Plant Signaling & Behavior 9, e28505; April; © 2014 Landes Bioscience

The *LNK1* night light-inducible and clock-regulated gene is induced also in response to warm-night through the circadian clock nighttime repressor in *Arabidopsis thaliana*

Takeshi Mizuno,[1] Aya Takeuchi,[1] Yuji Nomoto,[1] Norihito Nakamichi,[2] and Takafumi Yamashino[1,*]

[1]Laboratory of Molecular and Functional Genomics; School of Agriculture; Nagoya University; Furocho, Chikusa-ku, Nagoya, Japan;
[2]Institute of Transformative Bio-Molecules; Nagoya University; Furocho, Chikusa-ku, Nagoya, Japan

Keywords: *Arabidopsis thaliana*; circadian clock; pseudo-response regulator; temperature response; transcription circuitry

Abbreviations: CCA1, CIRCADIAN CLOCK ASSOCIATED 1; ChIP, chromatin immunoprecipitation; EC, Evening Complex; ELF3, EARLY FLOWERING 3; ELF4, EARLY FLOWERING 4; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYL; LUX, LUX ARRHYTHMO; PCL1, PHYTOCLOCK 1; PIF4, PHYTOCHROME-INTERACTING FACTOR 4; PRR, PSEUDO RESPONSE REGULATOR; TOC1, TIMING OF CAB EXPRESSION 1

Ambient temperature has two fundamental impacts on the *Arabidopsis* circadian clock system in the processes referred to as temperature compensation and entrainment, respectively. These temperature-related longstanding problems have not yet been fully clarified. Recently, we provided evidence that temperature signals feed into the clock transcriptional circuitry through the evening complex (EC) nighttime repressor composed of LUX-ELF3-ELF4, and that the transcription of *PRR9, PRR7, GI* and *LUX* is commonly regulated through the nighttime repressor in response to both moderate changes in temperature (Δ6 °C) and differences in steady-state growth-compatible temperature (16 °C to 28 °C). These temperature-associated characteristics of the core clock genes might be relevant to the fundamental oscillator functions. Here, we further show that the recently identified *LNK1* night light-inducible and clock-controlled gene, which actually has a robust peak at daytime, is induced also by warm-night through the EC nighttime repressor in a manner very similar to *PRR7*, which is also night light-inducible daytime gene. Based on these findings, a hypothetical view is proposed with regard to the temperature entrainment of the central oscillator.

In the flowering plant *Arabidopsis thaliana*, significant progress has been made in defining the molecular mechanism of circadian clock operation in plants.[1-3] The central oscillator that has been uncovered is composed of at least ten gene-products, including CCA1, LHY, PRR9, PRR7, PRR5, TOC1 (or PRR1), LUX (or PCL1), ELF3, ELF4, and GI, which all together constitute a multi-looped transcriptional circuitry that generates robust and free-running circadian rhythms.[4] This clock transcriptional circuitry has the capacity to integrate the external cues of light and temperature in order to not only accurately maintain the central oscillator functions in response to ever-changing seasonal conditions in natural habitats but also properly control a variety of output pathways. It has been postulated that *CCA1/LHY*, *PRR9*, and *GI* are implicated in light responses in the clock transcriptional circuitry.[5] Temperature has two mechanistic impacts on the plant oscillator system.[6-8] In a process referred to as temperature compensation, oscillator period resists changes in ambient temperature. On the other hand, in a process termed entrainment, temperature can act as a resetting cue. Molecular mechanisms underlying these temperature-related characteristics of circadian clock have not yet been fully clarified, despite the fact that they have been concerned in various studies of *A. thaliana*.[9-13] Recently, we provided evidence that temperature signals feed into the clock transcriptional circuitry through the evening complex (EC) nighttime repressor consisting of ELF3, ELF4, and LUX to commonly regulate transcription of *PRR9, PRR7, GI* and *LUX* in response to both moderate changes in temperature (Δ6 °C) and differences in steady-state growth-compatible temperature (16 °C to 28 °C).[14] Consequently, the expression of these EC-target genes is upregulated in response to a warm temperature specifically during the dark period, whereas they are reversibly downregulated in response to a cool temperature. It is suggested that EC nighttime repressor is inactivated under warm temperature

*Correspondence to Takafumi Yamashino; Email: yamasino@agr.nagoya-u.ac.jp
Submitted: 02/12/2014; Revised: 03/11/2014; Accepted: 03/11/2014; Published Online: 04/07/2014
Citation: Mizuno T, Takeuchi A, Nomoto Y, Nakamichi N, Yamashino T. The LNK1 night light-inducible and clock-regulated gene is induced also in response to warm-night through the circadian clock nighttime repressor in Arabidopsis thaliana. Plant Signaling & Behavior 2014; 9:e28505; PMID: 24690904; http://dx.doi.org/10.4161/psb.28505

conditions. As pointed out previously,[14] it might be puzzling that *LUX* is a target gene of the EC and is induced by a warm temperature that inactivates the EC nighttime repressor containing the gene product of *LUX* itself. However, it is conceivable that the induced LUX transcription factor may play a specific role in regulating a certain set of output genes without the need to form the EC under these conditions. It is also conceivable that the induced LUX transcription factor may compensate the EC inactivation by a temperature upshift. In any case, these temperature-associated characteristics of the core clock genes might be relevant to the fundamental oscillator functions such as temperature compensation and/or entrainment. Furthermore, transcription of another EC-target, the output *PIF4* gene, is modulated through the same thermoregulatory mechanism, thereby leading to the PIF4-dependent temperature-adaptive photoperiodic regulation of hypocotyl elongation.

During the last decade, a number of clock-associated genes have been reported for *A. thaliana* based on the fact that a miss-expression and/or loss-of-function mutation of each gene result in an altered property of circadian rhythm.[15] Hence, we wanted to look for additional EC-targeted and thermo-regulated clock-associated genes. In this study, we focused on the *RVE8*, *LNK1*, and *JMJ30* (also known as *JMJD5*) genes,[16-19] not only because the expression of these newly identified genes are directly controlled by the central oscillator, thereby showing robust free-running rhythms with their peaks at the daytime, but also because the clock functions are markedly compromised in their loss-of-function mutants. Among them, here we show that the *LNK1* night light-inducible and clock-regulated gene is also induced by warm-night through the EC nighttime repressor.

Transcription of LNK1 is Able to Respond to Changes in Ambient Temperature Specifically at Dark Period

To examine temperature responsiveness of the recently identified clock-associated genes, *RVE8*, *LNK1*, and *JMJ30*, in response to changes in growth-compatible ambient temperatures, wild-type (WT) seedlings (accession Col-0) were grown at 22 °C in light/dark cycles, and the temperature was raised to 28 °C at eight different zeitgeber time (ZT) points including the daytime and nighttime, as schematically shown in **Figure 1** (top). After 3 h, RNA samples were prepared and temperature responsiveness of *RVE8*, *LNK1*, and *JMJ30* was examined (**Fig. 1A, D, and G**, red). As a control, samples prepared from plants grown continuously at 22 °C were also analyzed (**Fig. 1A, D, and G**, green). The diurnal expressions of these genes robustly oscillated. *RVE8* showed a peak at morning, *LNK1* at daytime, whereas *JMJ30* displayed its peak at evening. It was then revealed that the transcription of *LNK1* was markedly upregulated following the temperature upshift, specifically during the dark period (red arrows in **Figure 1D**), while *RVE8* and *JMJ30* did not respond at any time (**Fig. 1A and G**). To examine these phenomena more closely, seedlings grown at 22 °C were upshifted to 28 °C at ZT = 12(early night) and ZT = 18 (midnight), respectively, and the temperature responses were followed at 1 h or 2 h intervals. Indeed, *LNK1* was upregulated in response to the temperature upshift at both ZT = 12 and ZT = 18 (**Fig. 1E and F**). Other genes (*RVE8* and *JMJ30*) appeared to be insensitive to changes in temperature (**Fig. 1B, C, H, and I**). Furthermore, the expression of *LNK1* was downregulated in response to a temperature downshift, when seedlings grown at 28 °C were downshifted to 16 °C at ZT = 18 (**Fig. 2A**). Taken together, it was concluded that the expression of *LNK1* is reversibly regulated in response to changes across a range of growth-compatible temperatures, specifically during the dark period. Essentially the same properties were seen for *LNK2* (a homolog of *LNK1*), although less strikingly (**Fig. 2B**). These properties are essentially same as those previously reported for a set of core clock genes, *PRR9*, *PRR7*, *GI*, and *LUX*.[14]

Temperature Response of LNK1 is Gated through the Clock Function

The above results suggested that the temperature response of *LNK1* is gated in a time-of-day (or dark period) specific manner through the clock function. To address this issue, we compared the temperature response of *LNK1* grown under light/dark (LD) cycles with that grown in continuous darkness (DD), as schematically illustrated (**Fig. 3**, top). First, as a control, a biologically independent experiment was replicated in LD and seedlings were subjected to temperature upshift at ZT = 15 (**Fig. 3A**) Then, seedlings grown at 22 °C under LD were transferred to DD, and exposed to 28 °C at appropriate timings during the first subjective daytime (**Fig. 3B**) and nighttime (**Fig. 3C**). *LNK1* responded to the temperature upshift at the subjective nighttime in DD as well as in LD (**Fig. 3C**), but not at the subjective daytime (**Fig. 3B**). These results suggested that the free-running circadian clock gates temperature signals in such a manner that the timing of temperature responses is confined to strictly during the dark period. This idea was further confirmed by analyzing the temperature response of *LNK1* under continuous light (LL) conditions (**Fig. 3**, bottom). Indeed, *LNK1* responded to the temperature upshift even in LL at the subjective nighttime (**Fig. 3E**).

The EC Nighttime Repressor is Implicated in the Temperature Responsiveness of LNK1

It was previously shown that the EC nighttime repressor is a common factor for the temperature responses of *PRR9*, *PRR7*, *GI*, and *LUX*.[14] To see whether this is the case also for *LNK1*, the temperature response of *LNK1* was examined through employing a set of EC loss-of function mutants, namely, *elf3–8*, *elf4–2,* and *pcl1–1*, in addition to wild-type Col-0 (**Fig. 4A to D**). These seedlings were upshifted from 22 °C to 28 °C at ZT = 18, as described above. Compared with Col-0 (**Fig. 4A**), the expression of *LNK1* in *elf3–8* was constitutively high both before and after the temperature upshift (**Fig. 4B**). As a result, the temperature responsiveness of *LNK1* was apparently abolished in this mutant. Essentially the same phenotypes were seen in both *elf4–2* and *pcl1–1* mutant seedlings

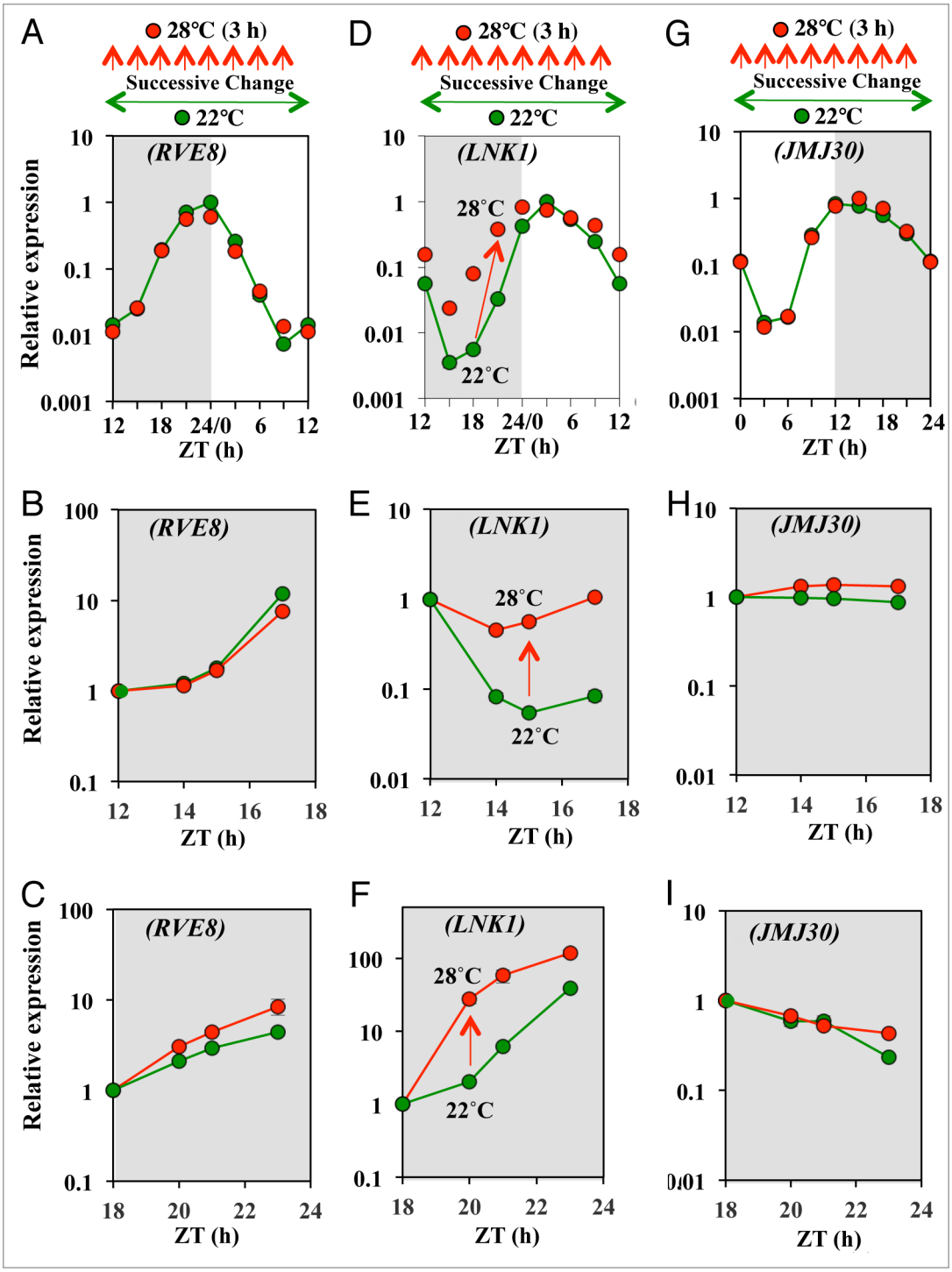

Figure 1. (Upper part, **A**, **D**, and **G**) Temperature responses of a set of clock genes. Seedlings (Col-0) were grown at 22 °C for 8 d in light/dark cycles, and the growth temperature was upshifted to 28 °C at different zeitgeber time (ZT) points, as indicated schematically. RNA samples were prepared after incubation for 3 h, and levels of transcripts were determined by qRT-PCR (red). RNA samples were also prepared from control plants grown continuously at 22 °C (green). Relative expression levels are shown as mean values ± SD (n = 3). The values were normalized to the maximum value of the samples at 22 °C. The shaded period corresponds to the dark. (Lower part, **B**, **C**, **E**, **F**, **H**, and **I**) Expression of a set of clock genes following temperature upshift. Seedlings (Col-0) grown at 22 °C under light/dark cycles were upshifted to 28 °C at ZT = 12 (**B**, **E**, **H**) and ZT = 18 (**C**, **F**, **I**), and the temperature responses of a set of indicated clock genes were examined. Values were normalized to the initial ones, and relative expression levels are shown as mean values ± SD (n = 3). Detailed methods were described previously.[14] The LNK1 primers for qRT-PCR used are 5′-GTCTTTGAAA CGAGAGCCAA CTTG-3′ and 5′- GGAGCAGCAT CAGGAAACACC-3′.[17]

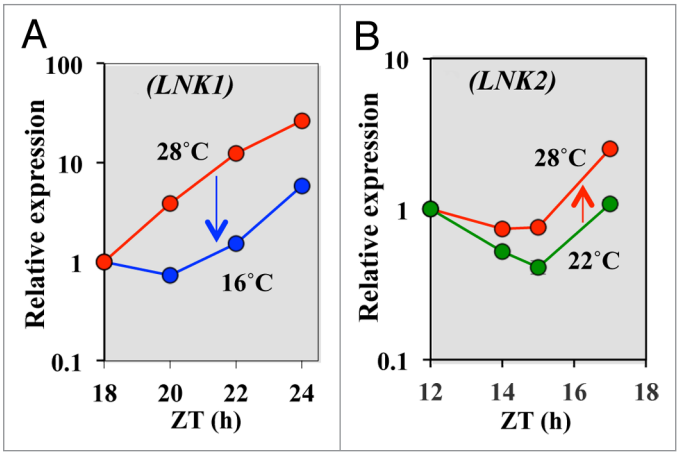

Figure 2. (**A**) Response of *LNK1* following a temperature downshift. Seedlings (Col-0) grown at 28 °C under light/dark cycles were downshifted to 16 °C at ZT = 18, and the temperature responses of *LNK1* were examined. Values were normalized to the initial ones, and relative expression levels are shown as mean values ± SD (n = 3). (**B**) Response of *LNK2* following a temperature upshift. Seedlings (Col-0) grown at 22 °C under light/dark cycles were downshifted to 28 °C at ZT = 12, and the temperature responses of *LNK2* were examined. Values were normalized to the initial ones, and relative expression levels are shown as mean values ± SD (n = 3).

(**Fig. 4B and C**, respectively). In other words, the temperature response of *LNK1* is severely compromised in the set of EC loss-of-function mutants. Hence, it was suggested that the EC nighttime repressor is crucially and commonly implicated in the mechanism underlying the temperature responses of not only a set of core clock genes (*PRR9*, *PRR7*, *GI*, and *LUX*) but also the clock-associated *LNK1* gene.

The EC Nighttime Repressor Binds to the LNK1 Promoter

To test the possibility that the EC nighttime repressor binds to the *LNK1* promoter, we conducted chromatin immunoprecipitation (ChIP) assays by employing a set of transgenic lines each carrying an appropriate composite transgene, namely, *ELF3*$^{-pro}$*-ELF3-YFP* and *LUX*$^{-pro}$*-LUX-GFP*, both of which have been established previously.[20,21] These transgenic lines have been successfully used for ChIP assays, the results of which demonstrated that the consensus LUX-binding site (designated LBS) is 5′-GAT(T/A)CG-3′, and that *PRR9*, *PRR7*, *GI*, *LUX*, and *PIF4* are the direct targets of the EC.[14,20-23] The promoter sequence of *LNK1* contains several perfect or near-perfect LBSs. Based on this fact, we selected appropriate candidate regions (amplicons designated a to d) in addition to a negative control amplicon designated cs which is derived from the coding-region of *LNK1*, as schematically shown (**Fig. 4**, middle). The amplicons a and b contain a single and a double LBS, respectively. The amplicons c and d contain a single near-perfect LBS (GATTCT), respectively. First, we conducted ChIP assays by employing *ELF3*$^{-pro}$*-ELF3-YFP* with special reference to these

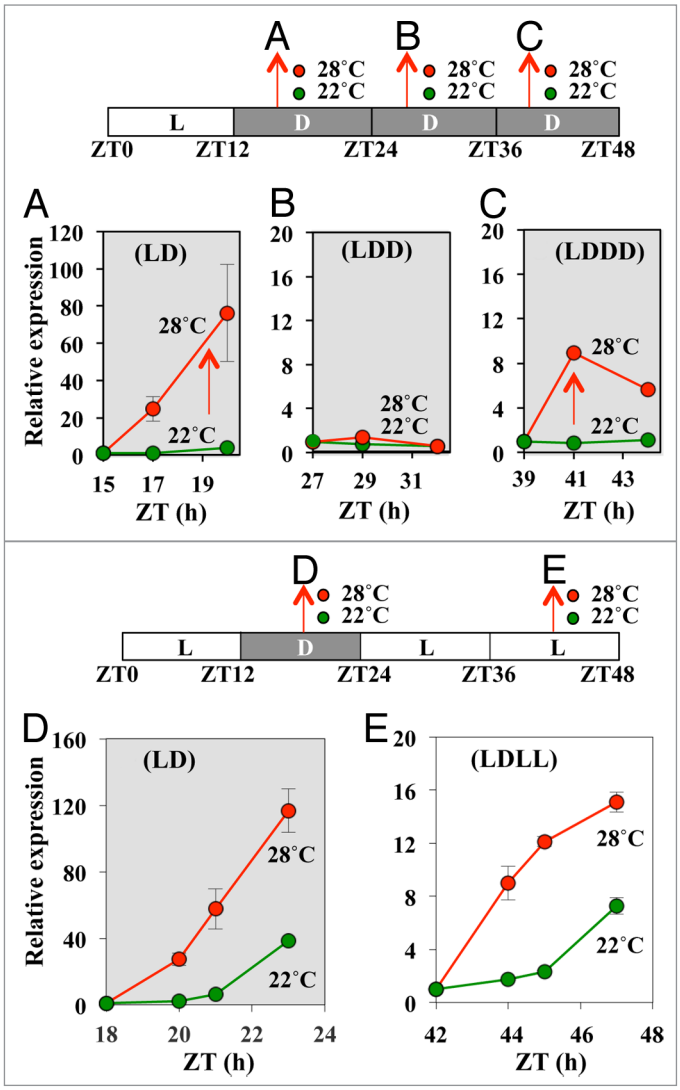

Figure 3. Response of *LNK1* following a temperature upshift under continuous dark and light conditions. Experiments were performed as schematically illustrated. Other details are the same as those given in **Figure 1**.

amplicons. ELF3-YFP efficiently binds to the region confined by the amplicon c, which is located around the transcription start site of *LNK1* (**Fig. 4E**). The result was confirmed by the replicated experiment with biologically independent samples (**Fig. 4F**). Concomitantly, ChIP assay employing *LUX*$^{-pro}$*-LUX-GFP* was also performed (**Fig. 4F**). Both ELF3-YFP and LUX-GFP preferentially bind to the same region located near the *LUX* transcription start site. These results are compatible with the idea that the *LNK1* gene is a direct target of the EC nighttime repressor, although ChIP assay employing an appropriate ELF4 probe still remains to be performed. Taken together with the genetic evidence shown in **Figure 4A–D**, it was concluded that the EC nighttime repressor is essential for the temperature-dependent repression of *LNK1* as well as a set of core clock genes (*PRR9*, *PRR7*, *GI*, and *LUX*).

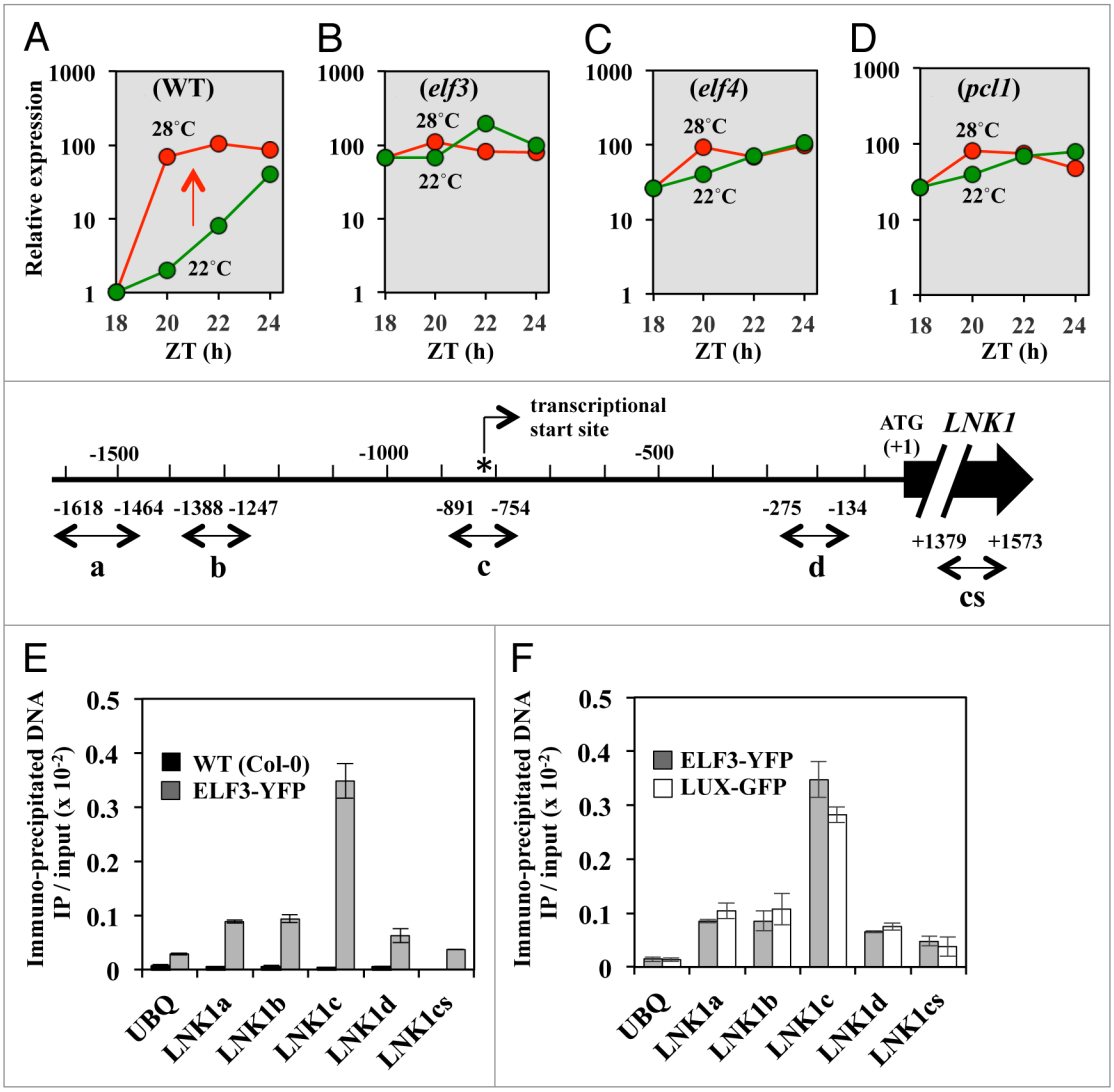

Figure 4. (Upper part, **A** to **D**) Response of *LNK1* to a temperature upshift in a set of clock mutants. The set of indicated mutant seedlings (*elf3*, *elf4*, *pcl1*), together with wild-type seedlings, were grown at 22 °C, upshifted to 28 °C at the indicated ZT points, and the temperature responses of a set of indicated clock genes were examined. Values were normalized to the initial ones of wild-type seedlings. Their relative expression levels are shown as mean values ± SD (n = 3). The shaded period corresponds to the dark. (Lower part, **E** and **F**) ChIP assays with the *LNK1* promoter. An upstream region of the *PRR7* gene is schematically depicted at the top. ChIP assays were performed with reference to the indicated amplicons denoted by a to d, together with cs by employing a set of transgenic lines carrying *ELF3*^{pro}*-ELF3-YF* and *LUX*^{pro}*-LUX-GFP*, as indicted. As a negative control, Col-0 seedlings were also analyzed. Detailed methods were described previously.[14] Primers used for ChIP assays are; amplicon a, 5′-CAAGTTCCCA AATTCACCG-3 and 5′-GAGCTTCTTT TCTTTGACTT TGATG-3′; amplicon b, 5′-GCTCGGAGAC GAGAGAAGC-3′ and 5′-GGGAGCGATG AGAGATGG-3′; amplicon c, 5′-CACGTGTTGA TATCATGGTC AC-3′ and 5′-GACTTTCTAG AGATGTTCGG CAG-3′; amplicon d, 5′-GGAATTCATG AATAGTTGAA AGAATG-3′ and 5′-CAGCTTCCAA TAATAGAGAA ATCG-3′; amplicon cs, 5′-GTTTCGTTAA GGGCAAGTGG-3′ and 5′-CCCATCCAGA TGATGAAGATG-3′.

Implications

The essence of this and previous studies was schematically summarized in **Figure 5A**. We showed that a set of core clock genes (*PRR9*, *PRR7, GI*, and *LUX*), a clock-associated light-inducible gene (*LNK1*), and a pair of clock output genes (*PIF4* and *PIF5*) are common targets of the EC nighttime repressor. Growth-compatible temperature signals (*e.g.*,changes in temperature of Δ6 °C, and differences in steady-state temperature from 16 °C to 28 °C) feed into the clock transcriptional circuitry through a common pathway in which a warm temperature antagonizes EC repressor-activity, whereas a cool temperature stimulates it. Consequently, these target genes are commonly regulated at the level of transcription, depending on ambient temperatures in such a way that a warmer temperature more efficiently induces their transcription specifically during the dark period, whereas a cooler temperature more strongly represses them. As noted previously,[14] we do not know whether the DNA-binding of EC to the target promoters itself is inhibited by a warm temperature, or a warm temperature inhibits the repressor ability of EC without affecting its DNA-binding ability. Althoguh it was suggested that the EC nighttime repressor

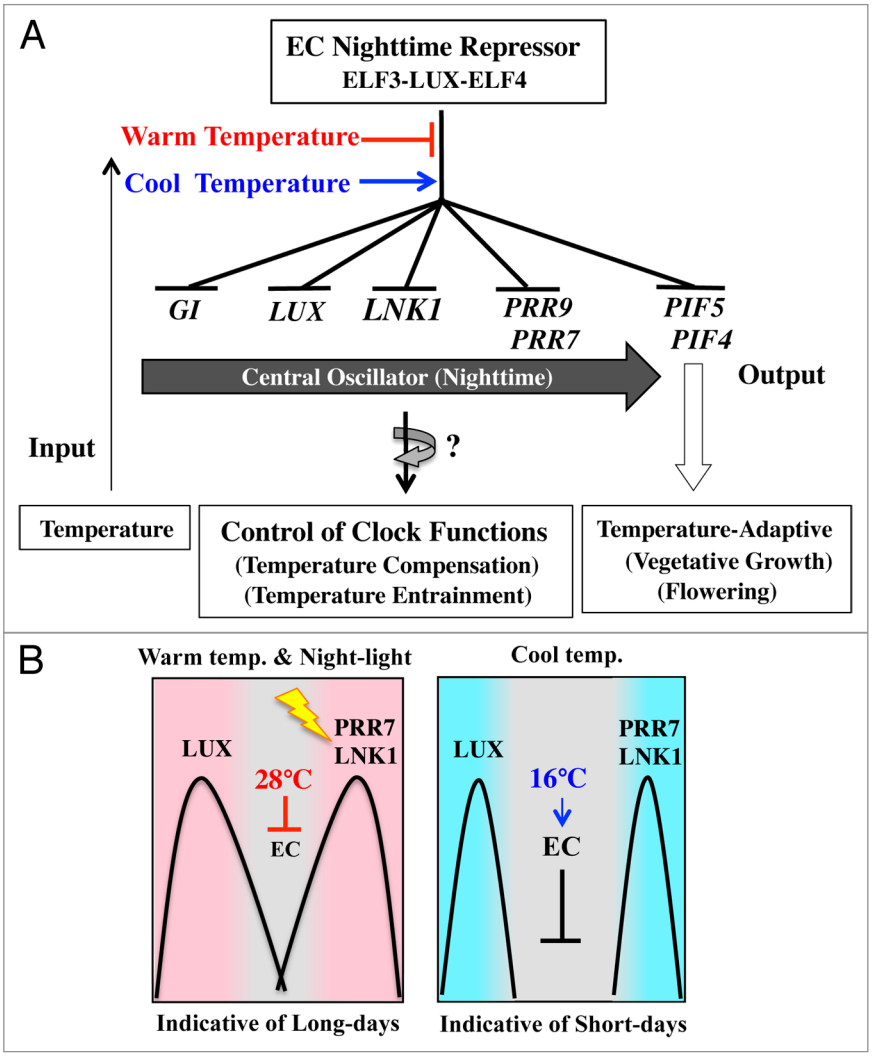

Figure 5. Models proposed in this study. (**A**) Schematic representation of the EC nighttime repressor-mediated temperature responses of the clock transcriptional circuitry. Details are given in the text. Briefly, both temperature signals (*i.e.*, changes in temperature and differences in growth temperature) feed into the clock transcriptional circuitry through the EC nighttime repressor so as to regulate its direct targets, namely, *PRR9*, *PRR7*, *GI*, *LUX*, and *LNK1* in such a manner that a warm temperature antagonizes EC activity, whereas a cool temperature stimulates it. These findings may be relevant to the longstanding problems referred to as temperature compensation and entrainment. Such a temperature signal also feeds as far as the PIF4/PIF5-mediated output pathway, thereby leading to the temperature-adaptive control of hypocotyl elongation. (**B**) Schematic illustration is shown to propose a hypothetical view that imply the importance of temperature-adaptive EC nighttime repressor to properly track seasonal changes in photo-cycles and thermo-cycles. Details are given in the text.

is subjected to the COP1-dependent degradation,[4] no siguificant effect on the stabilities of ELF3-YFP and LUX-GFP protiens was observed in response to changes in temperatures (data not shown).

Ambient temperature affects the fundamental clock functions at several aspects. The revealed EC-mediated and temperature-regulated signaling network of the clock transcriptional circuitry might impact strongly on these clock functions (see **Figure 5A**). (1) In a process referred to as temperature compensation, the oscillator period resists changes in ambient temperature. This mechanism ensures a constant oscillation period of about 24 h within a wide range of ambient temperatures. On the other hand, in a process termed entrainment, temperature can act as a resetting cue. Indeed, temperature fluctuations as small as Δ4 °C within a day can reset the plant circadian oscillator.[6-8] The EC-dependent coordinate thermoregulations of *LUX*, *GI*, *LNK1*, *PRR9*, and *PRR7* might be implicated in the mechanisms underlying temperature compensation and/or entrainment. (2) It was previously shown that a 1 h light-pulse at the nighttime effectively induced the transcription of *LNK1* that has a robust peak normally at the daytime.[17] Interestingly, it was also noted that the same characteristics (*i.e.*, night light-responsiveness) were seen for *PRR7* and *GI* as well.[17] Taken together with the results in this study, these light and warm temperature-inducible genes might integrate coordinately both light and temperature signals at the core oscillator to keep track of (or entrain) seasonal changes in photo-cycles and thermo-cycles. This might allow the central oscillator to regulate a

variety of output pathways in order to properly control the plant development and/or flowering time in response to changes in photoperiods and ambient temperatures. Indeed, another EC-controlled output gene, *PIF4* is crucially involved in the photoperiod- and temperature-dependent control of hypocotyl elongation and flowering time (see **Figure 5**).[24-27] In any case, the results of this study will shed new light on the longstanding problems with regard to the impact of ambient temperature on the plant circadian clock, as further discussed.

It may be worth noting that recent studies showed that the temperature-dependent alternative splicing of certain clock genes (e.g., *CCA1* and *LHY*) results in modulation of their transcriptional profiles, which occur usually at an extremely low temperature (e.g., 4 °C).[28,29] The temperature-induced events reported in this and previous studies are most likely not relevant to such effects of alternative splicing on the transcriptional circuitry of clock genes, because the events are observed within a range of growth-compatible moderate temperatures.

A Proposed Hypothesis

Finally, we would like to consider with regard to the above implications in further detail (**Fig. 5B**). For the dark period-dependent temperature responses of both the evening (e.g., *GI* and *LUX*) and daytime genes (e.*g., LNK1* and *PRR7*), the temperature-sensitive EC nighttime repressor plays a crucial role, as demonstrated in this and previous studies.[14] A warmer temperature at dusk is indicative of a prolonged evening. Accordingly, the evening gene, *GI* and *LUX*, should remain expressed. The same signal at dawn is indicative of the coming sunrise. Accordingly, the daytime genes, *LNK1* and *PRR7*, should promptly start to be expressed. As schematically shown, the time interval of phases of the evening and daytime genes would be shortened in a warmer day (**Fig. 5B**, left). This means that a warmer ambient temperature would be indicative of short-nights (or long-days). Vice versa, the time interval of phases of the evening and daytime genes would be lengthened in a cooler day (**Fig. 5B**, right). This means that a cooler ambient temperature, which activates the EC nighttime repressor, would be indicative of long-nights (or short-days). It is worth mentioning that light signal during the subjective night, which is indicative of the sunrise, also induces the early expression of *PRR7* as well as *LNK1*.[17] Taken together, it is tempting to hypothesize that through these EC-mediated regulatory mechanisms, temperature and light signals are integrated coordinately at the core oscillator to properly track seasonal changes in photo-cycles and thermo-cycles. This idea is consistent with the proposed functions of LNKs.[17] Together with the recent and related studies form other groups,[10-13,30,31] our results will shed new light on the longstanding temperature-associated subjects in this field.

Disclosure of Potential Conflicts of Interests

There were no potential conflicts of interests to disclose.

Acknowledgments

We would like to thank Dr. Onai and Dr. Ishiura (Nagoya University, Japan) for providing seeds of the *pcl1–1* mutant, Dr. Millar (University of Edinburgh, UK) for the *elf3–8* mutant and the *ELF3*:*ELF3-YFP elf3–4* transgenic line, and also Dr. Kay (USC Dana and David Dornsife College of Letters, Arts and Sciences, CA) for the *elf4–2* mutant and the *LUX*:*LUX-GFP lux-4* transgeneic line. This work was supported by Japan Society for the Promotion of Science [no. 23580133 to T.Y., no. 24380046 to T.M.].

References

1. McClung CR. The genetics of plant clocks. Adv Genet 2011; 74:105-39; PMID:21924976; http://dx.doi.org/10.1016/B978-0-12-387690-4.00004-0
2. Nagel DH, Kay SA. Complexity in the wiring and regulation of plant circadian networks. Curr Biol 2012; 22:R648-57; PMID:22917516; http://dx.doi.org/10.1016/j.cub.2012.07.025
3. Carré I, Veflingstad SR. Emerging design principles in the Arabidopsis circadian clock. Semin Cell Dev Biol 2013; 24:393-8; PMID:23597453; http://dx.doi.org/10.1016/j.semcdb.2013.03.011
4. Pokhilko A, Fernández AP, Edwards KD, Southern MM, Halliday KJ, Millar AJ. The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. Mol Syst Biol 2012; 8:574; PMID:22395476; http://dx.doi.org/10.1038/msb.2012.6
5. Pokhilko A, Hodge SK, Stratford K, Knox K, Edwards KD, Thomson AW, Mizuno T, Millar AJ. Data assimilation constrains new connections and components in a complex, eukaryotic circadian clock model. Mol Syst Biol 2010; 6:416; PMID:20865009; http://dx.doi.org/10.1038/msb.2010.69
6. Boikoglou E, Ma Z, von Korff M, Davis AM, Nagy F, Davis SJ. Environmental memory from a circadian oscillator: the Arabidopsis thaliana clock differentially integrates perception of photic vs. thermal entrainment. Genetics 2011; 189:655-64; PMID:21840862; http://dx.doi.org/10.1534/genetics.111.131417
7. McClung CR, Davis SJ. Ambient thermometers in plants: from physiological outputs towards mechanisms of thermal sensing. Curr Biol 2010; 20:R1086-92; PMID:21172632; http://dx.doi.org/10.1016/j.cub.2010.10.035
8. Wigge PA. Ambient temperature signalling in plants. Curr Opin Plant Biol 2013; 16:661-6; PMID:24021869; http://dx.doi.org/10.1016/j.pbi.2013.08.004
9. Edwards KD, Anderson PE, Hall A, Salathia NS, Locke JC, Lynn JR, Straume M, Smith JQ, Millar AJ. FLOWERING LOCUS C mediates natural variation in the high-temperature response of the Arabidopsis circadian clock. Plant Cell 2006; 18:639-50; PMID:16473970; http://dx.doi.org/10.1105/tpc.105.038315
10. Salomé PA, McClung CR. PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. Plant Cell 2005; 17:791-803; PMID:15705949; http://dx.doi.org/10.1105/tpc.104.029504
11. Gould PD, Locke JC, Larue C, Southern MM, Davis SJ, Hanano S, Moyle R, Milich R, Putterill J, Millar AJ, et al. The molecular basis of temperature compensation in the Arabidopsis circadian clock. Plant Cell 2006; 18:1177-87; PMID:16617099; http://dx.doi.org/10.1105/tpc.105.039990
12. Salomé PA, Weigel D, McClung CR. The role of the Arabidopsis morning loop components CCA1, LHY, PRR7, and PRR9 in temperature compensation. Plant Cell 2010; 22:3650-61; PMID:21098730; http://dx.doi.org/10.1105/tpc.110.079087
13. Gould PD, Ugarte N, Domijan M, Costa M, Foreman J, Macgregor D, Rose K, Griffiths J, Millar AJ, Finkenstädt B, et al. Network balance via CRY signalling controls the Arabidopsis circadian clock over ambient temperatures. Mol Syst Biol 2013; 9:650; PMID:23511208; http://dx.doi.org/10.1038/msb.2013.7
14. Mizuno T, Nomoto Y, Oka H, Kitayama M, Takeuchi A, Tsubouchi M, Yamashino T. Yamashino, T. Ambient temperature signal feeds into the circadian clock transcriptiuonal circuitry through the EC nighttime repressor in Arabidopsis thaliana. Plant Cell Physiol 2014; In press; PMID:24500967; http://dx.doi.org/10.1093/pcp/pcu030.
15. Hsu PY, Harmer SL. Wheels within wheels: the plant circadian system. Trends Plant Sci 2013; PMID:24373845; http://dx.doi.org/10.1016/j.tplants.2013.11.007
16. Hsu PY, Devisetty UK, Harmer SL. Accurate timekeeping is controlled by a cycling activator in Arabidopsis. Elife 2013; 2:e00473; PMID:23638299; http://dx.doi.org/10.7554/eLife.00473

17. Rugnone ML, Faigón Soverna A, Sanchez SE, Schlaen RG, Hernando CE, Seymour DK, Mancini E, Chernomoretz A, Weigel D, Más P, et al. LNK genes integrate light and clock signaling networks at the core of the Arabidopsis oscillator. Proc Natl Acad Sci U S A 2013; 110:12120-5; PMID:23818596; http://dx.doi.org/10.1073/pnas.1302170110
18. Jones MA, Covington MF, DiTacchio L, Vollmers C, Panda S, Harmer SL. Jumonji domain protein JMJD5 functions in both the plant and human circadian systems. Proc Natl Acad Sci U S A 2010; 107:21623-8; PMID:21115819; http://dx.doi.org/10.1073/pnas.1014204108
19. Lu SX, Knowles SM, Webb CJ, Celaya RB, Cha C, Siu JP, Tobin EM. The Jumonji C domain-containing protein JMJ30 regulates period length in the Arabidopsis circadian clock. Plant Physiol 2011; 155:906-15; PMID:21139085; http://dx.doi.org/10.1104/pp.110.167015
20. Dixon LE, Knox K, Kozma-Bognar L, Southern MM, Pokhilko A, Millar AJ. Temporal repression of core circadian genes is mediated through EARLY FLOWERING 3 in Arabidopsis. Curr Biol 2011; 21:120-5; PMID:21236675; http://dx.doi.org/10.1016/j.cub.2010.12.013
21. Helfer A, Nusinow DA, Chow BY, Gehrke AR, Bulyk ML, Kay SA. LUX ARRHYTHMO encodes a nighttime repressor of circadian gene expression in the Arabidopsis core clock. Curr Biol 2011; 21:126-33; PMID:21236673; http://dx.doi.org/10.1016/j.cub.2010.12.021
22. Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farré EM, Kay SA. The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature 2011; 475:398-402; PMID:21753751; http://dx.doi.org/10.1038/nature10182
23. Chow BY, Helfer A, Nusinow DA, Kay SA. ELF3 recruitment to the PRR9 promoter requires other Evening Complex members in the Arabidopsis circadian clock. Plant Signal Behav 2012; 7:170-3; PMID:22307044; http://dx.doi.org/10.4161/psb.18766
24. Nomoto Y, Kubozono S, Yamashino T, Nakamichi N, Mizuno T. Circadian clock- and PIF4-controlled plant growth: a coincidence mechanism directly integrates a hormone signaling network into the photoperiodic control of plant architectures in Arabidopsis thaliana. Plant Cell Physiol 2012; 53:1950-64; PMID:23037003; http://dx.doi.org/10.1093/pcp/pcs137
25. Nomoto Y, Kubozono S, Miyachi M, Yamashino T, Nakamichi N, Mizuno T. Circadian clock and PIF4-mediated external coincidence mechanism coordinately integrates both of the cues from seasonal changes in photoperiod and temperature to regulate plant growth in Arabidopsis thaliana. Plant Signal Behav 2013; 8:e22863; PMID:23154509; http://dx.doi.org/10.4161/psb.22863
26. Kumar SV, Lucyshyn D, Jaeger KE, Alós E, Alvey E, Harberd NP, Wigge PA. Transcription factor PIF4 controls the thermosensory activation of flowering. Nature 2012; 484:242-5; PMID:22437497; http://dx.doi.org/10.1038/nature10928
27. Yamashino T, Nomoto Y, Lorrain S, Miyachi M, Ito S, Nakamichi N, Fankhauser C, Mizuno T. Verification at the protein level of the PIF4-mediated external coincidence model for the temperature-adaptive photoperiodic control of plant growth in Arabidopsis thaliana. Plant Signal Behav 2013; 8:e23390; PMID:23299336; http://dx.doi.org/10.4161/psb.23390
28. James AB, Syed NH, Bordage S, Marshall J, Nimmo GA, Jenkins GI, Herzyk P, Brown JW, Nimmo HG. Alternative splicing mediates responses of the Arabidopsis circadian clock to temperature changes. Plant Cell 2012; 24:961-81; PMID:22408072; http://dx.doi.org/10.1105/tpc.111.093948
29. Park MJ, Seo PJ, Park CM. **CCA1** alternative splicing as a way of linking the circadian clock to temperature response in Arabidopsis. Plant Signal Behav 2012; 7:1194-6; PMID:22899064; http://dx.doi.org/10.4161/psb.21300
30. Thines B, Harmon FG. Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. Proc Natl Acad Sci U S A 2010; 107:3257-62; PMID:20133619; http://dx.doi.org/10.1073/pnas.0911006107
31. Hussain F, Gupta C, Hirning AJ, Ott W, Matthews KS, Josic K, Bennett MR. Engineered temperature compensation in a synthetic genetic clock. Proc Natl Acad Sci U S A 2014; 111:972-7; PMID:24395809; http://dx.doi.org/10.1073/pnas.1316298111