT and *det1-1* seedlings were grown in 12L12D, and harvested at ZT12 and ZT20 in which the level of *LHY* transcripts between WT and *det1-1* was greatly distinguishable. ChIP assays were performed with antibody specific to H3Ac and the enrichments of *LHY*, *CCA1* and *TOC1* loci were measured by using diverse sets of primers (Fig. 2A). H3Ac levels in LP1 and LX1 but not LX4 and L3U regions of *LHY* were higher at ZT20 than at ZT12 in both WT and *det1-1* (Fig. 2B and 2C). The change for H3Ac at *LHY* was similarly observed at the *CCA1* locus (Fig. 2B and 2D). In the case of *TOC1*, H3Ac levels of both WT and *det1-1* were higher at ZT12 than at ZT20 in TP2 and TX1 regions, whereas the differences were minor or undetectable in TX5 and T3U regions (Fig. 2B and 2E). Taken together, these results indicate that the transcription activity of all *Arabidopsis* central clock genes, *LHY*, *CCA1*, and *TOC1*, in 12L12D is positively correlated with the levels of H3Ac.

Expression of *LHY*, *CCA1* and *TOC1* in WT and *det1-1* seedlings under constant conditions

An important property of circadian rhythms is their ability to persist in the absence of initial input signals. This property reflects regulation by an endogenous and self-sustaining oscillator, the circadian clock. Expression of *LHY* has been known to keep oscillating in constant light (LL) and constant dark (DD) conditions [29,40,41]. To assay the effect of *DET1* on *LHY* expression in long intervals of light or dark treatments, WT and *det1-1* plants carrying the *LHY:luc* reporter gene were grown under 12L12D, then released to constant light (LL) and constant dark (DD). The luminescence of these

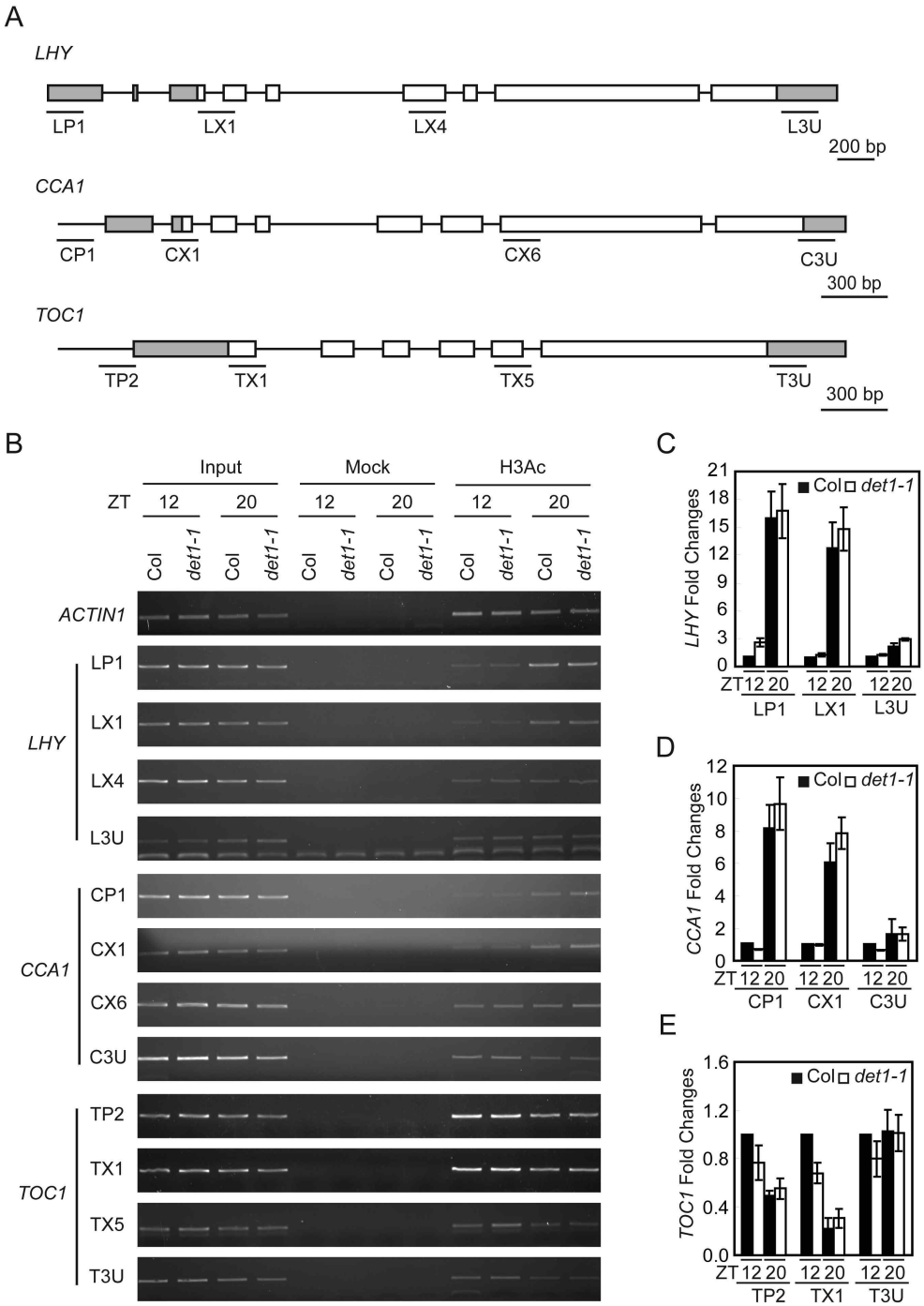

Fig. 2. Histone modifications of oscillator components, *LHY*, *CCA1* and *TOC1* in Col and *det1-1* plants grown in 12L12D by H3Ac. (A) Schematic representations of *LHY*, *CCA1* and *TOC1* genomic regions. The gray boxes in the front indicate exonic 5′UTRs, and the rear gray box represents the 3′UTR. Labeled lines indicate regions amplified by primers (Materials and Methods) during the ChIP experiments. (B) ChIP assay of *LHY*, *CCA1* and *TOC1* chromatin with antibody against H3Ac. Col and *det1-1* seedlings were grown under 12L12D conditions for 10days. Plants were harvested at ZT 12 and ZT 20 in 12L12D and used for ChIP analyses. *Actin1* was included as an expression control. "Input" indicates chromatin before immunoprecipitation. "Mock" refers to control samples lacking antibody. *Actin1* was used as an internal control. (C-E) Relative levels of H3Ac in *LHY* (C), *CCA1* (D), and *TOC1* (E) chromatins. The same immune precipitated elutes used in Figure 2B were evaluated by qPCR analysis. The wt Col levels at ZT 12 were set to 1 after normalization by input. Error bars represent SD of three technical replicates.

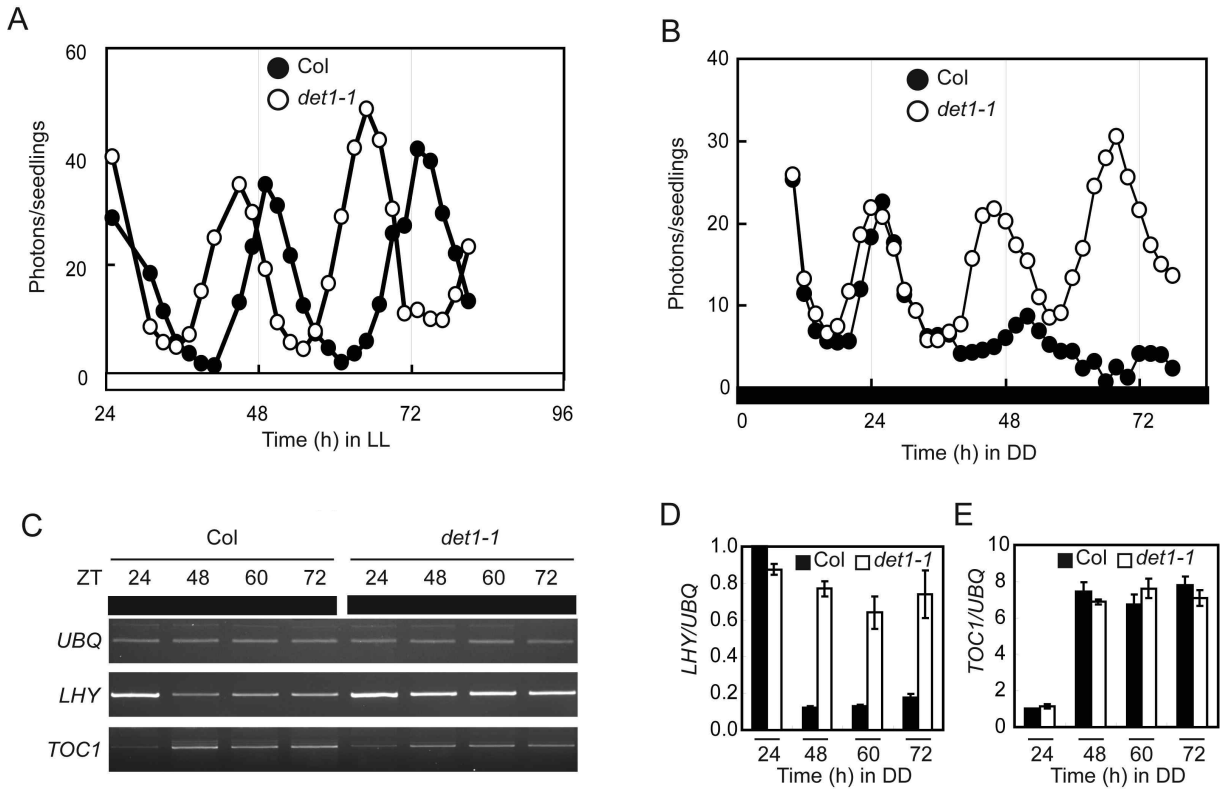

Fig. 3. Expression of *LHY* and *TOC1* transcripts in wild-type Col and *det1-1* seedlings under constant conditions. (A) Expression of *LHY:luc* in wild-type Col and *det1-1* seedlings in LL. Col and *det1-1* plants carrying *LHY:luc* reporter construct were grown under 12L12D cycles for 7 days and then transferred to LL at time zero (ZT 0). White bar indicates light phases. Data points represent average luminescence levels from 24 to 30 individual seedlings. (B) Expression of *LHY:luc* in wild-type Col and *det1-1* seedlings in LL. Col and *det1-1* plants carrying *LHY:luc* reporter construct were grown under 12L12D cycles for 7 days and then transferred to constant dark (DD) at ZT 0. Black bar indicates dark phases. Data points represent average luminescence levels from 24 to 30 individual seedlings. (C) Expression of *LHY* and *TOC1* transcripts in wild-type Col and *det1-1* seedlings grown in DD. Col and *det1-1* seedlings were grown under 12L12D conditions for 7 days, transferred to DD at ZT 0. Plants were harvested at ZT 24, ZT 48, ZT 60 and ZT 72, respectively, and used for RT-PCR analysis. *UBQ* was included as an expression control. (D, E) qPCR analysis of *LHY* (D) and *TOC1* (E) transcripts. The same RNAs used in Figure 3C were evaluated. *UBQ* was used as an expression control. The wt Col levels at ZT 24 were set to 1 after normalization by *UBQ*. Error bars represent SD of three technical replicates.

seedlings was imaged starting at subjective dawn (ZT0 or ZT24). In WT plants, *LHY:luc* expression oscillated with peaks at ZT24, 48 and 72 in LL while the *det1-1* mutant plants showed advancing rhythms with peaks at ZT 24, 38 and 58 (Figure 3A). However, differences in rhythms of WT and *det1-1* are more striking in DD conditions. In WT plants, *LHY:luc* expression oscillated and dampened after one or two cycles with peaks at ZT26, 52 and 76 in DD while the *det1* mutant plants showed much vigorous and advancing rhythms with peaks at ZT 24, 46 and 68 (Fig. 3B). To test whether *LHY:luc* reflects well the level of *LHY* mRNAs. WT and *det1-1* seedlings were grown in 12L12D, released to DD and then harvested at ZT24, 48, 60 and 72 for RT-PCR experiments. Like *LHY:luc* results (Fig. 3B), the levels of *LHY* transcripts were more highly expressed in *det1-1* than WT plants in DD (Fig. 3C and 3D). Little changes in *TOC1* expression between both Wt and *det1-1* were observed (Fig. 3C and 3D). Taken together, these results suggest that DET1 is required for proper regulation of clock function and for down-regulation of *LHY* transcripts in DD.

To elucidate whether high expression of *LHY* transcripts of *det1-1* in DD is correlated with high enrichment of H3Ac, ChIP assays were performed using the same tissues harvested at ZT 48 and 60 as used in Fig. 3C. H3Ac levels in LP1 and LX1 but not LX4 and L3U regions of *LHY* were higher in *det1-1* than in Wt at both ZT48 and ZT 60 (Fig.

4A and 4C). Higher enrichment of H3Ac in CP1, CX1 and CX6 but not C3U regions of *CCA1* were also observed in *det1-1* than in WT at ZT48 and ZT 60 (Fig. 4A and 4D). H3Ac levels in TX1 of *TOC1* locus were higher in *det1-1* than in Wt at ZT48 and ZT 60 whereas the differences were minor or undetectable in TP2, TX5 and T3U regions (Fig. 4A and 4E). These H3Ac results were consistent with the ChiP experiments with the antibody specific to H3K9Ac except that no differences in H3K9Ac levels at *TOC1* locus between Wt and *det1-1* were detected in all regions tested (Fig. 4B). These results imply that DET1 acts as a negative regulator of *LHY* expression through H3Ac.

Discussion

Light exerts a powerful effect on plant development,

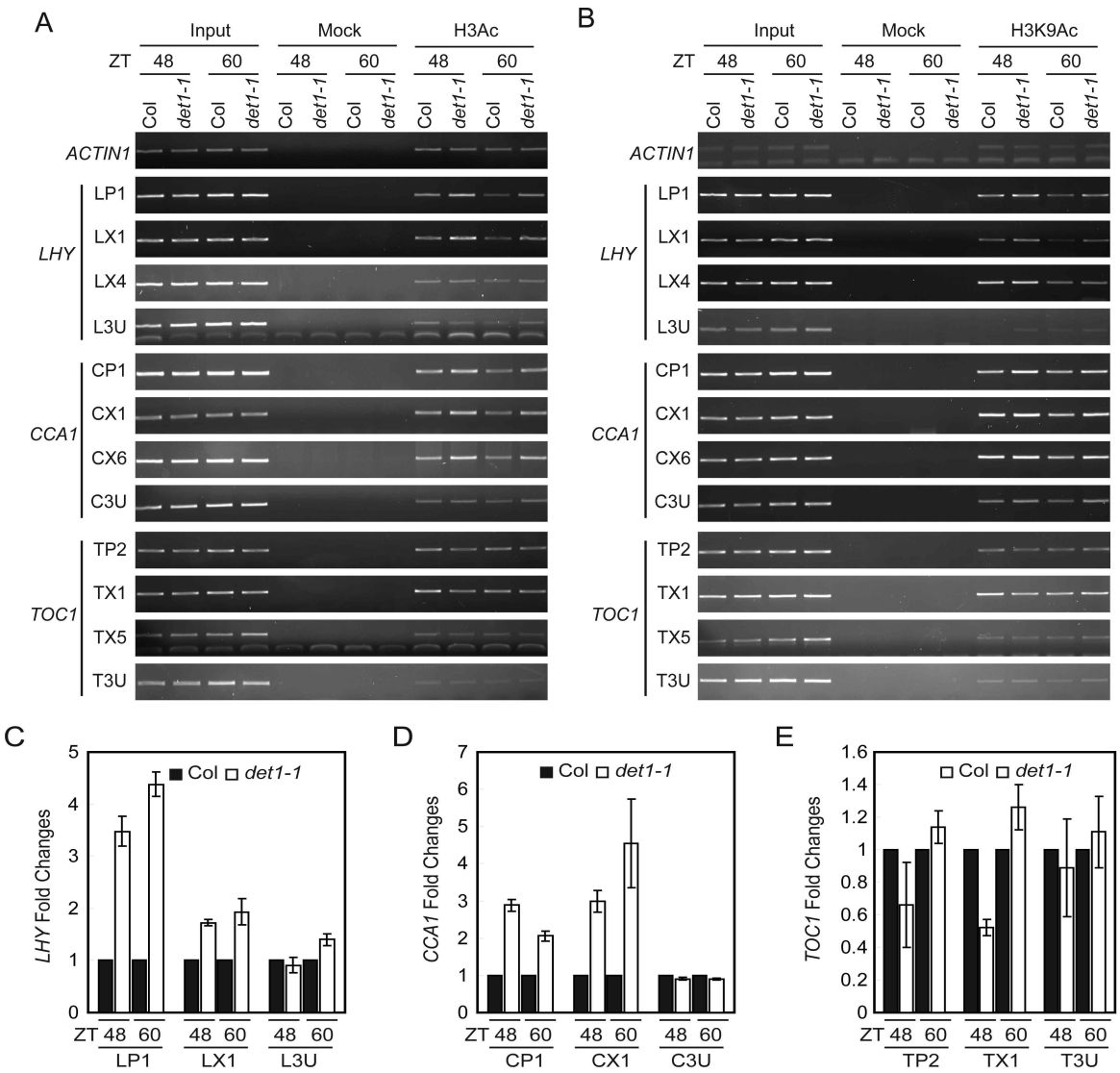

Fig. 4. Histone modifications of oscillator components, *LHY, CCA1* and *TOC1* in Col and *det1-1* plants grown in DD by H3Ac and H3K9Ac. (A) ChIP assay of *LHY, CCA1* and *TOC1* chromatin with H3Ac. Wild- type Col and *det1-1* seedlings were grown under 12L12D conditions for 7 days and transferred to DD at ZT 0. Plants were harvested at ZT 48 and ZT 60 in DD and used for ChIP analyses. *Actin1* was included as an expression control. (B) ChIP assay of *LHY, CCA1* and *TOC1* chromatin with H3K9Ac. Samples were identical as in Figure 4A but antibody against H3K9Ac was used for immunoprecipitation. (C-E) Relative levels of H3Ac in *LHY* (C), *CCA1* (D) and *TOC1* (E) chromatin by qPCR analysis. The same immune precipitated elutes used in Figure 4A were evaluated. The wt Col levels were set to 1 after normalization by input. Error bars represent SD of three technical replicates.

which is particularly striking during seedling development [22]. *Arabidopsis* seedlings grown in white light exhibit short hypocotyls, open cotyledons, and normal chloroplast development, while seedlings grown in the dark exhibit an etiolated phenotype, consisting of long hypocotyls, closed cotyledons, and lack of chloroplast development. Furthermore, the *det1* mutant showed constitutive expression of light-regulated genes, suggesting that the wild-type *DET1* gene may function as a negative regulator of light-induced gene expression [30]. Therefore, DET1 is thought to act as a repressor of photomorphogenesis in *Arabidopsis*. Similarly, expression of LHY in *det1-1* was constitutively elevated in DD conditions, implying that DET1 acts as a negative regulator for *LHY* expression. Under 12L12D, an early increase in *LHY:luc* expression in *det1-1* mutant was observed by luminescence assays, but not RT-PCR. Probably, luminescence assays are so sensitive that it is hard to detect an early increase in *LHY* transcripts through RT-PCR assays.

Recent studies have shown that the activation of a number of clock-regulated genes of diverse organisms including mammals, fly, and plants is coupled with changes in histone modifications such as acetylation [19,28,31,34]. These results indicate that despite of divergences in oscillator components, mechanisms by which the expression of oscillator genes regulated through chromatin modification are commonly applied to the circadian systems of mammals, fly, and plants. In *Neurospora*, an ATP-dependent chromatin-remodeling enzyme CLOCKSWITCH (CSW) was shown to be required for clock function [2]. CSW binds to the promoter of a central clock gene *FREQUENCY* (*FRQ*) and regulates *FRQ* expression through controlling the chromatin structure of its promoter. In mammals, the three key clock genes, *PERIOD 1* (*PER1*), *PER2*, and *PER3*, exhibit circadian rhythms in H3Ac, and the histone acetyltransferase p300 precipitates together with CLOCK protein in this process in a time-dependent manner [15]. The transcription factor protein CLOCK, which is a central component of the circadian clock, was reported to have an intrinsic HAT activity, suggesting that histone acetylation is important for core clock mechanisms [12]. Although most studies have focused on histone acetylation in the circadian system, histone methylation has also been suggested to be crucial for clock function. Mammalian histone methyltransferase (HMT) Enhancer of Zeste Homolog 2 (EZH2), a polycomb group enzyme that mediates the di- and tri-methylation of lysine 27 on histone H3 (H3K27me2 and H3K27me3, respectively) at the promoters of the *PER1* and *PER2*, was reported to be required for mammalian circadian clock function [16]. WDR5, a member of an HMT complex, was shown to interact directly with PER1 and mediate the rhythmic methylation of H3K4 and H3K9 at the promoters of PER1-regulated genes [5].

The *Arabidopsis* genome encodes 18 histone deacetylases (HDACs) that are divided into three different types: Reduced Potassium Deficiency 3, HD-tuin, and Sirtuin types [20]. 12 HATs that are encoded by the *Arabidopsis* genome can be grouped into four families based on sequence homologies and mode of actions: GNAT, MYST, p300/CBP, and TAFII250 families [8]. However, HDACs and HATs responsible for the dynamic acetylation of H3 within *LHY* and *CCA1* chromatin are yet to be identified. Simultaneously, based on the fact that DET1 has no HAT activity, any linker connecting both the HATs directly required for *LHY* histone acetylation and DET1 protein remains to be addressed.

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초록 : 히스톤 3 아세틸화(H3Ac)를 통한 De-Etiolated 1 (DET1)의 애기장대 생체시계 조절

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자기 현가적(self-sustaining) 조절 장치인 생체시계는 24시간 주기의 생체리듬을 조절하며 또한 생물체로 하여금 매일 변화하는 자연환경의 외부 신호를 인지할 수 있도록 해준다. 생체시계 유전자의 발현 조절은 전사/해독의 역환류 기작을 통해 이루어진다. 애기장대 *LATE ELONGATED HYPOCOTYL* (*LHY*)와 *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*)는 아침에 최고조로 발현되며 해독된 LHY and CCA1는 저녁에 최고로 발현되는 *TIMING OF CAB EXPRESSION1* (*TOC1*)의 발현을 억제한다. TOC1단백질은 *LHY*와 *CCA1* 발현을 촉진시킴으로써 생체시계의 핵심 진자(oscillator)를 형성한다. 동물에서 생체시계의 주요 전사 인자인CLOCK은 아세틸화효소 활성 기능을 가지며, 이는 생체시계의 기능 유지에 아세틸화의 중요함을 의미한다. 하지만 애기장대 생체시계에 아세틸화를 담당하는 인자에 대한 정보는 현재 보고된 바가 없다. 본 연구에서 DET1 (De-Etiolated1)는 암조건하에서 애기장대 생체시계 관련 핵심인자 중 하나인 *LHY*발현을 억제하는데 필요하며 이의 억제는 H3Ac 조절을 통해 이루어짐을 증명하였다. 하지만 *LHY* 아세틸화를 담당하는 효소의 발굴 및 이들 효소와 DET1과의 연결을 찾는 문제는 여전히 미재로 남아있다.