Daylength Measurements by Rice Plants in Photoperiodic Short-Day Flowering

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Plants set seed at appropriate seasons. One major mechanism responsible for this adaptation involves photoperiodic flowering. Most plants are classified as either long-day plants, which flower under a longer photoperiod, or short-day plants, which flower under a shorter photoperiod. A third group, day-neutral plants, is not responsive to changes in photoperiod. During the past decade, molecular analysis has revealed at the molecular level how the long-day plant *Arabidopsis thaliana* measures daylength in photoperiodic flowering. In contrast, the molecular mechanisms underlying the responses of short-day plants are still under investigation. Progress in understanding photoperiodic flowering in rice (*Oryza sativa*), a short-day plant, revealed unique, evolutionarily conserved pathways involved in photoperiodic flowering at the molecular level. Furthermore, the conserved pathways promote flowering under short-day conditions and suppress flowering under long-day conditions in rice, but promote flowering under long-day conditions in *Arabidopsis*. In this chapter, we discuss the molecular mechanisms responsible for short-day flowering in rice in comparison with long-day flowering in *Arabidopsis*.

KEY WORDS: Photoperiodic flowering, Rice, Short-day plants, Comparative biology.

I. Introduction: History of Studies on Photoperiodic Flowering Before Molecular Cloning

Photoperiodic flowering was first reported by Garner and Allard in the 1920s (Garner and Allard, 1920, 1923). Their research revealed that many plants flowered during appropriate seasons regardless of the timing of sowing. They

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subsequently demonstrated that flowering of these plants was controlled by photoperiod and categorized the plants into two groups: short-day plants and long-day plants (Table I). Subsequent physiological analysis of a range of species revealed that many short-day plants possess a critical threshold for the daylength (Thomas and Vince-Prue, 1997). For instance, some short-day plants, such as *Xanthium* spp., can detect differences of around 15 min/day and can use these differences to determine whether they should flower (Thomas and Vince-Prue, 1997). Further analysis led to speculation that short-day plants actually detected the length of the night rather than daylength. This idea arose from observations of a phenomenon termed the "night-break response," in which a short light pulse during the night clearly inhibited flowering in many short-day plants, suggesting that the duration of the dark period was the critical cue for detecting short days (Hamner and Bonner, 1938). Whether short-day plants recognize the length of the night is not yet known.

From the 1940s to the 1960s, many models were proposed to explain the mechanism for daylength measurement. Two major early types of models, the hourglass and clock models, were often discussed. The hourglass models were based on cumulative signaling mediated by photoreceptors such as phytochromes (Parker *et al.*, 1946). In contrast, the clock models were based on a hypothesized internal time-keeping mechanism, such as a circadian clock system, although the existence of such mechanisms was unproven at the time (Bünning, 1960). Currently, we know that neither model is fully supported by the modern molecular model of photoperiodic flowering. Toward the end of this period, Pittendrigh and his colleagues (Pittendrigh and Minis, 1964) refined the traditional Bünning clock model hypothesis (Bünning, 1960), and proposed a new mechanism named the "external coincidence" model (Fig. 1). In this model, signals mediated by photoreceptors are first gated by phases determined by circadian clocks and transmitted into actions that lead to induction or suppression of flowering. The circadian clock systems are also entrained (synchronized) by environmental cues, including

TABLE I
Three Types of Plants Based on Their Photoperiodic Flowering Responses

Class	Main types	Photoperiodic conditions that induce flowering	Thresholds on critical daylength	Night-break response
1	Short-day plants	Shorter days	Sharp	Sensitive
2	Long-day plants	Longer days	Generally, dull	Not sensitive
3	Day-neutral plants	No response	–	No response

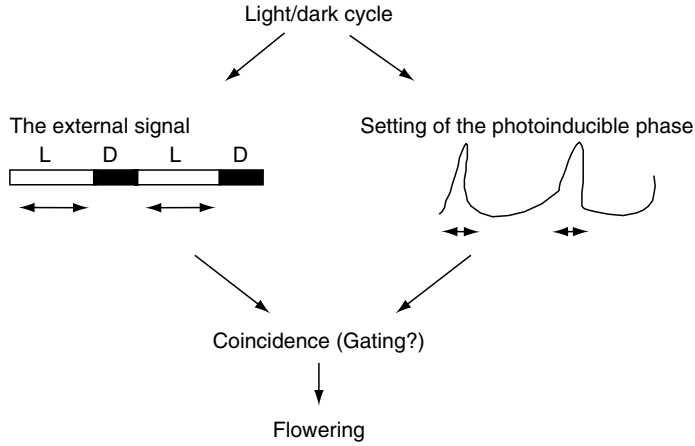

FIG. 1 The external coincidence model. In 1964, Dr. Pittendrigh and his colleagues proposed this model to explain how plants recognize daylength in their photoperiodic response. They proposed this model based on phase setting of the circadian clock in *Drosophila* by various light conditions. (A scheme based on the model in Pittendrigh and Minis, 1964.)

light or photoperiod. In this model, the interaction between acute light signals and circadian clock systems is the most important factor in detecting daylength. Molecular analyses have basically supported this model, as described later in this chapter. The main competing model at the time was named the ''internal coincidence'' model, in which two independent internal rhythms interact to detect daylength, although no molecular evidence has supported this model yet (Thomas, 1998).

In the mid-1980s, Lumsden and Furuya (1986) performed physiological experiments that demonstrated that two distinct responses to light, a kind of phase response and some acute response to light, were involved in flowering in *Pharbitis nil*, a short-day plant. This work clearly supported the external coincidence model. Therefore, biochemical, genetic, or combined approaches were needed to provide the molecular biological details behind the model.

II. Photoperiodic Flowering in Plants

A. Photoperiodic Flowering in *Arabidopsis*

To compare the molecular mechanisms responsible for photoperiodic flowering in rice, a short-day plant, with those in long-day plants, it is helpful to summarize progress toward understanding the molecular mechanisms

responsible for photoperiodic flowering in a well-studied model plant. In this section, I discuss our knowledge of these mechanisms in *Arabidopsis thaliana*, a long-day plant (Fig. 2).

Molecular genetics revealed four distinct flowering pathways in *Arabidopsis*: long-day promotion, autonomous promotion, vernalization repression, and gibberellic acid (GA)-mediated promotion (Mourdov *et al.*, 2002; Simpson and Dean, 2002). It should be noted that extensive screening of mutants before the molecular cloning era contributed tremendously to progress in understanding photoperiodism in *Arabidopsis* (Koorneef *et al.*, 1991). Here, photoperiodic flowering in *Arabidopsis* is mainly explained by the long-day flowering promotion pathway. In this pathway, a transcriptional activator named *CONSTANS* (*CO*) is a key controller. *CO* mRNA is mainly expressed at night under the control of the circadian clock system, regardless of photoperiod (Suarez-Lopez *et al.*, 2001). As a result, *CO* is considered to be an output of the circadian clock. Circadian clocks are believed to consist of a negative feedback loop between transcription and translation in *Arabidopsis* (Yanovsky and Kay, 2003). Currently, *TIMING OF CAB EXPRESSION 1* (*TOC1* or *APRR1*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*)/*LATE AND ELONGATED HYPOCOTYL* (*LHY*) genes are

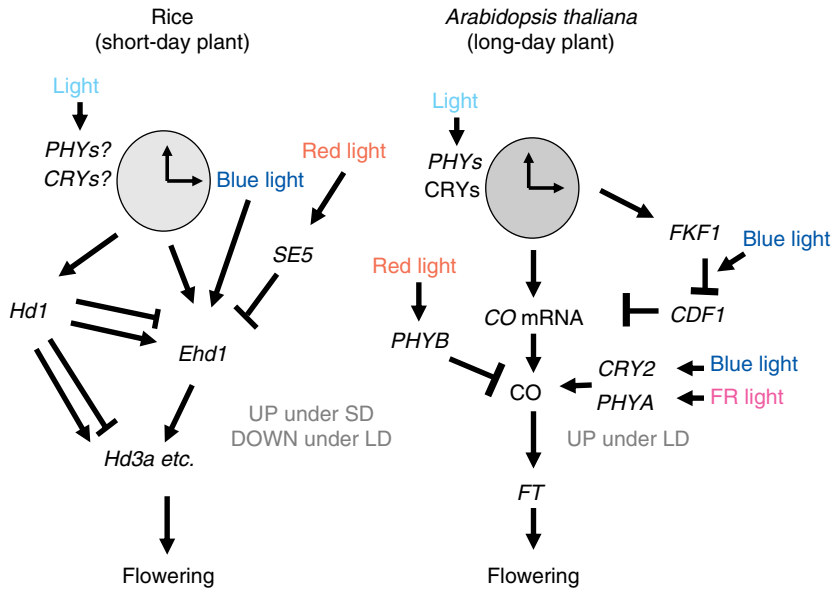

FIG. 2 A comparison of the gene network responsible for photoperiodic regulation of flowering in rice and *Arabidopsis*. The modes of action of light signals that have been identified thus far are highlighted.

known to be active in this feedback loop (Alabadi *et al.*, 2001; Green and Tobin, 2002; Strayer *et al.*, 2000). Other paralogous members of *TOC1* (*APRR3*, *5*, *7*, and *9*) have been shown to be involved in this loop (Mizuno and Nakamichi, 2005; Nakamichi *et al.*, 2005). A cloned Myb transcriptional factor, *LUX ARRHYTHMO* (*LUX*)/*PHYTOCLOCK1*(*PCL1*), may be involved in this loop (Hazen *et al.*, 2005; Onai and Ishiura, 2005). Among the genetic factors that surround the main loop, *EARLY FLOWERING 3* (*ELF3*) and *GIGANTEA* (*GI*) are expressed mainly at dusk and maintain the core circadian loop (Covington *et al.*, 2001; Fowler *et al.*, 1999; Hicks *et al.*, 2001; Parks *et al.*, 1999). *ELF3* is required for gating of the circadian clock, a process in which light signals are transmitted phase-dependently to control acute gene expression by light (McWatters *et al.*, 2000). This gating is also involved in entrainment of the circadian clock. The early flowering and photoperiod-independent phenotype of *elf3* mutants are explained by derepression of *CO* mRNA in the mutants (Suarez-Lopez *et al.*, 2001). *GI* is believed to be related more directly to *CO* expression, since *gi* mutants flowered late, were insensitive to photoperiodic changes, and exhibited severe reduction of *CO* expression. However, mechanisms for this gene expression have not yet been revealed. In another study, circadian clocks were entrained by environmental light signals mediated by phy and cry photoreceptors (Somers *et al.*, 1998). This indicates that *CO* expression is indirectly regulated by phys and crys through circadian clock phasing, a regulation that is relatively tolerant to genetic defects because phys and crys function redundantly.

CO can promote *FLOWERING LOCUS T* (*FT*) expression only at dusk under long-day conditions (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999; Yanovsky and Kay, 2002). In contrast, *FT* is not expressed under short-day conditions. This regulation of *FT* is the true molecular nature of photoperiodic flowering in *Arabidopsis*. This *FT* expression by *CO* is mediated by two molecular mechanisms. One is repression of *CO* mRNA transcription by the *CDF1* transcription factor, and *CDF1* interacts with the *FKF1* F-box with a LOV domain (a blue light reception domain) and is degraded to express *CO* only at dusk under long-day conditions (Imaizumi *et al.*, 2003, 2005). The second mechanism is regulation of CO protein stability (Valverde *et al.*, 2004). CO is rapidly degraded in darkness by unknown mechanisms, but degraded in the morning and day-time by phyB, however, stabilized by blue and far-red light signals mediated by *cry2* and *phyA,* respectively, only at dusk under long-day conditions. In contrast, under short-day conditions, *CO* is expressed only slightly due to repression by CDF1, and CO protein is rapidly degraded by phyB and the dark signals; however, the *cry2* and *phyA* signals do not function at dusk under short-day conditions.

Both *CO* and *FT* are expressed in the sieve elements of vascular bundles (An *et al.*, 2004; Takeda and Goto, 2003), but *FT* is not expressed in

provisional floral meristems (Abe *et al.*, 2005; Wigge *et al.*, 2005). Therefore, there should be some long-distance signals that cause transitions from an inflorescent meristem into a floral meristem. The *FD* gene has been cloned (Abe *et al.*, 2005; Wigge *et al.*, 2005). In the work, several lines of evidence show that *FD* functions with *FT* to induce flowering, and physical interactions between *FD* and *FT* suggest that *FD* is a direct target of *FT* products. However, the *FD* gene is expressed only at the shoot apical meristem (SAM), but not in the sieve elements. It has been suggested that *FT* could be a part of florigen, a proposed phytohormone that induces flowering, and that *FT* mRNA or protein may move to the SAM from the sieve elements (Abe *et al.*, 2005; Wigge *et al.*, 2005). Genetic analysis revealed that *FT* and *FD* together induce *APETALLA 1* (*AP1*) expression, which is an early factor in the ABC models, in which floral organ formation is genetically explained in *Arabidopsis*. *FT* mRNA has been shown to move into SAM through the sieve elements (Huang *et al.*, 2005) .

B. Quantitative Trait Locus (QTL) Analysis for Photoperiodic Flowering in Rice

When researchers try to clone a gene from a mutant line by map-based cloning, extra QTLs in the segregating populations may make it difficult to map the target gene. This happened when researchers tried to clone the flowering-time genes in *Arabidopsis* because popular parent accessions such as Col and L*er* unexpectedly contained major QTLs in vernalization pathways such as those for *FLC* and *FRIGDA* (*FRI*) (Lee *et al.*, 1993). In contrast, QTL analysis of flowering-time genes in rice performed by Dr. M. Yano's group in Tsukuba, Japan, contributed greatly toward identifying the genetic networks (Yano, 2001). In contrast, flowering-time genes in rice mutants have not yet been extensively screened. When plants have adapted to local regions, flowering time is a major trait involved in this adaptation, and the adaptation process results in natural genetic variation in the species. In addition, the rice breeding process may have increased the variation in flowering time to produce cultivars suitable for a wide range of cultivation conditions. Therefore, QTL analysis could reveal how natural selection, domestication, and breeding have proceeded in rice.

Dr. Yano and his colleagues identified at least 14 QTLs from a cross between *indica* and *japonica* rice subspecies. In the F_2 population, they identified five QTLs, *Hd1* through *Hd5* (Yamamoto *et al.*, 1998; Yano *et al.*, 1997). Subsequently, they identified another three QTLs (*Hd7*, *Hd8*, and *Hd11*) using BC_1F_5 lines (Lin *et al.*, 1997, 1998). Furthermore, they identified *Hd6*, *Hd9*, *Hd10*, *Hd12*, *Hd13*, and *Hd14* only by using advanced backcross progeny, such as BC_3F_2 or BC_4F_2 (Lin *et al.*, 2002, 2003; Yamamoto *et al.*, 2000). The epistatic relationship among QTLs may sometimes mask the contribution of the

interacting QTLs in certain genetic populations. Therefore, advanced backcross progeny should be carefully searched to find such QTLs (Yamamoto *et al.*, 2000). Chromosome segment introgression lines in which segments from the "Kasalath" cultivar (ssp. *indica*) have been introgressed into the "Koshihikari" cultivar (ssp. *japonica*) were established and made available to researchers (Ebitani *et al.*, 2005). Flowering-time analysis using these lines clearly revealed flowering-time QTLs that were consistent with previously identified QTLs. This kind of approach using introgressed lines could be useful in identifying novel QTLs and in using such materials to create new cultivars.

C. Cloning of Flowering-Time Genes in Rice and Orthologous Relationships with *Arabidopsis* Genes

A list of known flowering genes in rice and *Arabidopsis* and their orthologous relationships are shown in Table II. In this section, I discuss these genes in more detail.

1. *SE5* and *HY1*

The *SE5* gene was the first flowering-time gene that was cloned in rice (Izawa *et al.*, 2000). This gene encodes a key hemeoxygenase enzyme involved in phytochrome chromophore biosynthesis. Therefore, the *se5* mutant is severely deficient in phytochrome signaling. The *se5* mutant flowers considerably earlier than in the wild-type plant and slightly earlier under long-day conditions than under short-day conditions. This clearly indicates that phytochromes are involved in photoperiodic control of flowering in rice, especially in the repression of flowering under long-day conditions. The mutant phenotype suggests that rice plants can be a kind of long-day plant if phytochrome repression is removed from the wild-type plant. The *Arabidopsis* ortholog of *SE5* is *HY1* (Muramoto *et al.*, 1999). The *hy1* mutant exhibits an early-flowering phenotype, mainly under short-day conditions, but flowers as early as the wild-type under long-day conditions, indicating that floral repression by phytochrome signals is not required for long-day promotion in *Arabidopsis* (Goto *et al.*, 1991). In fact, *PHYA* promotes flowering under conditions rich in far-red light and is involved in photoperiodic flowering in *Arabidopsis* (Goto *et al.*, 1991; Reed *et al.*, 1994). Phytochrome double mutants were extensively analyzed in rice, and the results suggested that rice phyA plays an important role in floral repression under long-day conditions (Takano *et al.*, 2005). These results clearly demonstrate that differences in the photoreceptor functions are responsible for the different responses to photoperiod between the short-day rice plants and long-day *A. thaliana* plants. In *Arabidopsis*, cry2-mediated promotion

TABLE II
Orthologous Relationships in Flowering-Time Genes

	Rice gene	Accession no.	Functions	*Arabidopsis* gene	Accession no.	Functions	Evidence
1	*SE5*	Os06g0603000	Floral repression, mainly long days	*HY1*	At2g26670	Floral repression, mainly under short days	Genome, Phenotypes, Expression
2	*Hd1*	Os06g0275000	Floral promotion under short days, Severe floral inhibition under long days	*CO*	At5g15840	Floral promotion under long days	Genome, Phenotypes, Expression
3	*Hd6*	Os03g0762000	Severe floral inhibition under long days	Redundant genes?	At2g23070 At2g23080 At3g50000 At5g67380	No mutant reported yet	
4	*Hd3a*	Os06g015770	Flowering switch	*FT*	At1g65480	Floral switch	Genome, Phenotypes, Expression
5	*Ehd1*	No accession number assigned	Preferential floral promotion under short days	No ortholog	–	–	Genome
6	*OsMADS50*	Os03g0122600	Floral promotion	*SOC1*(*AGL20*)	At2g45660	Floral promotion	Genome, Phenotypes, Expression
7	*OsMADS14*/ *OsMADS15*	Os03g0752800/ Os03g0605200	No mutant reported yet	*FUL/AP1/CAL*	At5g60910/ At1g69120/ At1g26310	Floral organ formation	Genome, Expression
8	*OsLHY*	Os08g0517600	No mutant reported yet	*CCA1/LHY*	At2g46830/ At1g01060	Circadian clock core components	Genome, Expression
9	*OsPRRs*	Os02g0618200	No mutant reported yet	*TOC1*(*APRR1*)/ *APRRs*	At5g61380 etc.	Circadian clock core components	Genome, Expression
10	*OsGI*	Os01g0182600	No mutant reported yet	*GI*	At1g22770	Circadian clock core components	Genome, Expression
11	No ortholog	–	–	*FD*	At4g35900	Floral promotion with the FT floral switch	Genome
12	No ortholog	–	–	*FLC*	At5g10140	Severe floral repression released by vernalization	Genome
13	*OsFKF1*	Os11g0547000	No mutant reported yet	*FKF1*	At1g68050	Floral promotion through degradation of flowering repressors	Genome, Expression

of flowering is a key factor in *FT* activation by *CO* under long-day conditions (Guo *et al.*, 1998). In rice, blue light has a strong promotion effect, even in the *se5* mutant background (M. Katsumata *et al.*, unpublished observations). Therefore, floral promotion by blue-light signals may also be involved in short-day promotion of rice flowering, although no genetic evidence for blue-light receptors in rice has yet been reported.

2. *Hd1* and *CO*

Hd1 was the first rice QTL cloned from the cross between an *indica* cultivar, "Kasalath," and a *japonica* cultivar, "Nipponbare" (Yano *et al.*, 2000). Of the two, "Nipponbare" is a photoperiod-sensitive cultivar, whereas "Kasalath" is not very sensitive. *Hd1* is a major QTL that differs between the two cultivars. The cloning of *Hd1* revealed that *Hd1* encodes a *CO*-type transcription factor, and a genome-wide search confirmed that *Hd1* is the sole *CO* ortholog in rice (Izawa *et al.*, 2003). In the same clade, there are two other *CO*-like genes (*COL1* and *COL2*) in *Arabidopsis*, although their mutants did not produce clear phenotypes related to flowering time (Ledger *et al.*, 2001). A near-isogenic line that contains a homozygous "Kasalath" chromosome fragment of the *Hd1* region in the "Nipponbare" background has been produced, and its flowering time has been analyzed. The results indicate that *Hd1* can slightly promote flowering under short-day conditions and strongly repress it under long-day conditions (Yano *et al.*, 2000). This floral promotion by *Hd1* under short-day conditions was also confirmed at the gene-expression level for *Hd3a* (see Section II.C.4), which serves as a flowering switch gene in rice (Izawa *et al.*, 2002; Kojima *et al.*, 2002). An *hd1* mutant exhibited a clear reduction in *Hd3a* expression under short-day conditions. In contrast, the *Arabidopsis CO* gene promoted flowering only under long-day conditions. There has been no report of a repressor function of *CO* in *Arabidopsis*.

A novel rice flowering-time gene, *Early heading date 1* (*Ehd1*; see Section II.C.5) has been cloned and shown to promote flowering in rice mainly under short-day conditions (Doi *et al.*, 2004). The "Taichung 65" ("T65") cultivar, which contains defective alleles of both *Hd1* and *Ehd1*, flowered relatively late even under short-day conditions. Introduction of a functional *Hd1* allele into "T65" produced early flowering under short-day conditions (Doi *et al.*, 2004). This indicates that the promotion activity of *Hd1* under short-day conditions was masked in the functional *Ehd1* background by competition with the function of *Ehd1*. Therefore, in rice, *Hd1* has a dual function in flowering regulation, although the molecular mechanisms have not yet been revealed. The *Hd1* expression pattern was similar to those observed in *CO*, regardless of photoperiod. If we count the time from dawn, *CO* is expressed more strongly at dusk under long-day conditions

than at the same time under short-day conditions in *Arabidopsis*. This bimodal or "shouldered" *CO* expression is under long-day conditions by the *FKF1* blue-light receptor in *Arabidopsis* (Imaizumi *et al.*, 2003). In rice, this pattern of expression was not clearly observed under long-day conditions. There is one *FKF1* ortholog in the rice genome (see Section II.C.13), although its genetic function has not yet been analyzed (T. Izawa *et al.*, unpublished observations). Meanwhile, *Hd1* was significantly expressed at dawn under short-day conditions, but not under long-day conditions. This reduction in dawn mRNA transcription under long-day conditions was also observed in *Arabidopsis* (Saurez-Lopez *et al.*, 2000).

3. *Hd6*

Hd6 was originally isolated as a QTL in a cross between "Nipponbare" and "Kasalath" (Yamamoto *et al.*, 2000). Cloning the QTL revealed that the *Hd6* gene encodes a casein kinase II α subunit (Takahashi *et al.*, 2001). The "Nipponbare" allele contains a premature stop codon caused by a single nucleotide polymorphism (SNP) in the *Hd6* open reading frame (ORF) and is very likely a null allele. Clear epistasis between the *Hd2* and *Hd6* QTLs has been reported (Yamamoto *et al.*, 2000). Further genetic analysis has revealed that late flowering with the functional *Hd6* allele is caused by the presence of a functional *Hd1* gene (E. Ogiso *et al.*, unpublished observations). The sum of all epistasis may have caused delayed identification of the *Hd6* gene as a QTL in the backcrossed populations, not in the original F_2 population. Interestingly, this gene was previously identified as the *E3* gene in various cultivars by means of conventional genetic analysis (Y. Okumoto *et al.*, unpublished observations). The defective *e3* allele in tested cultivars was clearly consistent with the presence of a premature stop codon in *Hd6*. In *Arabidopsis*, the casein kinase II α subunit was analyzed using antisense technology (Lee *et al.*, 1999). The phenotypes of transgenic lines with reduced transcription of CKII α mRNA were pleiotropic and the effect on flowering time was not clear. In contrast, the casein kinase II β subunit gene was identified as a protein that interacts with CCA1 protein (Sugano *et al.*, 1998). Here, CCA1 is a core component of the circadian clock in *Arabidopsis*.

The CKII β subunit can phosphorylate the CCA1 protein *in vitro*. In addition, overexpression of CKII β caused defects in the circadian clock and affected flowering time in *Arabidopsis* (Sugano *et al.*, 1999). Therefore, CKII has been defined as a circadian clock controller and provides photoperiodic control of flowering through the circadian clock function in *Arabidopsis*. This type of regulation of the circadian clock by CKII has also been reported in other organisms such as *Neurospora*. How CKII controls photoperiodic flowering in rice is not yet known. The CCA1 sites that are phosphorylated by CKII have been mapped, and mutations for the sites have been shown to cause a loss of CKII regulation of the circadian clock (Daniel *et al.*, 2004).

In rice, a single ortholog of *CCA1*, *OsLHY* (see Section II.C.8), has been found (Izawa *et al.*, 2002), but two orthologous genes (*CCA1* and *LHY*) that function in circadian clock systems have been found in *Arabidopsis* (Izawa *et al.*, 2003). Some of the phosphorylated sites in CCA1 are not conserved in *OsLHY* (E. Ogiso *et al.*, unpublished observations). Therefore, it is possible that CKII controls flowering in rice by means of a different molecular mechanism.

4. *Hd3a* and *FT*

Hd3a was originally identified as a QTL by Yano *et al.* (1997), who demonstrated that *Hd3a* is a rice ortholog of *Arabidopsis FT* (Kojima *et al.*, 2002). Interestingly, there are two functionally related paralogous genes, *FT* and *TSF*, in *Arabidopsis*, but around 10 rice genes have been predicted to belong to the FT clade in the phylogenetic trees (Izawa *et al.*, 2002). Both *FT* and *TSF* promoted flowering in *Arabidopsis* under long-day conditions (Yamaguchi *et al.*, 2005), whereas *Hd3a* promoted flowering in rice under short-day conditions (Kojima *et al.*, 2002). The contribution to floral induction and the expression level of *FT* were both much larger than those of *TSF* in *Arabidopsis*. Analysis of *Hd3a* revealed that expression started several hours before dawn, reached a peak at dawn (sometimes two peaks, one before and the other after dawn), and continued throughout the day, stopping again around dusk under short-day conditions; the gene was not expressed under long-day conditions at the same development stages. In contrast, *FT* was expressed at dusk only under long-day conditions. The biological functions of *FT*-like genes other than *Hd3a* are not yet known. It has been demonstrated that overexpression of two *FT*-like genes (*FTL* and *RFT1*) induced ectopic floral transition just after regeneration from transformed calluses (Izawa *et al.*, 2002; Kojima *et al.*, 2002). In addition, Dr. Yano's group demonstrated that *RFT1* is also involved in floral induction in rice (S. Yamamoto *et al.*, personal communication). Work in *Arabidopsis* has demonstrated that *FT* mRNA is expressed in the sieve elements, may be involved in the production of long-distance transmissible signals known as florigens (Abe *et al.*, 2005; Wigge *et al.*, 2005), and is translocated to the meristem (Huang *et al.*, 2005). Conservation of floral pathways such as *CO/FT* and *Hd1/Hd3a* strongly suggests that *Hd3a* is also involved in transmissible signals in rice, although molecular confirmation of this role has not yet been achieved.

5. *Ehd1*

A novel flowering-time gene named *Ehd1* has been cloned and revealed to encode a B-type response regulator (Doi *et al.*, 2004). This gene promoted flowering in rice mainly under short-day conditions and slightly promoted

flowering under long-day conditions. Interestingly, there is no gene orthologous to *Ehd1* in *Arabidopsis*; therefore, this *Ehd1* pathway is unique to rice. In addition, *Ehd1* can promote flowering in rice in an *HD1*-deficient genetic background such as "T65." Therefore, there are at least two independent short-day promotion pathways in rice. Expression analysis of *Ehd1* revealed that *Ehd1* may be expressed through two distinct mechanisms: one by the circadian clock and the other by an acute response to light signals. Microarray analysis and subsequent quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assays revealed *FT*-like genes such as *Hd3a* and *RFT1* and several MADS-box genes such as *OsMADS1*, *OsMADS14*, and *OsMADS15* (Doi *et al.*, 2004; Jeon *et al.*, 2000; Kyozuka *et al.*, 2000). Therefore, it has become clear that *Ehd1* controls downstream flowering-time genes in rice. All B-type response regulators examined in *Arabidopsis* are involved in cytokinin signaling cascades (Heyl and Schmulling, 2003), even by exogenously applied cytokinin, although the relevant genes are not orthologous to *Ehd1*. In rice, exogenously applied cytokinin did induce some A-type response regulators and did not induce *Hd3a* and *OsMADS14*, which are genes located downstream from *Ehd1* (T. Izawa *et al.*, unpublished observations). Therefore, *Ehd1* response regulators may not transmit cytokinin signals. What kind of chemical signals can be transmitted by *Ehd1* is not yet known.

6. *OsSOC1/OSMADS50* and *SOC1/AGL20*

The role of *OsSOC1/OsMADS50*, a rice ortholog of *SOC1/AGL20* (Lee *et al.*, 2000; Onouchi *et al.*, 2000) involved in flowering-time regulation, has been analyzed using transgenic and mutant rice plants by Prof. An's group in Korea (Lee *et al.*, 2004). As is the case in *Arabidopsis*, *OsSOC1* promotes flowering in rice, but its function in the photoperiodic response has not been examined yet. *SOC1* was identified as a suppressor of *CO* expression in an overexpressing *Arabidopsis* line (Onouchi *et al.*, 2000). Subsequently, *SOC1/AGL20* was identified as a target of the *FLC* suppressor MADS-box gene, which is a key transcription factor in the vernalization response of *Arabidopsis* (Lee *et al.*, 2000). A vernalization treatment stably repressed the *FLC* mRNA trasncription. In addition, *SOC1* mRNA was detected mainly at the SAM. Therefore, whether the suppression of early-flowering phenotypes in *Arabidopsis* plants that overexpress *CO* was a direct effect or not is not yet known. The *OsMADS1*, *OsMADS14*, and *OsMAD15* genes, which belong to the SEP or the FUL clade, were identified as genes downstream of *OsSOC1* (Lee *et al.*, 2004). These results were consistent with the list of genes upregulated upon floral transition by *Ehd1* (Doi *et al.*, 2004). In addition, the SEP clade MADS-box genes were identified as basal MADS-box genes for flower formation, and FUL clade genes such as *AP1* were required for the development of floral organs such as sepals and petals (Honma and Goto, 2001).

Therefore, evolutionally conserved mechanisms could exist to stimulate flowering upon transition of meristem into floral tissues in both rice and *Arabidopsis*.

7. *OsMADS14/OsMADS15* and *FUL/AP1/CAL*

AP1 is required for the A function of flower formation in *Arabidopsis* in the ABC model (Gustafson-Brown *et al.*, 1994). *AP1* has not, however, been assigned as a flowering-time gene in *Arabidopsis*, because mutation of *AP1* did not change flowering time. *CAULIFLOWER* (*CAL*) is a paralog of *AP1* and functions redundantly with *AP1* (Kempin *et al.*, 1995). Another paralog of *AP1*, *FRUITFUL* (*FUL*), is pleiotropic and is mainly involved in carpel development (Gu *et al.*, 1998). However, *FUL* mRNA was detected at the floral meristems during floral transitions. The *ful ap1 cal* triple mutants showed no change in the timing of floral transitions, but never formed any flowers (Ferrandiz *et al.*, 2000). Therefore, these *Arabidopsis* MADS-box genes in the FUL clade are required for proper flower formation but do not switch as a switch in the transition to flowering. Extensive phylogenetic analysis of MADS-box genes in the FUL clade has revealed that the *AP1/CAL* genes contain a dicot-specific C-terminal domain that may have been produced by a frame-shift mutation (Cho *et al.*, 1999; Litt and Irish, 2003). Therefore, there is no critical gene orthologous to the *AP1/CAL* genes in monocot plants, including rice.

In the rice genome, three FUL clade MADS-box genes were found. Among them, two (*OsMADS14/RAP1B* and *OsMADS15/RAP1A*) were identified as genes downstream of the *Ehd1* gene (Izawa *et al.*, 2002), which suggested that *OsMADS14* and *OsMADS15* could be involved in flower formation in rice. However, no genetic evidence to support this hypothesis has been obtained. As is the case for *OsSOC1*, overexpression of *OsMADS14* and *OsMAD15* produced flower-like regenerated callus after transformation (Lee *et al.*, 2004). This is clearly different from the overexpression of *FT*-like genes, which usually produced several internodes after regeneration before producing ectopic florets at the tips (Izawa *et al.*, 2002). Therefore, FUL clade genes may be involved in downstream steps in flower formation in rice.

Interestingly, a cloned gene named *VRN1* in a diploid wheat cultivar encoded an *AP1*-like gene (Yan *et al.*, 2004). The *VRN1* allele of wheat was dominant and was responsible to the vernalization response in wheat. A mutation found in the promoter region of *VRN1* resulted in a lack of recognition of the wheat vernalization repressor and in a dominant spring growth habit. Since the wheat genome exhibited significant synteny at gene order levels in chromosome segment units among major cereals including rice, *OsMADS14* was able to be assigned as the gene corresponding to *VRN1* in rice (Yan *et al.*, 2004). Although a vernalization response has not been

reported in rice, it is quite possible that the *OsMADS14/RAP1B* gene is a flowering-time gene in rice, unlike the *Arabidopsis* FUL clade genes. Genetic analysis is needed to answer this question, although FUL clade genes other than *OsMAD14* and *OsMADS15* should also be considered.

8. *OsLHY* and *CCA1/LHY*

CCA1 was originally identified as a Myb transcription factor that binds to a region of the promoter of an *Arabidopsis* light-harvesting chlorophyll-*a/b* protein gene, *Lhcb1*3*, which is necessary for regulation of this gene by phytochrome (Wang and Tobin, 1998). *LHY* was originally identified in a transposon-induced activation tagging line of *Arabidopsis* that exhibited both late flowering and elongated hypocotyls (Schaffer *et al.*, 1998). *LHY* encodes a Myb protein with high similarity to CCA1. Overexpression of *CCA1* produced similar phenotypes in a dominant *lhy* line. In these overexpressors of *LHY/CCA1*, rhythmic expression of the *cab* gene was severely reduced. In addition, the *LHY/CCA1* expression was lost in the overexpressors, suggesting that these genes are part of the core circadian clock components in *Arabidopsis*. In the rice genome, there is only one orthologous gene, *OsLHY*. The expression of *OsLHY* was examined in detail and the expression pattern of *OsLHY* was found to be very similar to those of the *LHY/CCA1* genes (Izawa *et al.*, 2002). In *Arabidopsis,* the *CCA1/LHY* genes consist of a negative transcription-translation feedback with the *TOC1* gene (see Section II.C.9). CCA1/LHY proteins have been shown to bind to the promoter of *TOC1*, another circadian clock component, *in vitro* and to negatively regulate *TOC1* expression in *Arabidopsis* (Alabadi *et al.*, 2001). How *CCA1/LHY* regulates genes expressed in the early morning, such as *cab*, and genes expressed in the evening, such as *TOC1*, is not yet known. Since acute repression of *LHY* mRNA coincided with acute translation of *LHY* protein, some unique translational regulation of *LHY* was proposed (Kim *et al.*, 2003). In rice, functional analysis of *OsLHY* has not yet been done.

9. *OsPRR* and *TOC1* Plus Other *APRR*s

The *toc1* mutant in *Arabidopsis* was the first mutant identified for the circadian clock using the *cab::luc* reporter gene expression pattern for phenotypic screening (Millar *et al.*, 1995). Cloning of *TOC1* revealed that it encodes a pseudotype response regulator that contains an atypical amino acid residue in the conserved amino acids required for phosphorylation in the receiver domain (Strayer *et al.*, 2000). Although it is still unknown how the pseudotype response regulator regulates other core clock members such as *CCA1/LHY*, *TOC1* functions as a positive regulator for the early expression of *CCA1/LHY* in the morning (Alabadi *et al.*, 2001). *TOC1* mRNA was

transcribed during the evening. *Arabidopsis* genome information revealed five paralogous genes named *APRR1* (*TOC1*), *APRR3*, *APRR5*, *APRR7*, and *APRR9* (Mizuno and Nakamichi, 2005). The expression patterns of these genes were sequential, with five different phases present as peaks during the day and parts of the night. Genetic work has demonstrated that *APRR* genes other than *APRR1* (*TOC1*) are involved in circadian clock systems, with complicated redundant roles (Mizuno and Nakamichi, 2005). In rice, five *PSEUDO RESPONSE REGULATOR* (*PRR*) genes were found in the rice genome, but were not clearly sequentially expressed (Murakami *et al.*, 2003). The biological function of the rice *PRR* genes has not yet been examined. A QTL for flowering time in barley has been shown to encode a pseudotype response regulator (Turner *et al.*, 2005). This QTL affected the expression of a specific *CO* ortholog in barley. A *PRR* orthologous to this gene in rice was mapped in the *Hd2* QTL region (Murakami *et al.*, 2005), although whether *Hd2* is the *PRR* gene is not yet proven.

10. *OsGI* and *GI*

A *gi* mutant has been identified in a drastically late-flowering phenotype of *Arabidopsis*. The cloning of *GI* did not reveal its biochemical function since it contained no known domains, and this has led to speculation about its biological functions (Fowler *et al.*, 1999; Parks *et al.*, 1999). It is clear that *GI* is a member of the *Arabidopsis* circadian clock system based on expression analysis of circadian clock genes in *gi* mutants, although it is not essential for maintaining the circadian rhythms. *GI* is required for proper expression of *CO* mRNA in *Arabidopsis*, since *CO* mRNA was severely reduced in *gi* mutants (Suarez-Lopez *et al.*, 2001). In rice, there is only one highly conserved ortholog of *GI* (Hayama *et al.*, 2002). This suggests an important role for *GI* in plants. Since overexpression and RNAi of *OsGI* in rice plants affected flowering time, possibly through its effects on *Hd1* expression, the function of *OsGI/GI* is conserved between rice and *Arabidopsis* (Hayama *et al.*, 2003). How *OsGI/GI* genes control downstream genes remains unknown.

11. *Arabidopsis FD*

The *fd* mutant is a late-flowering mutant in *Arabidopsis* (Koorneef *et al.*, 1991). Cloning of *FD* revealed that this gene is required for promotion of flowering by *FT*, induces expression of *AP1*, that is an ABC model gene, interacts with the FT protein *in vitro*, and is expressed only in the SAM, not in the sieve elements (Abe *et al.*, 2005; Wigge *et al.*, 2005). These results suggest a molecular model in which *FT* mRNA and the FT protein may act as a florigen. *FD* encodes a bZIP-type transcription factor and some conserved motifs with a paralogous gene in *Arabidopsis*. Therefore, we searched

the rice genome information to find any bZIP genes orthologous to *FD*, and found a few corresponding genes. As observed earlier, there is a highly conserved flowering-time gene pathway that includes the circadian clock, *GI*, *CO*, and *FT*. Thus, *FD* orthologs may also exist in rice, although genetic analysis to identify the target of *Hd3a* is thus needed. The *AP1* gene could be a target of the FD bZIP protein in *Arabidopsis*, since *FD* controls *AP1* gene expression together with *FT*. As described earlier, there may also be no true *AP1* ortholog in rice. In contrast, a FUL clade MADS-box gene named *VRN1* was identified as a flowering-time gene in a wheat cultivar (Yan *et al.*, 2003). Therefore, *Hd3a* may be involved in controlling different downstream flowering-time genes in rice from those in *Arabidopsis*.

12. *Arabidopsis FLC*

FLC was originally identified as a QTL (Lee *et al.*, 1993), and encodes a MADS-box protein. Further analysis revealed that *FLC* is a key transcriptional (Michaels and Amasino, 1999; Sheldon *et al.*, 2000) repressor required in the vernalization response. Without vernalization treatment, *FLC* is expressed and severely suppresses the flowering of winter-annual *Arabidopsis* ecotypes. With vernalization treatment, *FLC* is repressed, and this repression is maintained epigenetically to produce early flowering. The downstream gene of *FLC* was identified as *SOC1*, a floral promoter in *Arabidopsis* (Lee *et al.*, 2000). In the *Arabidopsis* genome, several *FLC* paralogs have been found, and like *FLC*, they are involved in flowering-time regulation in *Arabidopsis*. In contrast, there is no FLC clade MADS-box gene in the rice genome. This is consistent with no report indicating that rice possesses a vernalization response. *OsSOC1* is a floral promoter in rice (Lee *et al.*, 2004); therefore, this regulation by *FLC* occurred after the establishment of the floral promotion function of *SOC1* during plant evolution.

13. *OsFKF1* and *FKF1*

FKF1 is an F-box protein (Imaizumi *et al.*, 2003) and is expressed at dusk by the circadian clock. Analysis has revealed that FKF1 may degrade a transcriptional repressor factor named CDF1 that affects *CO* transcription through its response to blue light and interacts with the FKF1 protein only at dusk under long-day conditions (Imaizumi *et al.*, 2005). This action produces a small short peak of *CO* mRNA at dusk under long-day conditions. In rice, *OsFKF1* is a clear *FKF1* ortholog (T. Izawa *et al.*, unpublished observations). Rice *OsFKF1* is expressed similarly to *FKF1* in *Arabidopsis*. Analysis of mutants of rice *FKF1* has not yet been done, although *Hd3a* is not expressed at dusk under

long-day conditions. It is possible that the rice *FKF1* ortholog may repress floral inhibition by *Hd1* under long-day conditions.

14. Wheat *VRN2*

VRN2 is required for a vernalization response to occur in wheat. It has been revealed that *VRN2* encodes a CCT motif protein (Yan *et al.*, 2004). This CCT motif has so far been found in the *CO/Hd1* and *APRR/OsPRR* genes and is believed to be involved in protein–protein interactions. Phylogenetic analysis using all possible CCT motif proteins in plants has revealed that *VRN2* belongs to a monocot-specific clade, in which some rice CCT motif genes have been found (Griffiths *et al.*, 2003). Whether these rice CCT motif genes are involved in flowering-time gene regulation in rice is a very interesting question. Cloning of *VRN1* and *VRN2* in wheat and the loss of FLC clade MADS-box genes in the rice genome (Izawa *et al.*, 2003) strongly suggest that the molecular nature of the vernalization response in some monocot plants may not have been conserved with that in *Arabidopsis* during plant evolution.

III. Photoperiodic Responses

A. External Coincidence Model in Rice

E. Bünning first proposed the involvement of circadian clocks in photoperiodic flowering in the 1930s (Bünning, 1960). Later, Pittendrigh and his colleagues refined Bünning's hypothesis and proposed the external coincidence model (Fig. 1) to explain how plants measure daylength (Pittendrigh and Minis, 1964). In this refined model, light signals play two distinct roles—one in the entrainment of circadian clocks and another as acute light signals that transmit the light conditions to downstream genes. In rice, phase response curves have been created based on the phase responses of gene expression using the *cab1R::luc* gene as a reporter gene, and the results demonstrated that light pulses can entrain the phase of circadian clocks, as has been observed in other plants (Sugiyama *et al.*, 2001). These results clearly show that light can control the phase of circadian clocks, and that this entrainment is important for photoperiodic flowering in rice. Subsequently, we examined the quantitative gene expression patterns of circadian clock-related genes and flowering-time genes in a phytochrome-deficient (*se5*) rice mutant, and demonstrated that phytochrome is responsible for photoperiodic control of flowering; this control is not exerted through phase control of circadian clocks in rice, but rather through direct responses to light signals (Izawa *et al.*, 2002). Furthermore, phase changes resulting from the application of

atypical daylength (e.g., 36 h/day) resulted in severe flowering delays in the wild-type plant, but not in *Hd1*-deficient plants. In this situation, *Hd1* mRNA transcription levels are regulated by circadian clocks. These results indicate that the external coincidence model does a good job of explaining photoperiodic flowering at the gene-regulation level in short-day rice plants (Izawa *et al.*, 2002). This report presented the first evidence that supported the external coincidence model at the level of molecular biology and explained how plants measure daylength. Soon after this publication, Yanovsky and Kay (2002) used circadian clock mutants to demonstrate that photoperiodic flowering was also explained by the external coincidence model in *Arabidopsis*.

The external coincidence model can be understood by considering the following analogy. Imagine yourself in a room with a window (Fig. 3). This window has a windowshade that opens or closes to control light entry into the room. In addition, there is a clock on the wall of the room. This hands of the clock must be adjusted every day so that the time corresponds to an external environmental signal such as sunlight. In this situation, measuring daylength requires you to record the times of sunrise and sunset each day. To do this, you must open the windowshade so you can determine the position of the sun in the sky. You must also be able to see the hands of the clock so you can record the time. When you close the windowshade to block the window, you can no longer measure daylength. On the other hand,

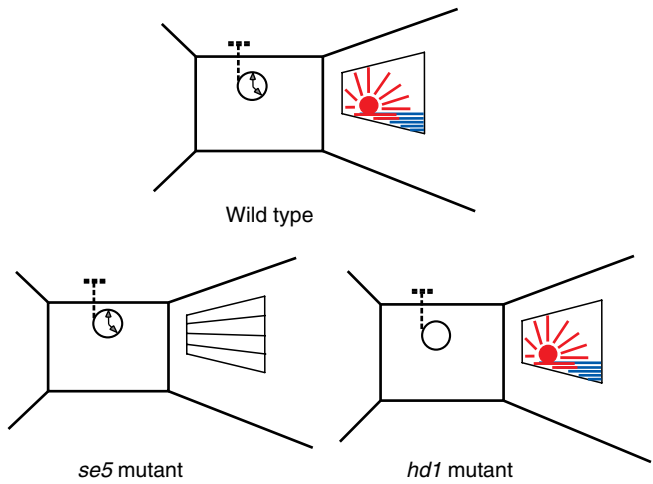

FIG. 3 An analogy for flowering-time mutants in rice. Assume that you are in a room and are required to measure daylength. The *se5* mutant resembles a room with a closed window; you cannot measure the daylength. The *hd1* mutant resembles a room in which the clock has no hands; you also cannot measure the daylengh.

if you break the hands of the clock, you also cannot measure daylength. In this analogy, the phytochrome-deficient *se5* mutant corresponds to the former case; it cannot measure daylength. In contrast, the *hd1* mutant corresponds to the latter case; it cannot reset its clock. Using artificial light–dark cycles would let you change the external signals to match the clock on the wall, but the result would be an erroneous measurement of daylength. This is reminiscent of the experiment with a 36-h daylength described in the previous paragraph.

The next important question concerns the natural signals used to transmit light signals capable of integrating the position of the clock's hands into floral induction activity. The position of the hands may correspond to the amount of mRNA for the hand protein (i.e., the *Hd1* gene product in rice). The integration step could result in activity (or an increased amount) of the Hd1 protein. One possible mechanism for this integration based on a comparison with the *Arabidopsis* system is that Hd1 product stability is controlled by light signals. If the same regulation of Hd1 product occurs in rice as occurs for CO (Valverde *et al.*, 2004), the Hd1 protein may accumulate at dusk only under long-day conditions, and this would be consistent with long-day inhibition of flowering. However, *Hd3a* was not expressed at dusk under long-day or short-day conditions (Izawa *et al.*, 2002; Kojima *et al.*, 2002). Therefore, it is not necessarily true that the Hd1 product accumulated only under long-day conditions. Furthermore, the CO product was degraded during the night (Valverde *et al.*, 2004), therefore this product did not exist at dawn, regardless of photoperiod, in *Arabidopsis*. If the same is true in rice, Hd1 would not exist at dawn under both long-day and short-day conditions, but this is not consistent with the promotion of *Hd3a* mRNA transcription by *Hd1* under short-day conditions. In addition, in *Arabidopsis*, phyA stabilizes the CO product at dusk under long-day conditions and promotes flowering under light rich in far-red radiation under long-day conditions (Goto *et al.*, 1991). However, rice phyA, working together with phyB and phyC, represses flowering (Takano *et al.*, 2005). Therefore, it is unlikely that phyA controls the *Hd1* gene product in the same way as it stabilizes the *CO* product. In addition, if rice *FKF1* is also involved in *Hd1* expression patterns under long-day conditions, as it is in *Arabidopsis*, this may not be sufficient to explain photoperiodic flowering in rice.

When we compared flowering times and the expression of flowering-time genes between *se5* and *hd1* mutants, we clearly observed derepression of gene expression in floral switch genes such as *Hd3a* in *se5* mutants under both long-day and short-day conditions, but did not observe this in *hd1* mutants even under long-day conditions (Izawa *et al.*, 2002; Kojima *et al.*, 2002). Therefore, an unknown floral repression system other than *Hd1* may exist in rice under long-day conditions. This unknown system should function independently of *Hd1* and should be regulated by phytochrome signals.

In contrast, it is likely that the *Hd1* product would be stabilized at dawn under short-day conditions. This is because the *Hd1* gene clearly promotes *Hd3a* gene expression early in the morning under short-day conditions in rice. How light signals control this stability under short-day conditions is not yet known.

In rice, the *Ehd1* promotion system plays an important role in promoting flowering, mainly under short-day conditions. This preferential promotion of flowering under short-day conditions by *Ehd1* could be attributed to greater expression of *Ehd1* under short-day conditions than under long-day conditions. We have demonstrated that *Ehd1* expression could be divided into two parts: one for the acute response to light and another regulated by the circadian clock (Doi *et al.*, 2004). Note that this type of *Ehd1* expression does not require *Hd1* function. It is likely that interaction between the acute response and the circadian clock could be responsible for the preferential expression of *Ehd1* under short-day conditions, although the relevant protein (other than Hd1) for *Ehd1* expression has not yet been identified. We found that *Ehd1* mRNA is regulated by blue light signals (T. Izawa *et al.*, unpublished observations). Therefore, blue light may play one or more important roles in controlling flowering time in rice, but may use mechanisms different from those in *Arabidopsis*.

B. Differences in Photoperiodic Responses Between Rice and *Arabidopsis*

Figure 2 compares the gene network involved in photoperiodic control of flowering time in rice and *Arabidopsis*. One of the greatest differences between rice and *Arabidopsis* is the distinct regulation of *Hd3a/FT* mRNA transcription by *Hd1/CO*. Under short-day conditions, rice *Hd3a* is expressed just before dawn, and continues to be expressed during the day but gradually decreases (Fig. 4). In contrast, no *Hd3a* mRNA is detected under long-day conditions. That is, *Hd3a* was not expressed at midnight under both short-day and long-day conditions. In the *hd1* mutant, the main defect in gene expression is a drastic reduction in *Hd3a* mRNA at dawn under short-day conditions. In the *Hd1*-deficient background, one peak before dawn and another peak during the day were often observed. This remaining expression of *Hd3a* mRNA under short-day conditions could be due to expression of *Ehd1* (Doi *et al.*, 2004). However, in *Arabidopsis*, *CO*-induced *FT* mRNA was present at dusk only under long-day conditions. No clear inhibitory action by *CO* has been reported for flowering-time regulation. For instance, there is no clear *co* phenotype under short-day conditions. In the *co* mutant, *FT* was not expressed even under long-day conditions; therefore, these mutants flowered very late under long-day conditions. These comparisons

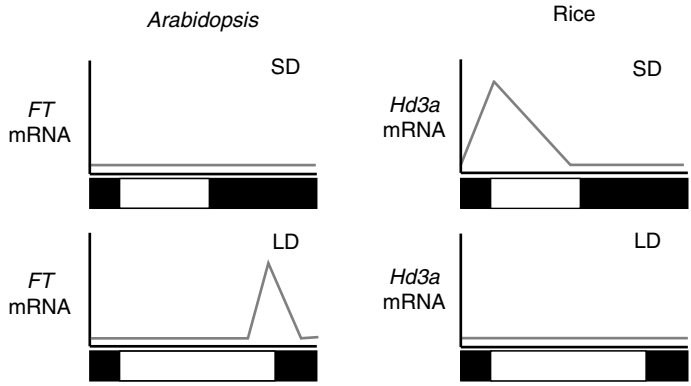

FIG. 4 Schematic representation of diurnal *FT* and *Hd3a* expression (mRNA production) in rice and *Arabidopsis*. In rice, *Hd3a* mRNA was detected before dawn and during the daytime only under short-day conditions. In *Arabidopsis*, *FT* mRNA was detected only at the end of the day and during early evening under long-day conditions. The different patterns clearly reveal the differences between short-day and long-day plants at the level of gene expression.

clearly indicate that *Hd1* functions as a transcriptional activator of *Hd3a* at dawn under short-day conditions, whereas *CO* functions as a transcriptional activator of *FT* at dusk under long-day conditions. Since *Hd3a/FT* and *Hd1/CO* are orthologous gene pairs, it is likely that similar *cis* elements could be involved in this activation. In my group, transient analysis using *Arabidopsis* seedlings led to the identification of two distinct *cis* elements that are required for *CO* expression in the *FT* promoter (H. Nakagawa *et al.*, unpublished observations). Therefore, we are currently investigating related *cis* elements found in *Hd3a* promoters.

In addition, *Hd1* also functions as a transcriptional repressor under long-day conditions. Since this kind of repression has not been reported for *CO* in *Arabidopsis*, this difference could represent a novel mechanism for changing the *Hd1* gene's function from an activator under short-day conditions to a repressor under long-day conditions through its effects on *Hd3a* expression, perhaps by forming a repressor complex with Hd1, although it is unknown whether these activators or repressors can recognize the same *cis* elements. Analysis of gene expression in *hd1* mutants has complicated the situation. Although *hd1* mutants flowered significantly earlier under long-day conditions, there was no drastic repression of *Hd3a* by *Hd1* at the transcriptional level under long-day conditions. This may indicate the existence of another mechanism of floral repression under long-day conditions in rice. This speculation is supported by the fact that *se5* mutants flowered drastically earlier than *hd1* mutants and *Hd3a* was significantly derepressed in *se5* under long-day conditions. Therefore, such unidentified repression systems appear to

have been derepressed in *se5* mutants, but not in *hd1* mutants (Izawa *et al.*, 2002; Kojima *et al.*, 2002).

The other primary difference between rice and *Arabidopsis* is that a two-component signaling system may be involved in photoperiodic flowering in rice but not in *Arabidopsis*. In all analyzed *Arabidopsis* mutants with A-type or B-type response regulators, and *Hpt* genes are members of the cytokinin signaling pathways (Heyl and Schmulling, 2003). In addition, closely related downstream genes of B-type response regulators in *Arabidopsis* have been identified as A-type response regulators. In contrast, we have demonstrated that a B-type response regulator named *Ehd1* is involved in photoperiodic flowering (Doi *et al.*, 2004). Closely related downstream genes have been identified as *FT* orthologs, including *Hd3a* and some MIKC-type MADS-box genes in SEP or FUL clades, such as *OsMADS1*, *OsMADS14*, and *OsMADS15*. In addition, a phylogenetic analysis of B-type response regulators in rice and *Arabidopsis* using the typical receiver domain for generating the phylogenetic tree revealed that *Ehd1* has evolved monophyletically in the B-type response regulators (Doi *et al.*, 2004). Because the expression of *Ehd1* could be regulated by light and the circadian clock and was induced preferentially under short-day conditions, we concluded that *Ehd1* is a photoperiodic flowering-time gene in rice. The kind of signal, possibly a small chemical, that could be transmitted using the *Ehd1* receiver domain is not yet known.

There is no unmistakable genetic evidence yet, but it appears that genes homologous to wheat *VRN2* exist in rice (Yan *et al.*, 2004). If so, they could provide a unique form of regulation of flowering time in rice, since they would consist of a monocot-specific CCT motif-protein clade and since no vernalization system has been reported in rice. Similarly, wheat *VRN1* was shown to encode a FUL clade MADS-box gene and was identified as a flowering-time gene in wheat (Yan *et al.*, 2003). Therefore, the gene orthologous to *VRN1* in rice (i.e., *OsMADS14/RAP1B*) could be involved in flowering-time regulation. It was shown that both *Ehd1* and *OsSOC1* promote *OsMADS14* expression upon floral induction (Doi *et al.*, 2004; Lee *et al.*, 2004).

Although loss of FLC clade MADS-box genes in the rice genome may not be related to photoperiodic flowering, it is still noteworthy (Izawa *et al.*, 2003). In *Arabidopsis*, release of floral repression by a vernalization treatment and floral promotion under long-day conditions were sequential processes in winter-annual ecotypes (Simpson and Dean, 2002; Sung and Amasino, 2004). Therefore, natural signals due to environmental changes have been integrated to control regulation of some key genes such as *FT*, *SOC1*, and *LEAFY* (*LFY*), the so-called floral pathway integrators. Therefore, it might be interesting to learn whether these orthologous genes also function as such integrators of environmental changes in rice. This could reveal another major difference between rice and *Arabidopsis* in the control of flowering time.

C. Number of Photosignals Controling Flowering Time in Rice

In the original version of the external coincidence model that was proposed to explain photoperiodic flowering (Fig. 1), light was hypothesized to have two distinct actions. Progress in understanding the molecular genetics of *Arabidopsis* has increased the number of light actions to at least three. In *Arabidopsis*, phytochromes and cryptochromes together adjust (or entrain) the phase of the circadian clock (Devlin and Kay, 2001; Somers *et al.*, 1998). The circadian clocks then control downstream flowering-time genes such as *CO* and *FKF1*. This is the first action by which light signals control circadian clocks in *Arabidopsis*.

The second action involves activation of the FKF1 F-box protein by blue light to degrade repressor proteins such as CDF1 only at dusk under long-day conditions (Imaizumi *et al.*, 2003, 2005). With this regulation of *CO* transcription, *CO* is preferentially expressed only at dusk under long-day conditions. Note that *GI* mediates the phase information of the circadian clock to regulate night-time expression of *CO* under both long-day and short-day conditions.

The third action could be light-dependent degradation or stabilization of the *CO* gene product (Valverde *et al.*, 2004). Light signals mediated by phyB may significantly reduce the *CO* gene product in the morning. However, cry2 and phyA stabilize the CO protein in the evening. How these photoreceptor controls are restricted at certain times of the day is not yet known. Note that the CO protein is also degraded during the night by an unknown regulation process in *Arabidopsis*.

In rice, phytochromes mediate the photoperiodic control of flowering (Izawa *et al.*, 2000). In the phytochrome mutant, the circadian clocks worked properly as in the wild-type plant (Izawa *et al.*, 2002). Therefore, we conclude that light also has at least two actions in photoperiodic flowering in rice. In addition, our work has demonstrated that blue light significantly promotes flowering in rice, despite a complete defect in the phytochrome signals of *se5* (M. Katsumata *et al.*, unpublished observations). Therefore, in addition to floral repression by phytochromes mainly under long-day conditions, and the entrainment of circadian clocks by light pulses, this promotion by blue light could represent a third action by light signals in the regulation of flowering time in rice. Our preliminary data suggest that *Ehd1*, the unique floral promoter in rice, was expressed only in the presence of blue light signals with an *Hd1*-deficient background (T. Izawa *et al.*, unpublished observations). In addition, *Ehd1* was expressed rhythmically based on the action of circadian clocks under constant darkness (T. Izawa *et al.*, unpublished observations). Therefore, a circadian clock mediator other than *Hd1* and activation by blue light signals are both involved in *Ehd1* transcription. Compared with the earlier described mechanisms for how light signals

control flowering time in *Arabidopsis*, molecular genetic and biochemical evidence for the actions of light in rice remain to be fully described.

D. Fine-Tuning of Flowering Time in Rice by Activators and Repressors

Evidence suggests the presence of a system for fine-tuning flowering time in rice under long-day conditions, since both *Hd1* and *Ehd1* can independently control flowering time (Doi *et al.*, 2004). As described previously, *Hd1* slightly promotes flowering under short-day conditions and represses it drastically under long-day conditions (Fig. 5). In contrast, *Ehd1* preferentially promotes flowering under short-day conditions but also promotes it under long-day conditions. With a functional *Hd1* gene and an *Ehd1*-deficient background, rice did not flower even 180 days after sowing under long-day conditions (14 h light, 10 h dark). When we restored functional alleles of the *Ehd1* gene, rice flowered at 100 days after sowing under these conditions. This flowering time under long-day conditions is similar to those of typical rice cultivars. In addition, "T65," the cultivar with both *hd1* and *ehd1* alleles, flowered around 110 days under long-day conditions, but flowered around 95 days under inductive short-day conditions such as 10 h of light and 14 h of dark (Doi *et al.*, 2004). Note that typical cultivars of rice flowered 60 days after sowing under short-day conditions. Therefore, the combination of *Hd1* and *Ehd1* and allelic differences may provide different flowering times, especially under long-day conditions.

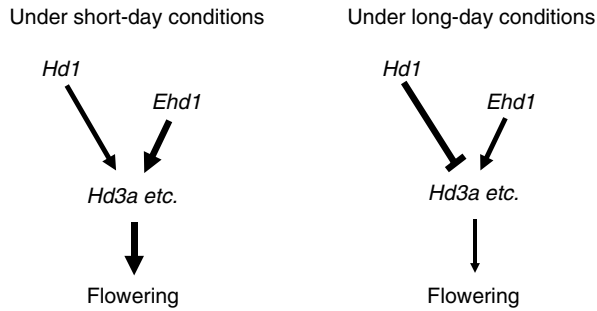

FIG. 5 Fine-tuning mechanisms for the control of flowering time in rice. Under short-day conditions, the mechanisms for promotion of flowering seem to be redundant. In contrast, the mechanism for control of flowering under long-day conditions consists of opposing actions of the *Hd1* and *Ehd1* genes, which may permit fine-tuning of flowering-time regulation, and thus more flexible adaptation to different environments.

Rice is grown as a major crop to provide a staple food around the world. In Japan, rice is usually transplanted into paddy fields in May and flowers in August in most areas. Since it takes around 1 month to flower after floral transition in rice, rice plants in paddy fields normally start to initiate floral transition just after the daylength begins to decrease. Therefore, the existence of two genes (*Hd1* and *Ehd1*) that respectively repress and promote flowering under long-day conditions appears to be significant for fine tuning of flowering time in the fields to adapt broader areas. Interestingly, both genes are involved in floral promotion under short-day conditions. Therefore, these genes may provide a more robust system to permit flowering and seed set under inductive short-day conditions, which usually coincide with decreasing ambient temperature in the autumn in temperate areas. If rice has not flowered before autumn, the harvest is lost and rice plants cannot survive. In northern Japan, some cultivars have been developed that have nearly lost their response to photoperiod during the breeding process; these cultivars flower in July in northern areas, before ambient temperatures decrease. Flowering time of these cultivars could be regulated by a different genetic system, although *Hd1* and *Ehd1* are still likely to be functional in these cultivars.

E. Molecular Nature of Rice Night-Break Responses

Night-break experiments have revealed many aspects of the mechanisms responsible for daylength measurement (Thomas and Vince-Prue, 1997). Based on these responses, researchers have long believed that the duration of darkness is a key factor in the photoperiodic control of flowering, especially in short-day plants, since many short-day plants exhibit a sensitive response to short pulses of light during the darkness (Thomas and Vince-Prue, 1997). Work using rice has demonstrated that *Hd3a* was repressed by light pulses in night-break experiments (Ishikawa *et al.*, 2005). This repression of *Hd3a* was not observed in *se5* and *phyB* mutants, but was observed in *phyA* and *phyC* mutants. This suggests that repression of flowering in rice by night-break treatments is mediated by phytochrome signals, as has been reported for other plants (Thomas and Vince-Prue, 1997). *Hd1* is involved in the promotion of flowering in these night-break experiments, but other genetic factors are required to fully explain repression of flowering by night-break light pulses in rice. These factors could include functioning of *Ehd1* (Doi *et al.*, 2004). Interestingly, night-break pulses affected *Hd3a* expression at the next dawn after the pulse. These results suggest that the Pfr active form of phyB produced by the light pulses at midnight may inhibit *Hd1* activator function at the next dawn under short-day conditions. This has not been observed in the regulation of CO activity in *Arabidopsis*.

TABLE III

Three Types of Adaptations to Seasonal Changes in Plant Kingdoms

Class	Main category	Responses	Winter form	Flowering time	Advantages	Disadvantages
1	Short-day plants	Short-day promotion; long-day repression	Seeds	Summer or autumn	Long vegetative phase	Possible loss of seed set during cold seasons
2	Long-day plants (winter annuals)	Vernalization derepression; long-day promotion	Plant form (such as Rossetta)	Spring or summer	Efficient seed setting	Survival in plant form during winter
3	Long-day plants (summer annuals)	Long-day promotion	Seeds	Spring or summer	Repeating generations per year	Short seed set; short phase for biomass

IV. Concluding Remarks

The survival strategy of plant species, including their adaptation to different areas, varies seasonally. There are three main types of adaptation in terms of the regulation of flowering time (Table III). Typical short-day plants, including rice, represent one class. In this class, plants germinate in the spring and grow during the summer; they initiate floral transition under appropriate daylength conditions (i.e., time points in decreasing daylength) and flower in the middle of the summer so they can set seed in the autumn. In this class, control of the amount of seed produced in each generation and the resulting local survival of the species become feasible if the plant can prolong its vegetative phase as much as possible in order to increase the biomass of individual plants and successfully set seeds before winter. A second class includes winter-annual *Arabidopsis* ecotypes and winter-annual wheat cultivars. In this class, plants germinate in the late summer or autumn and grow well, but do not flower before vernalization processes. During the winter, these plants become vernalized and able to respond to environmental changes such as increasing temperature and daylength in order to flower. When spring comes, the plants flower and set seed. The third class includes summer-annual *Arabidopsis* ecotypes and some wheat cultivars. In this class, plants germinate in the spring and flower in the summer. This class of plants may produce several generations in a year if growing conditions permit, and survive the cold season mainly as seeds. When spring comes, the increasing warmth may start germination. These three strategies and the corresponding adaptation mechanisms are controlled by various molecular mechanisms. Molecular genetics using rice and *Arabidopsis* has begun to provide a better understanding of how biodiversity at the molecular level allows plants to adapt to different growing conditions around the world. Further studies of rice will provide some of the missing details of the molecular mechanisms in this species and will let it serve as a model plant that provides further insights into other species in the short-day class.

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