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Mini Review

Pseudo-Response Regulators (PRRs) or *True* Oscillator Components (TOCs)

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In *Arabidopsis thaliana*, AUTHENTIC RESPONSE REGULATORS (ARRs) act as downstream components of the His-to-Asp phosphorelay (two-component) signaling pathway that is propagated primarily by the cytokinin receptor kinases, AUTHENTIC HIS-KINASES (AHK2, AHK3 and AHK4/CRE1). Thus, this bacterial type of signaling system is essential for responses to a class of hormones in plants. Interestingly, this higher plant has also evolved its own atypical (or unique) variants of two-component signal transducers, PSEUDO-RESPONSE REGULATORS (PRRs). Several lines of recent results suggest that the functions of PRRs are closely relevant to the plant clock (oscillator) that is central to circadian rhythms, the underlying mechanisms of which have long been the subject of debate. Through an overview of recent results, the main issue addressed here is whether or not the pseudo-response regulators (PRRs) are true oscillator components (TOCs).

Keywords: Biological clock — Circadiam rhythm — Flowering time — Photomorphogenesis — Pseudo-response regulator.

Abbreviations: CCA1, CIRCADIAN CLOCK-ASSOCIATED 1; HK, histidine kinase; LHY, LATE ELONGATED HYPOCOTYL; PRR, PSEUDO-RESPONSE REGULATOR; RR, response regulator; TOC1, TIMING OF CAB EXPRESSION 1.

Introduction

Circadian rhythms are endogenously generated in many organisms living on the spinning and revolving world (Loros and Dunlap 2001, Albrecht and Eichele 2003, Salome and McClung 2004). They maintain a period close to 24 h, corresponding to that of the rotation of the earth on its axis. These free-running oscillations are synchronized (or entrained) by certain environmental cues, such as daily light/dark and/or hot/cold cycles. These intrinsic (or genetically determined) biological mechanisms provide organisms with a 'clock and calendar' that make it possible for them to anticipate 'future'. In higher plants, such circadian rhythms are closely relevant to a wide range of biological processes, including movement of organs such as leaves and petals, stomatal opening and diurnal changes in photosynthetic activities (Bunning 1967, Barak et al. 2000, McClung 2000). Recent intensive studies on the model higher plant *Arabidopsis thaliana* have begun to shed light on the mechanisms underlying a variety of circadian-controlled biological events, including the photoperiodicity-dependent control of flowering time (Carre 2001, Eriksson and Millar 2003, Yanovsky and Kay 2003, Hayama and Coupland 2004, Salome and McClung 2004).

To such circadian rhythms, the clock (or oscillator) is central (Somers 2001, Young and Kay 2001). The current best candidates of *Arabidopsis* clock components are CCA1 (CIRCADIAN CLOCK-ASSOCIATED 1) and LHY (LATE ELONGATED HYPOCOTYL), which are homologous single Myb-containing transcription factors (Schaffer et al. 1998, Wang and Tobin 1998, Green and Tobin 1999, Alabadi et al. 2002, Mizoguchi et al. 2002). TOC1 (TIMING OF CAB EXPRESSION 1) is also believed to be another component of the central oscillator (Somers et al. 1998a, Strayer et al. 2000, Alabadi et al. 2001). These two types of clock components have been proposed to form an autoregulatory negative/positive feedback loop at the levels of transcription/translation (Alabadi et al. 2002, Mas et al. 2003a) (Fig. 1), which generates fundamental rhythms as has generally been demonstrated in many other model organisms, including fungi and mice (Loros and Dunlap 2001, Albrecht and Eichele 2003). More specifically, in plants, TOC1 is accumulated in late day and early night (pink line in Fig. 1A), and promotes the transcription of *CCA1* (and *LHY*) (black line, note that hereafter we will not mention the synonymous *LHY* gene for the purpose of clarity of this text). The subsequent rise of CCA1 protein level during early and midday acts to repress the transcription of *TOC1* through direct binding to *cis*-elements of the *TOC1* promoter (Fig. 1B). However, this proposed feedback loop is only a framework onto which other factors must be intensively incorporated.

Such circadian-associated factors include (for instance): photoreceptors (phyA/B and CRY1/2) (Somers et al. 1998b), ELF3 and ELF4 (EARLY FLOWERING 3 and 4) (Covington et al. 2001, Hicks et al. 2001, Doyle et al. 2002), GI (GIGANTEA) (Fowler et al. 1999, Huq et al. 2000), SRR1 (SENSITIVITY TO RED LIGHT REDUCED 1) (Staiger et al. 2003), TIC (TIME FOR COFFEE) (Hall et al. 2003) and a family of flavin-binding proteins including ZTL/ADO1 (ZEITLUPE/ADAGIO 1) and LKP2 (LOV KELCH PROTEIN 2)

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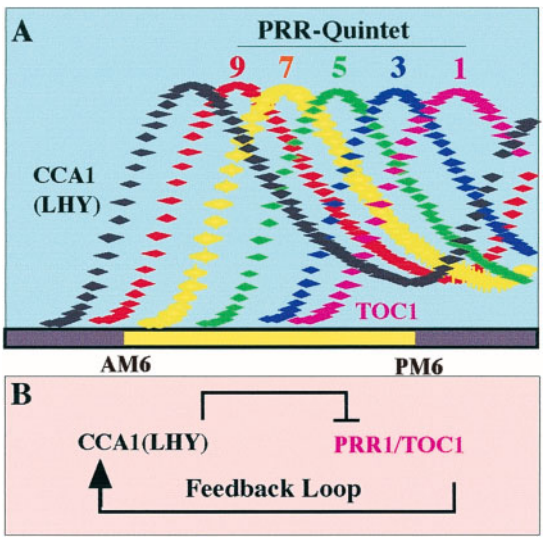

Fig. 1 Circadian waves of the PRR quintet. (A) The diurnal oscillation profiles of the *CCA1* clock gene, and the PRR quintet including the *TOC1* clock gene are shown (these profiles were intended to be solely schematic). (B) The currently consistent model of the CCA1–TOC1 negative/positive feedback circuitry.

(Somers et al. 2000, Jarillo et al. 2001, Schultz et al. 2001, Somers et al. 2004, Yasuhara et al. 2004). Certain enzymes were also implicated as such clock-associated factors, which include a regulatory subunit (CKB3) of casein kinase II (CK2) (Sugano et al. 1999), and poly (ADP-ribo)glycohydrolase (named TEJ) (Panda et al. 2002). Mutational lesions in any one of these clock-associated components somehow (and to various extents) affect clock-controlled biological events (see the references cited above). Nevertheless, the molecular functions of these factors are not yet clear, except for those of the ZTL family of F-box proteins that are involved in degradation of certain target proteins including TOC1 (Mas et al. 2003b, Somers et al. 2004), and the CK2 kinase that is involved in the CCA1 protein phosphorylation (Daniel et al. 2004). It is also certain that a number of as yet unidentified factors are still

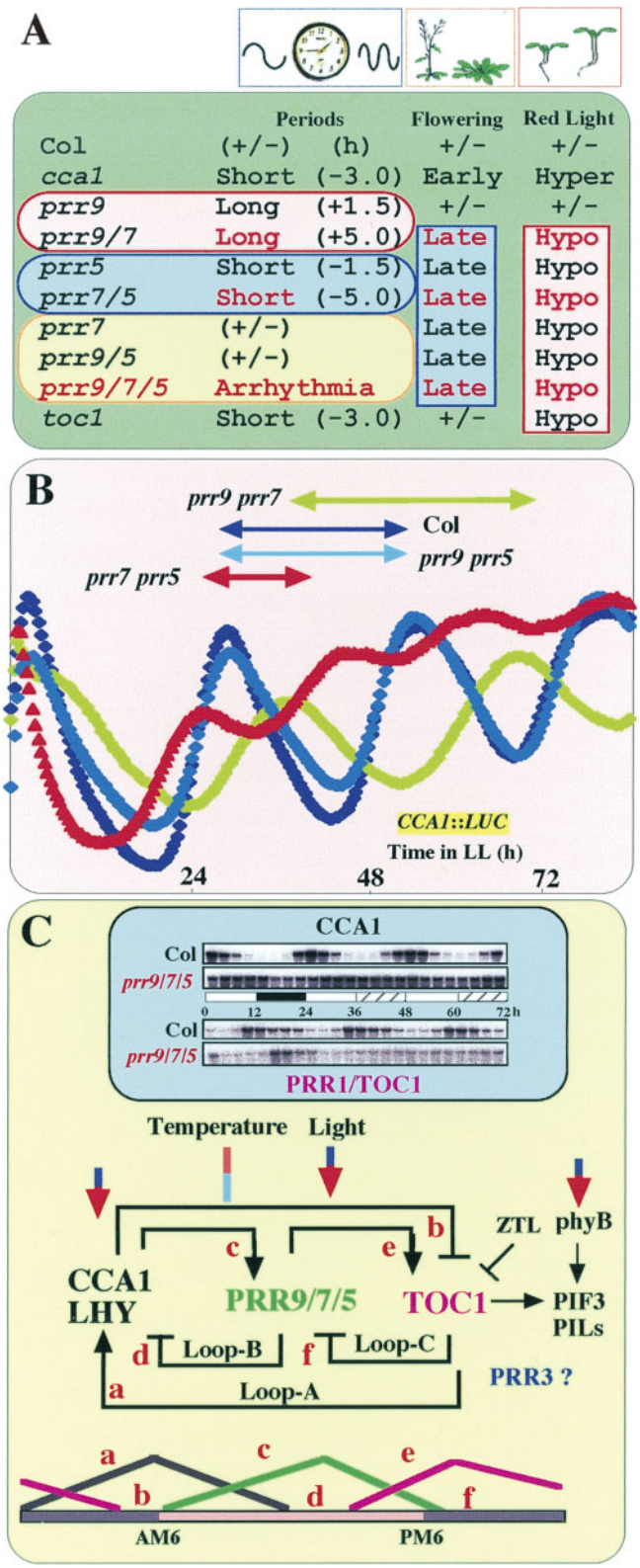

Fig. 3 Proposed views with regard to the PRR9/PRR7/PRR5 circuitry that is interlocked with the CCA–TOC1 clock circuitry. (A) A summarized view of the current genetic data for a set of *prr* mutants (these overviews are intended to be solely schematic). The parental plant is Columbia ecotype (Col). Periods (short or long in LL); flowering (early or late flowering in terms of photoperiodicity); red light (hyper- or hyposensitivity to red light during de-etiolation). (B) Schematized free-running rhythms of *CCA1* in the *prr9 prr7* double mutant, *prr9 prr5* double mutant and *prr7 prr5* double mutant, respectively, which were monitored with transgenic plants carrying *CCA1*::*LUC*. (C) A proposed multi-loop model, into which the PRR9/PRR7/PRR5 circuitry is integrated (details are given in the text). In the upper inset, the expression profiles of CCA1 and PRR1/TOC1 in the *prr9 prr7 prr5* triple mutant are shown. The results clearly showed: (i) arrhythmia in LL; (ii) anomalous phasing in diurnal oscillation in LD; and (iii) that the *CCA1* gene is constitutively transcribed, whereas the transcription of *TOC1* is severely attenuated.

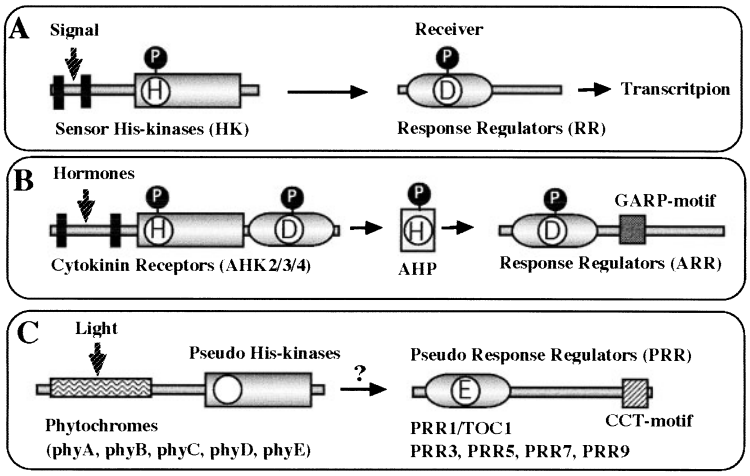

Fig. 2 Conceptual views of two-component signal transducers. (A) A generalized view of a two-component system (histidine kinases and response regulators). (B) A specialized view of the cytokinin-mediated His–Asp phosphorelay in *Arabidopsis thaliana*. AHPs serve as intermediates of the phosphorelay between AHKs and ARRs (Suzuki et al. 1998, Suzuki et al. 2002). (C) A presumed view with regard to pseudo two-component signal transducers, such as phytochromes and the PRR family members of higher plants.

missing from the current list of these clock-associated factors (Onai et al. 2004).

Among putative clock-associated factors, the PRR (PSEUDO-RESPONSE REGULATOR) family of proteins is interesting because TOC1 belongs to this small family (Makino et al. 2000). The PRR family consists of five members (PRR9, PRR7, PRR5, PRR3 and PRR1/TOC1) (Fig. 1A) (Matsushika et al. 2000, Nakamichi et al. 2003, Nakamichi et al. 2004). Another interesting fact is that these PRR proteins are very similar to the so-called bacterial response regulator (RR) in their structural designs (Mizuno 1998) (Fig. 2). Here we will focus only on the PRR family members, which we have mainly been studying. Through an overview of recent studies on PRRs, the main issue addressed here is whether or not the PRRs are true oscillator components (TOCs). It should be noted that there are many excellent reviews, in which the current view as to the CCA1–TOC1 feedback model, the clock-associated components mentioned above and the circadian-controlled biological events have already been discussed extensively (Barak et al. 2000, Somers 2001, Young and Kay 2001, Eriksson and Millar 2003, Yanovsky and Kay 2003, Hayama and Coupland 2004, Salome and McClung 2004).

What are Response Regulators (RRs)?

The so-called two-component system (or histidine-to-asparatate phosphorelay) is a widespread signal transduction mechanism in prokaryotes (Mizuno 1998). A typical two-component system consists of two common signal transducers: a sensor histidine kinase (HK) that serves as a phospho-donor, and a response regulator (RR) containing a common phospho-accepting receiver (Fig. 2A). In general, an HK phosphorylates its cognate RR in response to a certain stimulus, and the resulting phospho-RR acts as an on–off molecular switch to regulate a certain cellular event mainly at the level of transcription. According to this principle, every RR should invariably contain a receiver of about 120 amino acids in which an invariant phospho-accepting aspartate (D) residue is located (Fig. 2A). To date, numerous instances of such two-component signal transduction systems have been uncovered for a wide variety of microorganisms. For instance, the model bacterium *Escherichia coli* has 30 distinctive HK–RR systems, each of which somehow manages a certain cellular response to a given harsh circumstance (Mizuno 1997). Such a classical example is the EnvZ–OmpR two-component system that responds to an external osmotic stress in *E. coli* (Mizuno and Mizushima 1990). Interestingly, not only prokaryotic species but also many eukaryotes have come to employ such two-component systems through the course of evolution, except for vertebrates. Indeed, the model higher plant *A. thaliana* has 11 HKs and 23 RRs (Hwang et al. 2002, Mizuno 2004), among which the ETR1 ethylene receptor (HK) is such a founding example (Chang and Shockey 1999). More recently, three other HKs (AHK2, AHK3 and AHK4/CRE1) were proven to serve as the sensors for a class of hormones, cytokinins (Inoue et al. 2001, Suzuki et al. 2001, Ueguchi et al. 2001, Yamada et al. 2001). Results of recent extensive studies conclusively demonstrated that the AHK-mediated His-to-Asp phosphorelay is the main tactic implicated in the primary responses of this higher plant to cytokinins (Fig. 2B) (Hwang and Sheen 2001, Sheen 2002, Kakimoto 2003, Kiba et al. 2005, and references therein). In this typical phosphorelay in the higher plant, the downstream phospho-accepting RR components are a set of type-B ARRs (*ARABIDOPSIS* RESPONSE REGULATOR) consisting of 11 members (Imamura et al. 1999, Sakai et al. 2000), which function as DNA-binding transcriptional factors (Sakai et al. 2001, Imamura et al. 2003, Tajima et al. 2004). These plant RRs have a common structural design, in which a DNA-binding domain (named the GARP motif) follows an N-terminal receiver domain (Hosoda et al. 2002). Thus, these ARRs are quite authentic in that they look like bacterial RRs (see Fig. 1A, B). In short, the bacterial type of two-component systems appear to be widespread in plants, and AHKs and ARRs are major players implicated in sophisticated signal transduction pathways in response to plant hormones (Mizuno 2004).

What are Pseudo-Response Regulators (PRRs)?

When the entire genome sequences were inspected in order to compile all of the *Arabidopsis* RRs (Imamura et al. 1998, Imamura et al. 1999), it was immediately noticed that this higher plant has a set of genes, each of which at a glance was predicted to encode an RR-like protein (Makino et al. 2000). Nonetheless, these RR-like proteins should be discriminated from authentic RRs in the strict sense that they lack the invariant phospho-accepting Asp residue (replaced by a glutamate residue) (Fig. 2C). These gene products were thus collectively named 'PSEUDO-RR (PRR)'. In this connection, higher plants have a common set of genes, each of which appears to encode a 'PSEUDO-HK' (Fig. 2C) (Schneider-Poetsch 1992). In fact, they are already well known as light signal receptors, named phytochromes (actually PHYs), although they apparently have no HK activity (Quail 2002). Some bacterial species (e.g. cyanobacteria) do indeed employ authentic HKs as certain photo-signal receptors that are capable of phosphorylating their cognate RRs (Kehoe and Grossman 1996, Mizuno et al. 1996). One can thus envisage that higher plants have evolved their own unique variants of two-component signal transducers, which are now adopted for certain light signal transduction pathways. Therefore, it was not surprising when it was revealed that a set of PRRs are implicated in circadian rhythms that are closely relevant to light signal transduction, as will be discussed.

What do PRRs Look Like, and What Are They Doing?

As mentioned above, *Arabidopsis* has a small family of PRRs consisting of five members, whose structural designs are very similar (PRR1, PRR3, PRR5, PRR7 and PRR9) (Matsushika et al. 2000), of which PRR1 is identical to TOC1 (Strayer et al. 2000) (Fig. 2C). They have a receiver-like (or pseudo-receiver) domain at their N-terminal end followed by a long intervening sequence specific for each, which is followed by another common motif of about 50 amino acids at the very C-terminal end (termed the CCT motif: CONSTANS, CONSTANS-like and TOC1). This CCT motif is a plant-specific and widespread motif that is found in many apparently unrelated plant proteins, including the CONSTANS family of proteins (Putterill et al. 1995). We do not know the common molecular function of the PRR/TOC family members, although they are apparently localized in the nuclei (the CCT motif contains a nuclear localization signal) (Makino et al. 2000, Strayer et al. 2000). No evidence has been provided to support the assumption that PRRs are DNA-binding transcription factors. The function of pseudo-receiver domains is also not known, and results of in vitro experiments suggested that they do not undergo Asp phosphorylation (Makino et al. 2000). In short, the molecular functions of PRRs are entirely unknown; however, their biological roles have been well established in connection with circadian rhythms, as will be discussed. It may be noted that *Arabidopsis* has four more PRRs with no CCT motif (e.g. PRR2 and PRR4) (Makino et al. 2000), but they will not be discussed here because there is no evidence that they (PRR-even) are relatives of the PRR-odd family.

A Sign of Clock: the Temporal Transcriptional Profiles of PRRs Are Impressive

When the PRR family genes were uncovered, it was soon examined whether or not the expression of these genes is induced by cytokinins, because the expression of some ARRs (type-A) was known to be markedly and rapidly induced in response to cytokinins (Bradstatter and Kieber 1998, Kiba et al. 1999, Kiba 2005). The results were not reproducible in the sense that the basal levels of PRR transcripts varied considerably from one experiment to another in a cytokinin-independent manner (Makino et al. 2000). Everybody knew that the experimental skill of this investigator was extraordinary reliable, which meant that the expressions of PRRs themselves must keep changing temporarily in plants. This was a sign of diurnal oscillation (or circadian rhythm) in gene expression, which indeed led them to the demonstration that all of these five PRR members are subjected to robust circadian rhythms at the levels of transcription (Matsushika et al. 2000). The transcripts of *PRRs* start accumulating after subjective dawn one after another in the order *PRR9–PRR7–PRR5–PRR3–PRR1* with 2–3 h intervals, and the resulting overall profile was impressive no matter what its meaning (Fig. 1A). Among these *PRRs*, *PRR9* is unique in that its expression in etiolated seedlings was rapidly and transiently induced by white light (or red light) (Makino et al. 2001), the process of which was dependent on phytochromes (Ito et al. 2003, Ito et al. 2005). Such sequential transcriptions are preceded by the rhythm of *CCA1*, resulting in the sequential expression of *CCA1-PRR97531* (Fig. 1A). These phenomena were referred to as 'circadian waves of the PRR quintet', in the hope of finding their biological roles with special reference to the circadian clock. Such an assumption immediately was realized, at least in part, when Kay's group reported that PRR1 is identical to TOC1 (Strayer et al. 2000, Alabadi et al. 2001, Mas et al. 2003a). Thus, the circadian waves are started by the CCA1 clock component, and ended by another clock component TOC1 (Fig. 1) (Alabadi et al. 2002). However, the close relatives of TOC1, namely, PRR9, PRR7, PRR5 and PRR3, are currently not believed to be clock components. However, now this view must be changed slightly.

Recent Genetic Results Tell us Something Important About PRRs

Results of genetic studies provided us with a first insight into whether or not PRRs are implicated in the clock function. Indeed, plants harboring a severe lesion in the *TOC1* gene (*toc1-2* in C24 ecotype) display striking phenotypes with regard to circadian-associated events: short period in constant white light (LL), and arrhythmia in the dark (DD) or in red light (Alabadi et al. 2001, Mas et al. 2003a). Likewise, the circadian clock in plants carrying *cca1 lhy* double lesions is almost out of order (Mizoguchi et al. 2002). During the last few

years, intensive efforts have been made to produce comprehensive pictures as to the phenotypes of certain *prr* mutants (Eriksson et al. 2003, Ito et al. 2003, Kaczorowski and Quail 2003, Michael et al. 2003, Yamamoto et al. 2003, Farre et al. 2005, Nakamichi et al. 2005a, Nakamichi et al. 2005b, Salome and McClung 2005). The phenotypes of transgenic plants each aberrantly overexpressing (or misexpressing) a given *PRR* gene were also examined extensively (Makino et al. 2002, Matsushika et al. 2002a, Matsushika et al. 2002b, Sato et al. 2002, Murakami et al. 2004, Fujimori et al. 2005). These intensive genetic studies were done in accordance with the general idea that mutational lesions in any one of the clock-associated components (if not all) affect not only circadian rhythms at the level of transcription of clock-controlled genes, but also photomorphogenic responses (i.e. sensitivity to red light of the elongation of hypocotyls during de-etiolation) (Deng and Quail 1999) and/or photoperiodicity-dependent control of flowering time (Mouradov et al. 2002). As a result, quite (if not perfectly) consistent genetic data are now available for all kinds of single *prr* mutants, several double and triple mutants, and also all types of misexpressing transgenic lines. These results were somewhat complicated, but these do not need to be explained in detail here because they have already been summarized in previous and accompanying reports (Murakami et al. 2004, Nakamichi et al. 2005b). To support the ideas discussed in the next section, a brief and schematic summary of the phenotypes of *prr* mutants is presented (Fig. 3A). In short, the genetic results revealed that mutational lesions in any one of the five *PRR* genes result in perturbations (more or less) of the circadian-associated biological events, including free-running rhythms at the level of transcription, control of flowering time and photomorphogenic responses. So, the question is: are they PSEUDO-response regulators (PRRs) or TRUE oscillator components (TOCs)?

Is the PRR9/7/5 Circuitry Essential for the Clock Function per se?

The results of recent genetic studies have begun to provide a naive answer (Fig. 3), i.e. the plants carrying *prr9 prr7 prr5* triple lesions showed the severe phenotypes with regard to circadian rhythms (Nakamichi et al. 2005b; see also the inset in Fig. 3C): (i) arrhythmia in LL and DD; (ii) anomalous phasing in diurnal oscillation of certain circadian-controlled genes even under the entrainment conditions (in both the light cycle and temperature cycle); and (iii) the *CCA1* gene is constitutively transcribed, and the transcription of *TOC1* is severely attenuated. However, it was also true that PRR9, PRR7 and PRR5 are dispensable (or not essential) in the sense that the circadian-associated phenotypes were marginal in each single mutant (*prr9*, *prr7* or *prr5*) and even in the *prr9 prr5* double mutant (Eriksson et al. 2003, Ito et al. 2003, Yamamoto et al. 2003). These genetic data could formally be explained by assuming that the circadian-associated functions of PRR9/PRR7/PRR5 are essentially redundant. However, a more complicated scenario needed to be envisaged when it was found that the *prr9 prr7* double mutant showed a marked phenotype of long period (Farre et al. 2005, Nakamichi et al. 2005b, Salome and McClung 2005), whereas the *prr7 prr5* double mutant showed a striking phenotype of short period (Nakamichi et al. 2005a) (Fig. 3B). In other words, the mutational lesions of *prr9* and *prr5* were respectively exaggerated in the absence of the PRR7 function, despite the fact that the *prr7* single lesion itself showed a subtle phenotype (if any) with regard to the period (Nakamichi et al. 2005b). These findings suggest that the roles of PRR9/PRR7/PRR5 are overlapping and distinctive. The partially overlapping and clearly distinctive roles of PRR9/PRR7/PRR5 appear to be tightly coupled with each other, cooperatively, complementarily and temporally. It was reasonable to assume that these PRRs coordinately act as 'period-controlling factors'. In other words, the PRR9/7/5 circuitry might serve as a pacemaker that finely tunes the periods of rhythms by either shortening or lengthening depending on certain conditions (for further discussion about this issue, see Fig. 8 in the accompanying paper by Nakamichi et al. 2005b). Furthermore, this presumed PRR9/PRR7/PRR5 circuitry must be tightly coupled to (or interlocked with) the main clock consisting of CCA1 and TOC1 because the circadian clock is almost out of order in plants lacking the PRR9/PRR7/PRR5 circuitry. These ideas are intriguing, when considered with the current view that the positive/negative transcription cycle through *CCA1* and *TOC1* is only a framework onto which other period-controlling factors must be incorporated in order to make it possible for the central oscillator to incorporate time lags of many hours to culminate in circadian 24 h rhythm (see Fig. 3B). Indeed, the PRR9/PRR7/PRR5 circuitry has the ability to control the periods in a very wide range (from ~19 h in *prr7 prr5* to ~30 h in *prr9 prr7*) (Fig. 3).

A Modified Model for the Clock

Here the PRR9/PRR7/PRR5 circuitry was incorporated into the current CCA1–TOC1 single-loop model (Fig. 1). Although we do not know the modes of interaction among the PRR9/PRR7/PRR5 circuitry, it must be tightly coupled with the CCA1–TOC1 feedback loop, as discussed above. Based on the timetable of transcription of these genes (Fig. 1), we assume that the PRR9/PRR7/PRR5 circuitry interlocks with the main loop (loop-A) in such a way that the PRR9/PRR7/PRR5 circuitry forms two other positive/negative loops (loop-B and loop-C). This model is also based on the fact that the *CCA1* gene is constitutively transcribed in the *prr9 prr7 prr5* triple mutant, and the transcription of *TOC1* is severely attenuated (Fig. 3C). The logic behind these multiple positive/negative feedback loops is principally the same as that explained earlier for the CCA1–TOC1 single-loop model (see Introduction), so that one can easily envisage that the sequential transcriptional events (up and down) would occur as schematically illustrated (Fig. 3C, bottom, follow the events denoted by a, b, c, so on). Briefly, TOC1 activates the transcription of *CCA1* (arrow-a),

the accumulated CCA1 represses the transcription of *TOC1* (T bar-b), and consequently the repression of *PRR9/7/5* by TOC1 (T bar-f) is released (or derepressed), concomitantly the derepressed *PRR/9/75* is activated by CCA1 at the level of transcription (arrow-c). The accumulated PRR9/7/5 then represses the transcription of *CCA1* (T bar-d), and thus the repression of *TOC1* by CCA1 is released (T bar-b), and the released TOC1 gene is now activated by PRR9/7/5 (arrow-e). The accumulated TOC1 then represses *PRR9/7/5* (T bar-f) and indirectly derepresses *CCA1* (T bar-d). The derepressed *CCA1* gene is now ready to be activated by TOC1, and then the first event will be repeated once again after ~24 h. These closed and interlocked loops would generate the sustainable rhythms of *CCA1*, *PRR9/7/5* and *TOC1* at the level of transcription. In this way, the PRR9/PRR7/PRR5 circuitry might serve as a pace-maker, which finely tunes circadian rhythms by shortening and/or lengthening the period. Although we do not know the molecular bases of the interlocking loops (B and C), loop-B is compatible with that proposed recently by Farre et al. (2005), in which they demonstrated in vitro that CCA1 binds to the promoters of *PRR9* and *PRR7*. In short, the comprehensive genetic results are best and consistently explained by assuming that the PRR9/PRR7/PRR5 circuitry is tightly interlocked with the main CCA1–TOC1 oscillator.

Does the Multi-loop Model Improve the Original Single-loop Model?

(i) One can predict from the original model that the transcription of *CCA1* would be attenuated in a *toc1* null mutant, whereas it would become constitutive in a TOC1-overexpressing transgenic line (Fig. 1). Nonetheless, we experienced that the transcription of *CCA1*was only partially down-regulated in *toc1-2*, and it was severely repressed in TOC1-overexpressing transgenic plants (Makino et al. 2002). (ii) Based on the single-loop model, the transcription of *CCA1* is expected to be up-regulated in *ztl*, in which TOC1 is accumulated, because ZTL promotes the degradation of TOC1 (Fig. 3B). In fact, *CCA1* was markedly down-regulated in *ztl* (Somers et al. 2004). According to the interlocking multi-loop model, the transcript of *CCA1* may or may not be accumulated in TOC1-overexpessing plants (it is not easy to foresee the meta-stable consequence because in the multi-loop model TOC1 also indirectly activates the repressor of *CCA1*, i.e. PRR9/PRR7/PRR5). (iii) The transcription of *CCA1* is completely derepressed in the *prr9 prr7 prr5* triple mutant, while the transcription of *TOC1* is severely attenuated in the mutant, as mentioned above (Fig. 3C). Such a tight coupling of transcription between the PRR9/PRR7/PRR5 circuitry and the CCA1–TOC1 feedback loop is not predictable from the original model. (iv) Despite the fact that CCA1 and TOC1 apparently play antagonistic roles with each other in the single-loop model, both the *cca1* and *toc1* null mutations each result in the same phenotype of short period (Fig. 3A). This could be explained by assuming that the remaining feedback loop-B in the *toc1* null mutant might be able to generate rhythms with a short period; likewise, the feedback loop-C alone might also be able to do so in the *cca1* null mutant. (v) Finally, the multi-loop model provides more interfaces through which variable signals possibly come into, and go out from the central clock (as indicated in Fig. 3C, see also the following section). For instance, both the *prr9 prr7* double mutant and the *prr7 prr5* double mutant display altered rhythms even under the temperature cycle entrainment conditions (Nakamichi et al. 2005a, Salome and McClung 2005), suggesting that the multi-loop clock might provide such an interface through which the temperature signal is also integrated.

The PRR Family Members are Very Busy Throughout the Life Cycle

In general, mutational lesions in any one of the clock-associated components also affect photomorphogenic responses and/or photoperiodicity-dependent control of flowering time. These pleiotropic phenotypes may be attributed (at least partly) to defects in the circadian clock per se, as has been generally considered (Deng and Quail 1999, Yanovsky and Kay 2003). In accordance with this general view, the plants carrying *prr9 prr7 prr5* triple lesions showed remarkable phenotypes with regard to certain circadian-controlled events (Fig. 3A): (i) they showed a phenotype of late flowering that was no longer sensitive to the photoperiodicity; and (ii) they were blind to red light in the photomorphogenic responses during de-etiolation. These suggest that the PRR9/PRR7/PRR5 circuitry acts positively in the control of flowering time under long-day conditions, and also in the light signal transduction during de-etiolation under red light. In these processes, PRR7 appears to play a prominent role, despite the fact that the *prr7* single mutant itself showed only a marginal phenotype with regard to the period of rhythm per se (Nakamichi 2005b). More puzzlingly, the long period (or delayed phase) *prr9 prr7* double mutant plants and the short period (or advanced phase) *prr7 prr5* double plants showed essentially the same phenotypes (late flowering and hyposensitivity), suggesting that there is no apparent correlation between the intrinsic periods of the clock and the consequences of certain output pathways (Fig. 3A). There are well-documented precedents for such a paradox: the *cca1-1* mutant shows the phenotypes of short period and hypersensitivity, whereas the *toc1-2* mutant displays the phenotypes of short period and hyposensitivity (see Fig. 3A). As mentioned above, the *prr9 prr7 prr5* triple mutans appear to be blind to red light in the photomorphogenic responses, and their photomorphology was very similar to that observed for the photoreceptor *phyB* null mutants (Halliday et al. 1994). Again puzzlingly, the triple mutant shows a phenotype of late flowering, while the *phyB* mutant displays a phenotype of early flowering. Therefore, the molecular mechanisms underling these circadian-associated events are quite complicated, and clarification of these general problems must await further extensive examinations. Meanwhile, it may be noted that the phenotypes of *prr9 prr7*

pr5 are very similar to those (arrhythmia, late flowering and hyposensitivity) of *CCA1*-overexpressing transgenic lines (Daniel et al. 2004) (note that this is consistent with the multi-loop model). Also, the phenotypes of *prr9 prr7 prr5* are similar to those (late flowering, and hyposensitivity) of certain *gi* mutants (Fowler et al. 1999, Huq et al. 2000). Therefore, the PRR9/PRR7/PRR5 circuitry might indirectly affect these output regulatory pathways through interactions with other factors, such as CCA1, GI and TOC1, and/or some other interacting factors (ZTL, LKP2 and PIF3, etc., see below). Alternatively, the PRR9/PRR7/PRR5 circuitry might be bi-functional in the sense that the family members act together as clock components in certain situations, and also individually play roles close to certain output pathways under other circumstances. In any case, the PRR family members are very busy, and have to keep working daily from morning to night and annually from spring to winter throughout the life cycle.

What Are the PRR Family Members Doing Close to or Within the Circadian Clock?

No matter what they are doing, the molecular functions of PRR9/PRR7/PRR5 must be similar to that of TOC1 because their amino acid sequences (or protein structural designs) are very similar to each other. One can at least envisage that these PRRs including TOC1 play overlapping (or common) roles close to the central clock. To provide a hint, it would thus be worth listing some miscellaneous facets with regard to PRRs. (i) The first interesting facet is that ZTL (an F-box protein with an LOV domain: light, oxygen and voltage domain) physically interacts with PRR1/TOC1 and promotes its degradation in a proteasome-dependent manner (Somers et al. 2000, Mas et al. 2003b, Somers et al. 2004). Note also that LKP2 (ZTL homolog) interacts with PRR5 as well as PRR1/TOC1 (Yasuhara et al. 2004). (ii) PRR1/TOC1 has the ability to interact with a small subset of bHLH (basic helix–loop–helix) transcription factors including PIF3/4 (PHYTOCHROME INTERACTING FACTOR 3 and 4) (Ito et al. 2003, Yamashino et al. 2003, Fujimori et al. 2004), both of which in turn interact with phyB (Bauer et al. 2004, Monte et al. 2004). PRR1/TOC1 also interacts with four other homologous bHLH factors, PIL1/2/5/6 (PIF3-LIKE 1/2/5/6), which are implicated in certain circadian-associated light signal transduction pathways (e.g. shade avoidance and control of chlorophyll synthesis) (Salter et al. 2003, Kim et al. 2004). (ii) Finally, results of yeast two-hybrid assays suggested that PRR1/TOC1 and PRR9 form hetero-oligomers, and these interactions are via the homologous pseudo-receiver domains (Ito et al. 2003). It would be of interest to examine whether or not other PRRs form hetero-oligomers (or complexes) in certain combinations in plants. Taken together with another fact (iv) that the transcription of PRR9 is markedly induced by light in a phy-dependent manner (Ito et al. 2003, Ito et al. 2005), these miscellaneous facets suggest that PRRs, phytochromes, light-regulated F-box proteins and light-regulated bHLH transcription factors might coordinately play roles close to light signal transduction (input/output) pathways through formation of a network of protein–protein interactions (see Fig. 3C). This network might also be important for the clock function per se.

Lonely and Poor PRR3

Among the PRR family members, PRR3 is the least characterized. It was observed that the phenotype of a *prr3* allele was subtle (Michael et al. 2003), but that in fact it carries a T-DNA insertion at the very 3′ end of the coding sequence (more appropriate *prr3* alleles are needed). Meanwhile, PRR3-overexpressing transgenic plants were characterized, showing that they displayed the phenotypes of late flowering and hyposensitivity (Murakami et al. 2004), which were quite in contrast to those (early flowering and hypersensitivity) observed for PRR5-overexpressing plants (Sato et al. 2002). In contrast to other PRR members, PRR3 thus appears to serve as a negative regulator in the relevant signaling pathways, suggesting that PRR3 might play a crucial role distinctive from (or antagonistic to) those of other PRR members. In this respect, it may also be noted that PRR3 is specifically phosphorylated by a novel protein kinase belonging to the WNK family (Murakami-Kojima et al. 2002). Thus, the lonely PRR3 must eventually be integrated into the drama played by the PRR family members.

Catch the Family in the Rye Before the Sun also Sets

Recent results strongly support the view that dicotyledonous and monocotyledonous (e.g. rice and rye) plants share the evolutionarily conserved molecular mechanism underlying the photoperiodicity-dependent control of flowering (or heading) time (Hayama and Coupland 2004, and references therein). This implies that the clock function per se might also be conserved in both types of distantly related species. Indeed, rice also has exactly five members of the OsPRR family (*Oryza sativa* pseudo-response regulator) (Murakami et al. 2003). The expression of these evolutionarily conserved *OsPRR* genes is also under the control of circadian rhythm in such a manner that they are expressed in the order *OsPRR73* (*OsPRR37*), *OsPRR95* (*OsPRR59*) and finally *OsTOC1*. When the rice *TOC1* gene was aberrantly expressed in *A. thaliana*, the circadian rhythms in the weeds were severely perturbed (our unpublished data), suggesting that the OsPRR family members most probably play important roles close to the crop circadian clock, which might also be relevant to the control of heading date (Murakami et al. 2005). It may also be noted that an authentic rice type-B ARR (named Ehd1) is implicated in the control of heading date (Doi et al. 2004).

Conclusions

According to the spirit (but not the philosophy) of Zen, if TOC one is one of the TOCs, PRRs are also really TOCs; if PRRs are not TOCs, TOC one is solely one of the PRRs. According to the philosophy of modern sciences, such an ori-

ental rhetoric is nonsense. Therefore, the scientific questions are: what is the common molecular function of PRRs? How do they exert their overlapping and distinctive roles coordinately, complementarily and temporally? How do they interact with other clock-associated components, CCA1/LHY, ELF3/4, GI, PHYs, PIFs, ZTL/LKP2, etc?. Finally, how do they regulate the period of circadian rhythms, the timing of flowering and the sensitivity to light? Clarification of these problems must await further examinations. Meanwhile, the multi-loop model, into which PRRs are integrated on purpose, might provide us with a platform on which we can address the issues mentioned above. The spirit of Zen also implies that a simple model is best (Fig. 1), but also two alternatives are better than only one (Fig. 3).

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References

Alabadi, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Mas, P. and Kay, S.A. (2001) *Science* 293: 880–883.

Alabadi, D., Yanovsky, M.J., Mas, P., Harmer, S.L. and Kay, S.A. (2002) *Curr. Biol.* 12: 757–761.

Albrecht, U. and Eichele, G. (2003) *Curr. Opin. Genet. Dev. Dev.* 13: 271–277.

Barak, S., Tobin, E.M., Andronis, C., Sugano, S. and Green, R.M. (2000) *Trends Plant Sci.* 5: 517–522.

Bauer, D., Viczian, A., Kircher, S., Nobis, T., Nitschke, R., et al. (2004) *Plant Cell* 16: 1433–1445.

Bradstatter, I. and Kieber, J.J. (1998) *Plant Cell* 10: 1009–1019.

Bunning, E. (1967) The Physiological Clock. The Heidelberg Science Library, Springer-Verlag, New York.

Carre, I.A. (2001) *J. Biol. Rhythms* 16: 415–423.

Chang, C. and Shockey, J.A. (1999) *Curr. Opin. Plant Biol.* 2: 352–358.

Covington, M.F., Panda, S., Liu, X.L., Strayer, C.A., Wagner, D.R. and Kay, S.A. (2001) *Plant Cell* 13: 1305–1316.

Daniel, X., Sugano, S. and Tobin, E.M. (2004) *Proc. Natl Acad. Sci. USA* 101: 3292–3297.

Deng, X.W. and Quail, P.H. (1999) *Semin. Cell Dev. Biol.* 10: 121–129.

Doi, K., Izawa, T., Fese, T., Yamanouchi, U., Kubo, T., Shimatani, Z., Yano, M. and Yoshimura, A. (2004) *Genes Dev.* 15: 926–936.

Doyle, M.R., Davis, S.J., Bastow, R.M., McWatters, H.G., Kozma-Bognar, L., Nagy, F., Millar, A.J. and Amasino, R.M. (2002) *Nature* 419: 74–77.

Eriksson, M.E., Hanano, S., Southern, M., Hall, A. and Millar, A.J. (2003) *Planta* 218: 159–162.

Eriksson, M.E. and Millar, A.J. (2003) *Plant Physiol.* 132: 732–738.

Farre, M.E., Harmer, S.L., Harmon, F.G., Yanovsky, M.J. and Kay, S.A. (2005) *Curr. Biol.* 15: 47–54.

Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Morris, B., Coupland, G. and Putterill, J. (1999) *EMBO J.* 18: 4679–4688.

Fujimori, T., Sato, E., Yamashino, T. and Mizuno, T. (2005) *Biosci. Biotechnol. Biochem.* 69: 426–430.

Fujimori, T., Yamashino, T., Kao, T. and Mizuno, T. (2004) *Plant Cell Physiol.* 45: 1078–1086.

Green, R.M. and Tobin, E.M. (1999) *Proc. Natl Acad. Sci. USA* 96: 4176–4179.

Hall, A., Bastow, R.M., Davis, S.J., Hanano, S., McWatters, H.G., et al. (2003) *Plant Cell* 15: 2719–2729.

Halliday, K.J., Koorneef, M. and Whitelam, G.C. (1994) *Plant Physiol.* 104: 1311–1315.

Hayama, R. and Coupland, G. (2004) *Plant Physiol.* 135: 677–684.

Hicks, K.A., Albertson, T.M. and Wagner, D.R. (2001) *Plant Cell* 13: 1281–1292.

Hosoda, K., Imamura, A., Katoh, E., Hatta, T., Tachiki, M., Yamada, H., Mizuno, T. and Yamazaki, T. (2002) *Plant Cell* 14: 2015–2029.

Huq, E., Tepperman, J.M. and Quail, P.H. (2000) *Proc. Natl Acad. Sci. USA* 97: 9789–9794.

Hwang, I., Chen, H.-I. and Sheen, J. (2002) *Plant Physiol.* 129: 500–515.

Hwang, I. and Sheen, J. (2001) *Nature* 413: 383–389.

Imamura, A., Hanaki, N., Nakamura, A., Suzuki, T., Taniguchi, M., Kiba, T., Ueguchi, C., Sugiyama, T. and Mizuno, T. (1999) *Plant Cell Physiol.* 40: 733–742.

Imamura, A., Hanaki, N., Umeda, H., Nakamura, A., Suzuki, T., Ueguchi, C. and Mizuno, T. (1998) *Proc. Natl Acad. Sci. USA* 95: 2691–2696.

Imamura, A., Kiba, T., Tajima, Y., Yamashino, T. and Mizuno, T. (2003) *Plant Cell Physiol.* 44: 122–131.

Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., Tabata, S., Shinozaki, K. and Kakimoto, T. (2001) *Nature* 409: 1060–1063.

Ito, S., Matsushika, A., Yamada, H., Sato, S., Kato, T., Tabata, S., Yamashino, T. and Mizuno, T. (2003) *Plant Cell Physiol.* 44: 1237–1245.

Ito, S., Nakamichi, N., Matsushika, A., Fujimori, T., Yamashino, T. and Mizuno, T. (2005) *Biosci. Biotechnol. Biochem.* 69: 382–390.

Jarillo, J.A., Capel, J., Tang, R.H., Yang, H.Q., Alonso, J.M. and Cashmore, A.R. (2001) *Nature* 410: 487–490.

Kaczorowski, K.A. and Quail, P.H. (2003) *Plant Cell* 15: 2654–2665.

Kakimoto, T. (2003) *Annu. Rev. Plant Biol.* 54: 605–627.

Kehoe, D.M. and Grossman, A.R. (1996) *Sience* 273: 1409–1412.

Kiba, T., Naitou, T., Koizumi, N., Yamashino, T., Sakakibara, H. and Mizuno, T. (2005) *Plant Cell Physiol.* 46: 339–355.

Kiba, T., Taniguchi, M., Imamura, A., Ueguchi, C., Mizuno, T. and Sugiyama, T. (1999) *Plant Cell Physiol.* 40: 767–771.

Kim, O.-E., Park, J., Kim, J.-I., Kan, C. and Choi, G. (2004) *Plant Cell* 16: 3045–3058.

Loros, J.J. and Dunlap, J.C. (2001) *Annu. Rev. Physiol.* 63: 757–794.

Makino, S., Kiba, T., Imamura, A., Hanaki, N., Nakamura, A., Suzuki, T., Taniguchi, M., Ueguchi, C., Sugiyama, T. and Mizuno, T. (2000) *Plant Cell Physiol.* 41: 791–803.

Makino, S., Matsushika, A., Kojima, M., Oda, Y. and Mizuno, T. (2001) *Plant Cell Physiol.* 42: 334–339.

Makino, S., Matsushika, A., Kojima, M., Yamashino, T. and Mizuno, T. (2002) *Plant Cell Physiol.* 43: 58–69.

Mas, P., Alabadi, D., Yanovsky, M.J., Oyama, T. and Kay, S.A. (2003a) *Plant Cell* 15: 223–236.

Mas, P., Kin, W.Y., Somers, D.E. and Kay, S.A. (2003b) *Nature* 426: 567–570.

Matsushika, A., Imamura, A., Yamashino, T. and Mizuno, T. (2002b) *Plant Cell Physiol.* 43: 833–843.

Matsushika, A., Makino, S., Kojima, M. and Mizuno, T. (2000) *Plant Cell Physiol.* 41: 1002–1012.

Matsushika, A., Makino, S., Kojima, M., Yamashino, T. and Mizuno, T. (2002a) *Plant Cell Physiol.* 43: 118–122.

McClung, C.R. (2000) *Physiol. Plant.* 109: 359–371.

Michael, T.P., Salome, P.A., Yu, H.J., Spencer, T.R., Sharp, E.L., McPeek, M.A., Alonso, J.M., Ecker, J.R. and McClung, C.R. (2003) *Science* 302: 1049–1053.

Mizoguchi, T., Wheatley, K, Hanzawa, Y., Wright, L., Mizoguchi, M., Song, H.-R., Carre, I.A. and Coupland, G. (2002) *Dev. Cell* 2: 629–641.

Mizuno, T. (1997) *DNA Res.* 4: 161–168.

Mizuno, T. (1998) *J. Biochem. (Tokyo)* 123: 555–563.

Mizuno, T. (2004) *Curr. Opin. Plant Biol.* 7: 499–505.

Mizuno, T., Kaneko, T. and Tabata, S. (1996) *DNA Res.* 3: 407–414.

Mizuno, T. and Mizushima, S. (1990) *Mol. Microbiol.* 4: 1077–1082.

Monte, E., Tepperman, J.M., Al-Sady. B., Kaczorowski, K.A., Alonso, J.M., Ecker. J.R., Li, J.R., Zhang, Y. and Quail, P.H. (2004) *Proc. Natl Acad. Sci. USA* 101: 16091–19098.

Mouradov, A., Cremer, F. and Coupland, G. (2002) *Plant Cell* 14: S111–S130.

Murakami, M., Ashikari, M., Miura, K., Yamashino, T. and Mizuno, T. (2003) *Plant Cell Physiol.* 44: 1229–1236.
Murakami, M., Matsushika, A., Ashikari, M., Yamashino, T. and Mizuno, T. (2005) *Biosci. Biotechnol. Biochem.* 69: 410–414.
Murakami, M., Yamashino, T. and Mizuno, T. (2004) *Plant Cell Physiol.* 45: 645–650.
Murakami-Kojima, M., Nakamichi, N., Yamashino, T. and Mizuno, T. (2002) *Plant Cell Physiol.* 43: 675–683.
Nakamichi, N., Ito, S., Oyama, T., Yamashino, T., Kondo, T. and Mizuno, T. (2004) *Plant Cell Physiol.* 45: 57–67.
Nakamichi, N., Kita, M., Ito, S., Sato, E., Yamashino, T. and Mizuno, T. (2005a) *Plant Cell Physiol.* 46: 609–619.
Nakamichi, N., Kita, M., Ito, S., Yamashino, T. and Mizuno, T (2005b) *Plant Cell Physiol.* 46: 686–698.
Nakamichi, N., Matsushika, A., Yamashino, T. and Mizuno, T. (2003) *Plant Cell Physiol.* 44: 360–365.
Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-Ike, N. and Ishiura, M. (2004) *Plant J.* 40: 1–11.
Panda, S., Poirier, G.G. and Kay, S.A. (2002) *Dev. Cell* 3: 51–61.
Putterill, J., Robson, F., Lee, K., Simon, R. and Coupland, G. (1995) *Cell* 80: 847–857.
Quail, P.H. (2002) *Nat. Rev. Mol. Cell. Biol.* 3: 85–93.
Sakai, H., Aoyama, T. and Oka, A. (2000) *Plant J.* 24: 703–11.
Sakai, H., Honma, T., Aoyama, T., Sato, S., Kato, T., Tabata, S. and Oka, A. (2001) *Science* 294: 1519–1521.
Salome, P.A. and McClung, C.R. (2004) *J. Biol. Rhythms* 19: 425–435.
Salome, P.A. and McClung, C.R. (2005) *Plant Cell* 17: 791–803.
Salter, M.G., Franklin, K.A. and Whitelam, G.C. (2003) *Nature* 426: 680–683.
Sato, E., Nakamichi, N., Yamashino, T. and Mizuno, T. (2002) *Plant Cell Physiol.* 43: 1374–1385.
Schaffer, R., Ramsay, N., Samach, A., Corden, S., Putterill, J., Carre, I.A. and Coupland, G. (1998) *Cell* 93: 1219–1229.
Schneider-Poetsch, H.A. (1992) *Photochem. Photobiol.* 56: 836–846.
Schultz, T.F., Kiyosue, T., Yanovsky, M., Wada, M. and Kay, S.A. (2001) *Plant Cell* 13: 2659–2670.
Sheen, J. (2002) *Science* 296: 1650–1652.
Somers, D.E. (2001) *Philos. Trans. R. Soc. Lond. Ser. B* 356: 1745–1754.
Somers, D.E., Devlin, P.F. and Kay, S.A. (1998b) *Science* 282: 1488–1490.
Somers, D.E., Kim, W.-Y. and Geng, R. (2004) *Plant Cell* 16: 769–782.
Somers, D.E., Schultz, T.F., Milnamow, M., and Kay, S.A. (2000) *Cell* 101: 319–329.
Somers, D.E., Webb, A.A.R., Pearson, M. and Kay, S.A. (1998a) *Development* 125: 485–494.
Staiger, D., Allenbach, L., Salathia, N., Fiechter, V., Davis, S.J., Millar, A.J. Chory, J. and Fankhauser, C. (2003) *Genes Dev.* 17: 256–269.
Strayer, C., Oyama, T., Schultz, T.F., Raman, R. Somers, D.E., Mas, P., Panda, S., Kreps, J.A. and Kay, S.A. (2000) *Science* 289: 768–771.
Sugano, S., Andronis, C., Org, M.S., Green, R.M. and Tobin, E.M. (1999) *Proc. Natl Acad. Sci. USA* 96: 1362–12366.
Suzuki, S., Miwa, K., Ishikawa, K., Yamada, H., Aiba, H. and Mizuno, T. (2001) *Plant Cell Physiol.* 42: 107–113.
Suzuki, T., Imamura, A., Ueguchi, C. and Mizuno, T. (1998) *Plant Cell Physiol.* 39: 1258–1268.
Suzuki, T., Ishikawa, K., Yamashino, T. and Mizuno, T. (2002) *Plant Cell Physiol.* 43: 123–129.
Tajima, Y., Imamura, A., Kiba, T., Amano, Y., Yamashino, T. and Mizuno, T. (2004) *Plant Cell Physiol.* 45: 28–39.
Ueguchi, C., Koizumi, H., Suzuki, T. and Mizuno, T. (2001) *Plant Cell Physiol.* 42: 231–235.
Wang, Z.-Y. and Tobin, E.M. (1998) *Cell* 93: 1207–1217.
Yamada, H., Suzuki, T., Terada, K., Takei, K., Ishikawa, K., Miwa, K. and Mizuno, T. (2001) *Plant Cell Physiol.* 42: 1017–1023.
Yamamoto, Y., Sato, E., Shimizu, T., Nakamich, N., Sato, S., Kato, T., Tabata, T., Nagatani, A., Yamashino, T. and Mizuno, T. (2003) *Plant Cell Physiol.* 44: 1119–1130.
Yamashino, T., Matsushika, A., Fujimari, T., Sato, S., Kato, T., Tabata, S. and Mizuno, T. (2003) *Plant Cell Physiol.* 44: 619–629.
Yanovsky, M. and Kay, S.A. (2003) *Nat. Rev. Mol. Cell. Biol.* 4: 265–276.
Yasuhara, M., Mitsui, S., Hirano, H., Takanabe, R, Tokioka, Y., Ihara, N., Komatsu, A., Seki, M., Shinozaki, K. and Kiyosue, T. (2004) *J. Exp. Bot.* 55: 2015–2027.
Young, M.W. and Kay, S.A. (2001) *Nat. Rev. Genet.* 2: 702–715.

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