

# The plant circadian clock looks like a traditional Japanese clock rather than a modern Western clock

Takeshi Mizuno and Takafumi Yamashino\*

Laboratory of Molecular and Functional Genomics; School of Agriculture; Nagoya University; Nagoya, Japan

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**Abbreviations:** CCA1, circadian clock associated 1; EC, Evening Complex; ELF3, early flowering 3; ELF4, early flowering 4; GI, gigantea; LHY, late elongated hypocotyl; LNK1, night light-inducible and clock-regulated 1; LUX, lux arrhythmo; RVER8, reveille 8; PCL1, phyto clock 1; PIF4, phytochrome-interacting factor 4; PRR, pseudo response regulator; TOC1, timing of cab expression 1.

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\*Correspondence to: Takafumi Yamashino; Email: yamasino@agr.nagoya-u.ac.jp

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**L**ife cycle adaptation to seasonal changes in photoperiod and ambient temperature is a major determinant of the ecological success behind the widespread domestication of flowering plants. The circadian clock plays a role in the underlying mechanism for adaptation through generating endogenous rhythms that allow plants to adapt and adjust to both the 24 h diurnal rotation and 365 d seasonal revolution. Nevertheless, the mechanism by which the circadian clock tracks seasonal changes in photoperiod and temperature is a longstanding subject in the field. Recently, we have begun to understand the question of how the light and ambient temperature signals feed into the circadian clock transcriptional circuitry in day-night cycles in order to track seasonal changes in photoperiod and ambient temperature.<sup>1-4</sup> Our results collectively indicate that the evening complex (EC) nighttime repressor consisting of LUX-ELF3-ELF4 plays a crucial role in this respect. Here, we discuss about these recent studies to add further implications.

## Introduction

In *Arabidopsis thaliana*, significant progress has been made in defining the molecular mechanism of circadian clock operation.<sup>5-10</sup> The central oscillator that has been uncovered is composed of mainly 3 interlocking transcriptional loops. The core loop is composed of a pair of morning-phased genes, *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*); and an evening-phased gene, *TIMING OF CAB EXPRESSION 1* (*TOC1*). The

level of *TOC1* gene product is modulated by the *GIGANTEA* (*GI*) gene product. The morning loop is composed of *CCA1/LHY* and 3 members of a small family of *PSEUDO-RESPONSE REGULATOR* (*PRR9*, *PRR7*, and *PRR5*) genes. Lastly, the evening loop is composed of *TOC1* (also known as *PRR1*) and a protein complex of evening-phased gene products including *LUX ARRHYTHMO* (*LUX*) (also known as *PCL1*, *PHYTOCLOCK 1*), *EARLY FLOWERING 3* (*ELF3*) and *ELF4*. Recently, a positive transcription loop mediated by a morning-phased *RVE8* (*REVEILLE 8*) gene and a small *LNK* (*NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED*) family of day-phased genes was reported.<sup>10-13</sup> This interlocking transcriptional circuitry is also called as “the repressilator model,” in which the morning-phase *CCA1/LHY* genes are repressed by the day-phased *PRR9/7/5* gene products; the day-phased *PRR9/7/5* gene are repressed by the evening complex (EC) nighttime repressor consisting of *LUX/ELF3/ELF4*; and the transcription of the EC nighttime repressor are in turn inhibited by the morning-phase *CCA1/LHY* gene product. Among these plant core oscillator components, we focus here on the physiological role for the EC nighttime repressor by proposing the view that the EC nighttime repressor plays a crucial role in tracking seasonal changes in both photoperiod and temperature under day-night cycles.<sup>1-4</sup> In the originally report,<sup>1</sup> we argued that this property of the EC nighttime repressor might be relevant to the circadian clock-associated longstanding issues, namely, temperature compensation and temperature entrainment.<sup>13-17</sup> However, it should be noted that we do not intend to address

these issues in this addendum, because the observed EC's characteristics under day-night cycles may or may not be relevant to the classical temperature-associated properties of the free-running central oscillator.

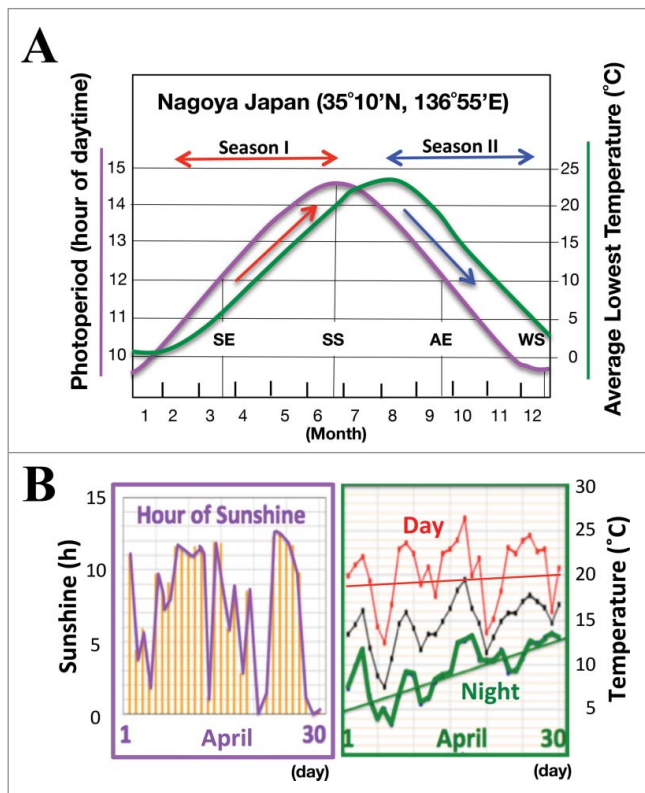
## Plant Circadian Clock Must Properly Track Local and Seasonal Changes in Photoperiod and Ambient Temperature

Here, we consider growth of *Arabidopsis thaliana* in the field of Nagoya (Japan), located at 35°10'N, 136°55'E. As has been noted previously,<sup>4</sup> the longest day (summer solstice) in Nagoya is approximately 14.5 h, while the shortest day (winter solstice) is 9.5 h (purple profile, data from <http://eco.mtk.nao.ac.jp>). The change in average lowest temperature (*i.e.*, night temperature) of each month during

2014 is also shown (Fig. 1A, green profile, data from <http://www.data.jma.go.jp>). Seasonal change in Nagoya can be divided into 3 periods: Season I (red arrows), during which both the daytime length and ambient temperature rapidly increase by about 65 min of daylight time and 6°C of ambient temperature per month, respectively; Season II (blue arrows), during which both the daylight time and ambient temperature quickly decrease. During the period between these seasons, the daylight time length decreases while the temperature increases, although the changes in both the photoperiod and ambient temperature during this period are relatively modest compared with Seasons I and II.

How does the plant circadian clock perceive the signal of seasonal change in photoperiod? The plant might measure the duration of sunshine of a given day. However, the duration of sunshine

dramatically changes within a few days depending on weather (Fig. 1B, left). Hence, bright sunlight alone in the daytime appears not to be the external light signal. Bünning and Pittendrigh have already addressed this issue (*i.e.*, photoperiodism) in early 20<sup>th</sup> century, and they proposed “an external coincidence model.” They hypothesized the coincidence of a phase of intrinsic rhythm and an input of external signal.<sup>18,19</sup> Nevertheless; its molecular bases are longstanding subjects in the field. On the other hand, how does the plant circadian clock perceive the signal of seasonal change in ambient temperature? Within a month, the average night temperature more markedly changes than the daytime temperature (*e.g.*, April) (Fig. 1B right, compare green and red lines). Therefore, the night temperature appears to be the reliable signal of change in season. However, the real world is more complex, because the night temperature markedly fluctuates within a few days (Fig. 1B, right). The plant circadian clock must ignore this type of daily variation in temperature within a short interval. How does the plant circadian clock overcome these problems in order to properly track seasonal changes in photoperiod and ambient temperature? Our recent study series provide us with insight into this subject in the field, as summarized below.<sup>1-4</sup>

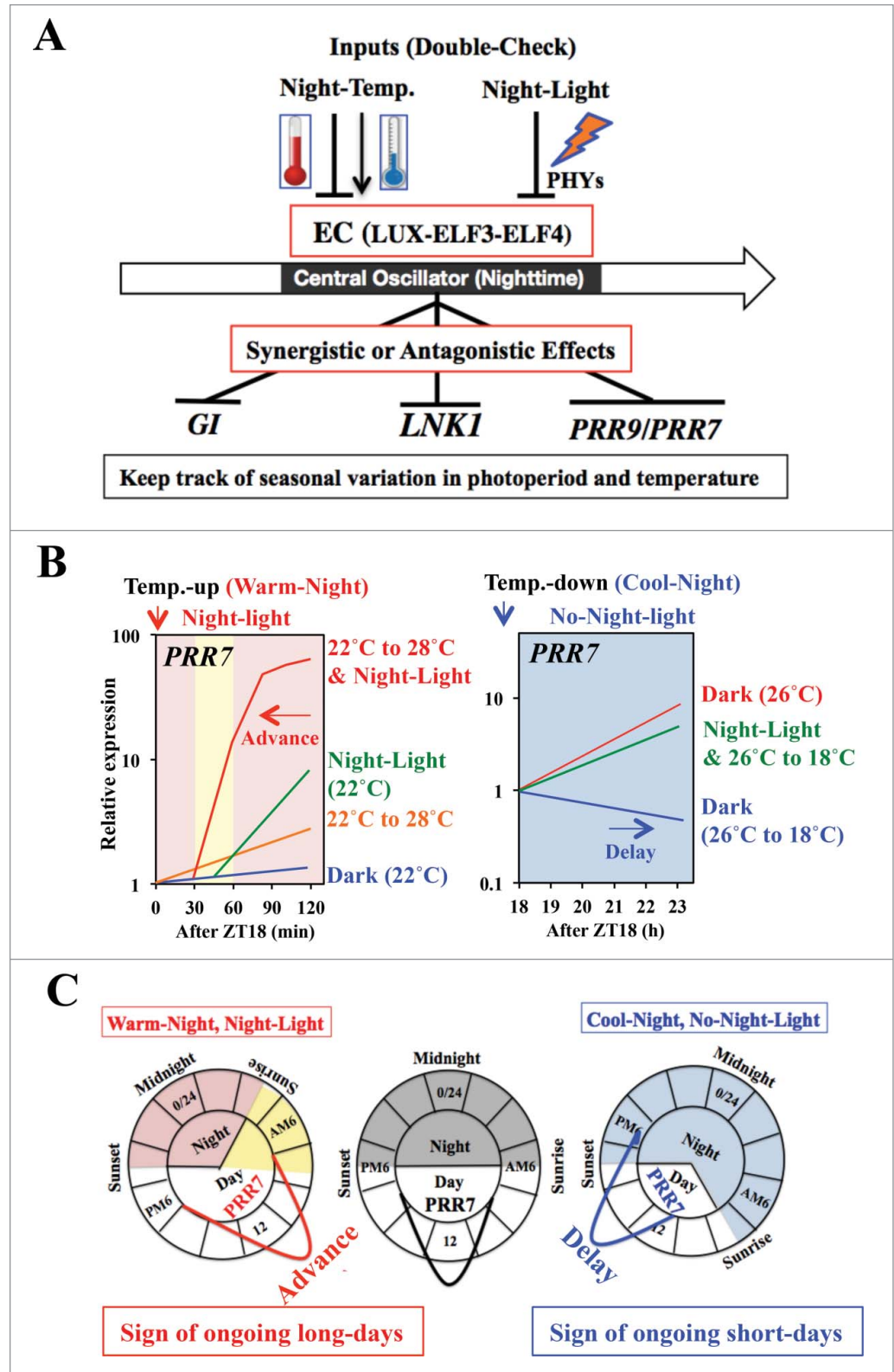


**Figure 1.** Schematic representation of daily and seasonal changes in photoperiod and ambient temperature in Nagaya, Japan. These illustrations are modified from those presented previously as Supplementary Fig. S10 and S11 in the earlier article,<sup>4</sup> and the data are from public database of Ephemeris Computation Office Public Relations Center, National Astronomical Observatory of Japan (<http://eco.mtk.nao.ac.jp/koyomi/index.html>) and Japan Meteorological Agency (<http://www.jma.go.jp/jma/menu/menureport.html>). Details are given in the section entitled “Plant Circadian Clock Must Track Local and Seasonal Changes in Photoperiod and Ambient Temperature.”

## The EC Nighttime Repressor Plays a Crucial Role in Tracking Seasonal Changes in Both Photoperiod and Ambient Temperature

Our proposal for a role of the EC nighttime repressor is schematically summarized in Fig. 2A. (1) The EC nighttime repressor represses the transcription of a set of core clock genes including *GI*, *LNK1*, *PRR9*, and *PRR7* through binding directly to their promoter regions. (2) The night temperature signal feeds into the circadian clock transcriptional circuitry through the EC nighttime repressor in such a manner that a growth-compatible warmer night temperature (*e.g.*, 28°C) inhibits EC function more, whereas a cooler night temperature (*e.g.*, 16°C)

stimulates it more. Consequently, the expression of these EC target genes is up-regulated in response to a warm temperature specifically during the dark, whereas they are reversibly down-regulated in response to a cool temperature. (3) The nighttime light signal also feeds into the circadian clock transcriptional circuitry through the EC nighttime repressor in concert with phytochromes in such a manner that the nighttime light signal inhibits EC function so that the same set of EC target genes is upregulated by the nighttime light. The effect is caused even through a dim light pulse in the dark (less than  $1 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 30 min), which may be a sign of changes in photoperiod. (4) More importantly, both the warm-night and night-light signals negatively modulate the activity of the EC nighttime repressor synergistically. In other words, an exponential burst of transcription (or markedly advanced expression) of the EC target genes is observed only when these signals are simultaneously fed into the repressor. (5) On the other hand, a night-light pulse antagonizes the positive effect of a cool-night signal on EC activity, thereby preventing the down-regulation (or significantly delayed expression) of the EC target genes during the cool night. These experimental data are also schematically illustrated in Fig. 2B. In short, the EC nighttime repressor has the bidirectional ability to cause both advanced and delayed expression of its target genes through double-checking ambient temperature and dim light in the night. This double-checking mechanism (*i.e.*, the coincidence of both the light and temperature



**Figure 2.** Schematic representations of the implications from our recent studies. These illustrations are based on our recent studies.<sup>1-4</sup> Details are given in the section entitled "The EC Nighttime Repressor Plays a Crucial Role in Tracking Seasonal Changes in Both Photoperiod and Temperature."



and an input of external signal.<sup>18</sup> Our results might suggest that the hypothesized intrinsic rhythm might be that of the EC nighttime repressor and the hypothetical external signal might be not only light but also ambient temperature during night. Although we do not know whether this is the case, our results at least suggest that the plant circadian clock has the abilities to recognize not only the absolute photoperiod but also the relative photoperiod (*i.e.*, an ongoing photoperiod compared with the past).

### Plant Circadian Clock Looks Like a Traditional Japanese Clock Rather Than a Modern Western Clock

To understand the plant circadian clock more conceptually, here we would like to introduce the mechanism of a Japanese clock, which tells us the traditional Japanese time. Unlike the modern Western (or universal) clock, the traditional Japanese (or domestic) clock was designed to track seasonal changes in photoperiod. Hence, we could imagine that the plant circadian clock might look like the traditional Japanese clock in the sense that both the biological and mechanical clock have the ability to track seasonal changes in domestic photoperiod. According to traditional Japanese time, a day (ca. Twenty-four h) is divided into 12 dials, each of which is assigned by a Zodiac symbol or name of an animal (Fig. 3A). Whatever the current season is, the “Rabbit” always assigns the time of sunrise, whereas “Rooster” exclusively assigns the time of sunset. Similarly, “Rat” assigns the midnight, whereas “Horse” assigns the time of noon. This means that each of 6 divisions during the daytime in the long-day season is longer than in the short-day season, whereas each of 6 divisions during the nighttime in the short-day season is longer than in the long-day season (compare Fig. 3A-a and -c). Unlike the modern Western clock, it was not so easy to make a mechanical Japanese clock that is able to tell us the traditional Japanese time throughout a whole year, because the relative speeds of clock hand should be different between daytime and

nighttime, also depending on season. However, workmen of the Edo period invented such a clock, with which the disk-shaped or linear dial-plate is replaced manually by every 15-days at the proper timing corresponding to 24 ends of seasons (or Solar term) during a year, so as to put the proper dial-plate fitting to the current season (Fig. 3A-d and Fig. 3B-a). Workmen invented a more sophisticated clock, which equips 2 independent pendulums with a fixed dial-plate (Fig. 3B-b). One of the pendulum ticks the time of night (upper pendulum), the other ticks the time of day. Even so, the positions of balance weights should be moved by every 15-days. In this respect, we human being have the ability to count and memorize the number of past days with help of calendar in order to change the dial-plate at the proper timing, while plants most likely does not have such an ability. Without need to count the number of past days, instead, the plant circadian clock has the ability to sense change in ambient temperature, so as to recognize turn of the season. Furthermore, take advantage of the EC’s ability to double-check both the night-light and night-temperature signals, the plant circadian clock is conservative enough to ignore the daily fluctuation of temperature. In other words, the plant circadian clock is sophisticated enough to conservatively track changes in season. The Japanese clock is equipped with many mechanical wheels, whereas the plant circadian clock is equipped with multiple transcriptional wheels. Among them, the EC nighttime repressor appears to play a curtail role in tracking seasonal variation in photoperiod and ambient temperature. Finally, we would like to speculate that the plant circadian clock in domestic habitats, like Japanese clock, would be practically reset only a few times in month, namely, only when both the night temperature and photoperiod are simultaneously and significantly changed.

### Concluding Remarks

Life cycle adaptation to seasonal variation in photoperiod and ambient temperature is a major determinant of the widespread ecological success of flowering

plants. In this addendum, we mainly considered above the intrinsic properties of the plant circadian clock. However, the intrinsic ability must somehow be linked to physiological outputs in the natural field. Otherwise, the sophisticated circadian clock would be useless. In this respect, other well-known EC targets are *PHYTOCHROME-INTERACTING FACTOR 4 (PIF4)* and *PIF5*, both of which play a positive role in the elongation of hypocotyls.<sup>20</sup> Considering that hypocotyl elongation is regulated by PIF4/PIF5 in a manner dependent on variation in both photoperiod and temperature,<sup>21–26</sup> together with the involvement of PIF4 in warm temperature-induced early flowering in short-day conditions,<sup>27</sup> the EC nighttime repressor appears to be central to ecological adaptation to seasonal variation in photoperiod and temperature by telling not only the diurnal time but also the seasonal time for developmental transition.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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