

RID1, encoding a Cys2/His2-type zinc finger transcription factor, acts as a master switch from vegetative to floral development in rice

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Transition from the vegetative phase to reproductive phase is a crucial process in the life cycle of higher plants. Although the molecular mechanisms of flowering regulation have been extensively characterized in a number of plant species, little is known regarding how the transition process initiates. Here, we show that the *Rice Indeterminate 1 (RID1)* gene acts as the master switch for the transition from the vegetative to reproductive phase. *RID1* encodes a Cys-2/His-2-type zinc finger transcription factor that does not have an ortholog in *Arabidopsis* spp. A *RID1* knockout (*rid1*), mutated by T-DNA insertion, never headed after growing for >500 days under a range of growth conditions and is thus referred to as a never-flowering phenotype. This mutation-suppressed expression of the genes is known to be involved in flowering regulation, especially in the *Ehd1/Hd3a* pathway and a series of *RFT* homologs. *RID1* seems to be independent of the circadian clock. A model was proposed to place *RID1* in the molecular pathways of flowering regulation in rice, for which there are two indispensable elements. In the first, *RID1* is controlling the phase transition and initiation of floral induction. In the other, the *Hd3a/RFL1/FTL* complex acts as the immediate inducer of flowering. Loss of function in either element would cause never-flowering. Once the phase transition is induced with the activation of *RID1*, flowering signal is transduced and regulated through the various pathways and eventually integrated with *FT*-like proteins to induce flowering.

never flowering | phase transition | rice indeterminate 1 | flowering time

The transition of the shoot apical meristem (SAM) from vegetative to reproductive growth is a critical developmental switch in plants that is regulated by both environmental and endogenous factors (1, 2). Extensive molecular genetic analyses in *Arabidopsis* spp. have identified a set of flowering-time mutants that have been assigned to four major genetic pathways based on their functions. The autonomous and gibberellin (GA) pathways respond to endogenous factors, such as plant age or leaf number, and are largely independent of environmental signals. The photoperiod and vernalization pathways respond to environmental factors (3, 4).

Among the environmental factors, photoperiod is one of the most important regulators of flowering. Long-day (LD) or short-day (SD) photoperiod-sensitive plants can be induced to flower with the appropriate day length. Comparison of flowering pathways in *Arabidopsis*, a LD plant, and rice, a SD plant, has revealed that major genes involved in photoperiod flowering are highly conserved between these two species. For instance, *OsGI*, *Hd1*, and *Hd3a* in rice, like their orthologs of *Arabidopsis*, *GI*, *CO*, and *FT*, respectively, play important roles in flowering (5–7). The *Arabidopsis* homolog of *Hd6*, a QTL that controls photoperiod sensitivity in rice and encodes a subunit of protein kinase CK2, is also involved in the photoperiod pathway by regulating the circadian rhythm in *Arabidopsis* (8, 9). *RCN1* and *RCN2*, which delay flowering in rice when overexpressed, have conserved functions similar to that of their *Arabidopsis* homolog *TFL1* (10). However, distinct effects of some regulators were also observed. For instance, *Hd1* activates the expression of *Hd3a* and promotes flowering under SD conditions,

which is similar to *CO* under inductive LD conditions in *Arabidopsis*. Under noninductive LDs, *Hd1* suppresses the expression of *Hd3a* and delays flowering in rice, whereas *CO* has no effect on flowering in *Arabidopsis* under noninductive SDs (11–13). Furthermore, some genes are unique to the flowering pathway in rice or *Arabidopsis*. It was shown that *Ehd1*, a B-type response regulator, is specific to floral induction in rice (6), whereas the MADS box gene, *FLC*, which plays a central role in integration of the autonomous and vernalization pathways in *Arabidopsis* (14), has no ortholog in rice (6, 15). It was recently reported that *OsMADS51* and *Ghd7* involved in flowering-time regulation in rice lacks homologs in *Arabidopsis* (16, 17).

Although a large number of flowering-time mutants have been identified in both *Arabidopsis* spp. and rice (15, 18), all of the individual mutants can only affect flowering time quantitatively by either accelerating or delaying flowering. An interesting question is whether there exists an upstream master regulator that controls the phase transition such that inactivating the master regulator would block the main flowering pathways for flowering transition and, thus, would cause a never-flowering phenotype.

The transition to flowering in maize is affected by *indeterminate1 (id1)*. Unlike *Arabidopsis* spp. and rice, maize is determinate, producing a set number of leaves before flowering. The *id1* mutant has prolonged vegetative growth and retains vegetative features in the inflorescence (19). *ID1* encodes a Cys-2/His-2 zinc-finger protein (19), one of the largest classes of transcription factor families in eukaryotes, playing roles in diverse biological processes (20, 21). Expression of *ID1* is limited to developing leaves, indicating a noncell autonomous effect on the vegetative-to-reproductive state of the apical meristem (19, 22, 23). The ID1 protein defines a new family of zinc-finger proteins named the ID-domain (IDD) gene family, which is found in both monocots and dicots. Biochemical analysis showed that IDD proteins can bind to an 11-bp DNA sequence with a consensus motif TTTGTCG/CT/CT/aT/aT (24). However, apart from *ID1*, the biological function of the IDD genes and the potential role of these genes in flowering time have not been reported to date.

In this study, we identified a rice mutant (*rid1*) caused by a T-DNA insertion at the *RID1* locus that encoded an IDD protein. Plants homozygous for the mutant allele produced a never-flowering phenotype. Expression of the genes known to be involved

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Data deposition: The *RID1* sequences reported in this paper have been deposited in the GenBank database [accession nos. FJ009578 (cDNA) and FJ009579 (genomic)].

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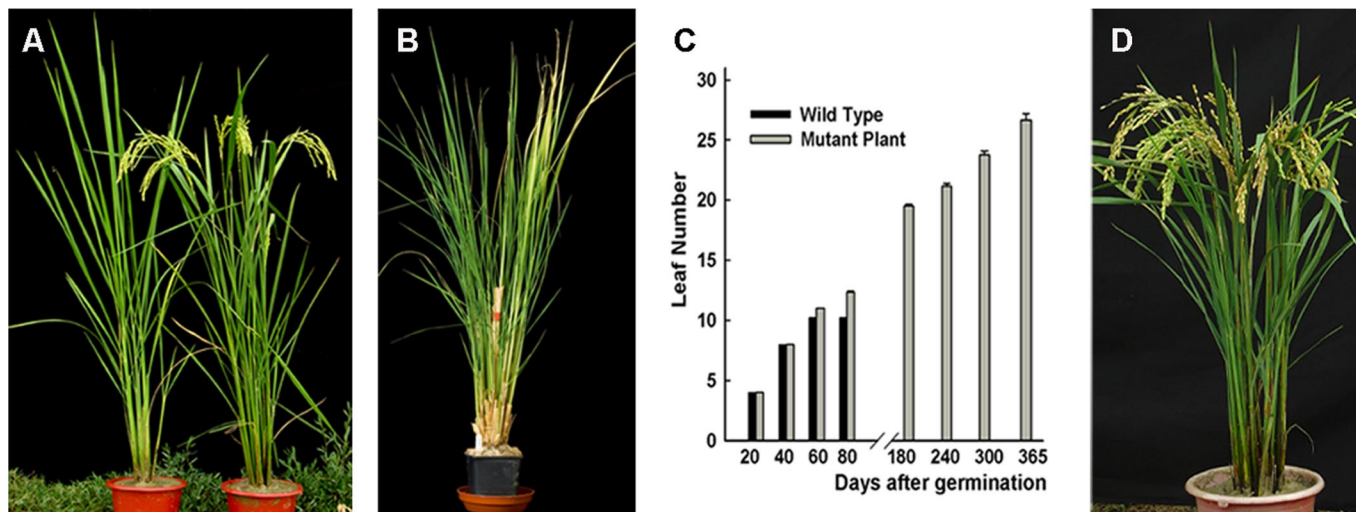


Fig. 1. Phenotype and complementation of the *rid1* mutant. (A) The phenotypes of *rid1* mutant (Left) and WT (Right) plants at maturity stage. (B) The *rid1* plant at 370 d after germination. (C) Numbers of leaves in the main culm of WT and *rid1* plants (average of six plants for both WT and *rid1*) during a whole year. (D) A phenotype-rescued *rid1* plant by genetic complementation.

in rice-flowering pathways was largely suppressed in the *rid1* mutant. The results suggest that *RID1* functions as a master switch for flowering induction in rice.

Results

Identification of a Never-Flowering Mutant. We generated an enhancer trap mutant library of rice for functional analysis of the rice genome (25, 26), from which progenies of 2,000 independent T_0 plants (primary transformants) were screened for heading time in 2004 at Wuhan, China. Progenies from eight families showed flowering-time segregation compared with the WT plants, and some plants from one family failed to head in the rice growing season (Fig. 1A).

The latter mutant plants were maintained by ratooning and then planted on Hainan Island (South China Sea) in mid-November of 2004, where they were exposed to natural SDs, inducers of flowering in rice. The mutant plants still had vegetative growth and did not head during this season until early May, 2005. Two artificial day-length treatments were also applied: neutral day-length (12 h light/12 h dark) and SD (10 h light/14 h dark), under which WT plants headed at ≈ 60 d and 55 d after germination, respectively. However, the mutant did not head after growing for 370 d (Fig. 1B) and instead continued to produce leaves with more than 30 leaves produced on the main culms of mutant plants (Fig. 1C). We designated this never-flowering mutant as *rid1*.

***RID1* Encodes a Cys-2/His-2 Zinc-Finger Transcription Factor.** Using the thermal asymmetric interlaced PCR (TAIL-PCR) method (27), we isolated genomic fragment flanking of the T-DNA insertion site from the *rid1* mutant plant. BLAST search of the flanking sequence against the TIGR Rice Genome Annotation database (<http://rice.plantbiology.msu.edu/index.shtml>) retrieved a sequence that was annotated as a Cys-2/His-2 zinc finger gene (LOC_Os10g28330), which we named *RID1*. *RID1* consisted of three exons, and the T-DNA was inserted into the second intron (Fig. 2A). The *RID1*-encoded protein showed 58% amino-acid-sequence identity with the maize ID1 (Fig. 2B). DNA blot hybridization showed that there is only one copy of T-DNA insertion in this mutant line (data not shown).

To test whether this T-DNA insertion was responsible for the mutant phenotype, 20 T_1 plants were assayed for cosegregation between the flowering phenotype and T-DNA insertion by PCR, using primers P1, P2, and P3 to amplify the T-DNA insertion site

(Fig. 2C). All of the seven plants homozygous for T-DNA insertion showed the never-flowering phenotype, whereas the remaining 13 plants, either homozygous for WT or hemizygous, headed at the same time as the WT. The cosegregation result strongly suggested that the *rid1* mutant phenotype was caused by the T-DNA insertion.

To further verify that the never-flowering phenotype is because of the loss of function of *RID1*, a 5.7-kb KpnI-BamHI fragment harboring the entire *RID1* coding region and a 1.9-kb 5'-upstream sequence were introduced into a *rid1* mutant background. Of 143 plants regenerated, 117 headed with varying heading dates (Fig. 1D), whereas no heading was observed among the 14 plants transformed with the empty vector (the negative control). DNA blot hybridization analysis of 12 T_0 *RID1*-transgenic plants chosen at random revealed that six plants contained one copy of the transgene, and the remaining plants carried two or more copies. All of the T_1 progenies produced by self-pollination of the six single-copy transformants showed segregation of heading date under both SDs and LDs. As expected, all of the transgene-positive segregants headed, whereas all of the negative plants never headed (data not shown). We therefore concluded that loss of function of *RID1* is the cause of the never-flowering phenotype.

Expression Pattern of *RID1* and Subcellular Localization of Its Protein.

To examine the temporal and spatial expression pattern of *RID1*, we performed RT-PCR analysis [with the primers in supporting information (SI) Table S1] with total RNA prepared from blades and sheaths of mature leaves and immature leaves, roots, shoot apices, and floral meristems from Zhonghua 11 WT plants grown under neutral-day-length conditions. The analysis revealed a low but detectable level of *RID1* transcript in immature leaves, but none in the root, mature leaves, SAM, or other tissues examined (Fig. S1). This expression pattern was similar to the previous result in maize (19, 22).

To further examine the expression pattern of *RID1*, the N-terminal sequence was fused in frame with a GFP, which was then fused with a 1.9-kb *RID1* upstream fragment (amplified with RG-F primers in Table S2) (*RID1::RID1::GFP*). This construct was introduced into rice by *Agrobacterium*-mediated transformation. GFP signal was clearly detected in the outer epidermal cell of immature leaves and in the region immediately beneath the meristem where internodes are visible (Fig. 3E and I-K) but not in other tissues.

The construct used for generating the mutant library carried a *GUS* reporter (25). It is anticipated that insertion of the T-DNA in

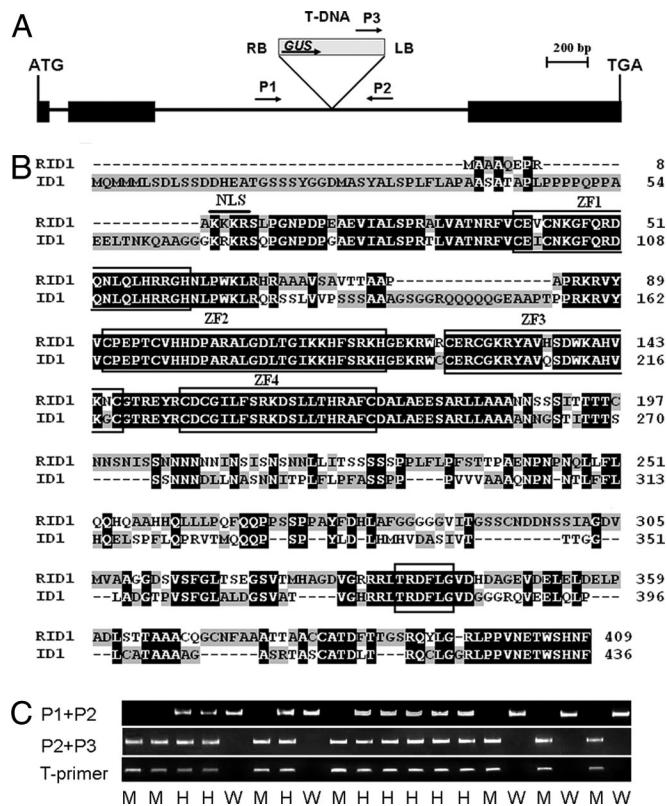


Fig. 2. Molecular features of *RID1*. (A) Structure of *RID1* and T-DNA insertion site. Three exons (filled boxes) and two introns (lines between the filled boxes) are shown. T-DNA was inserted into the second intron. Arrows indicate the primers used for analyzing the insertion site. LB and RB represent the left and right borders of T-DNA. (B) Alignment of amino acid sequences of *RID1* and *ID1* proteins. Clustalx software was used for the alignment. The identical amino acids are shown with white text on a black background. The "TRDFLG" motif and four zinc-finger motifs are boxed. A thick bar at the N-terminal region shows the putative nuclear localization signal motif. (C) PCR genotyping *RID1* segregants with primers as indicated in A (Table S2): M, homozygous for T-DNA insertion; W, WT; H, hemizygous. Primers P1 and P2 flank the T-DNA insertion and amplify a product from homozygous or WT allele. PCR-positive plants with P2 and P3 indicate T-DNA insertion in the examined site. Presence of a product with P2 and P3 and not with P1 and P2 indicates a plant homozygous for the insertion. PCR-positive plants amplified by T-primers indicate T-DNA insertion.

certain manners would result in expression of the *GUS* gene in the same pattern as the target gene. In the *rid1* mutant, the T-DNA was inserted in such a way that *GUS* was in the same orientation as *RID1* (Fig. 2A). We therefore assayed the pattern of *GUS* expression in this line. Indeed, *GUS* expression in the *rid1* mutant was only detected in immature leaves, similar to that of GFP (Fig. 3G).

We also assayed the subcellular localization of the RID1 protein. There is a putative nuclear localization signal motif near the N terminus of RID1 (22). Therefore, a construct was made to include the 112 amino acids at the N terminus of RID1, which was fused to the GFP protein and driven by the maize Ubiquitin promoter (*Ubi::ΔRID1::GFP*). We also made a construct for the control that contained GFP alone under the control of the Ubiquitin promoter. In the onion epidermal cells bombarded with the *Ubi::ΔRID1::GFP* construct, the GFP signal was observed in the nuclei (Fig. S2A–C). But in the cells bombarded with the *Ubi::GFP* construct, the GFP signal was in the cytosol and plasma membrane (Fig. S2D and E). This result confirmed the nuclear localization of the RID1 protein and is consistent with the presence of a predicted nuclear localization signal in the N terminus of ID1-like proteins (22).

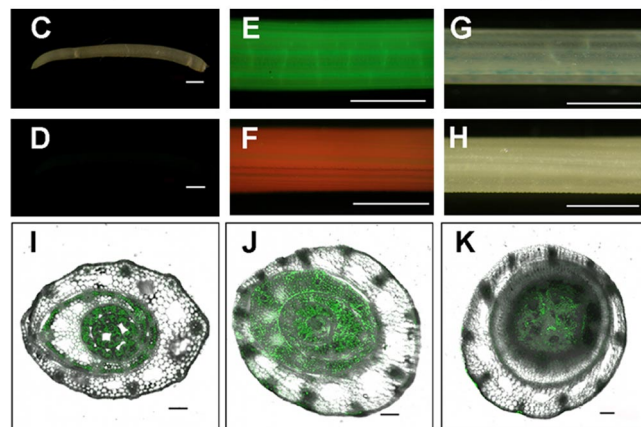
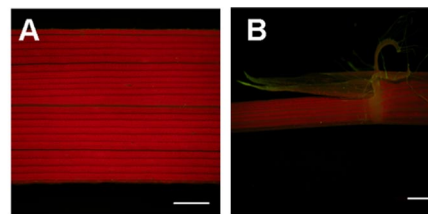


Fig. 3. Expression of *RID1* detected by GFP and *GUS* reporters. GFP assay of *RID1::RID1::GFP* transgenic plants in mature leaf blade (A), mature leaf ligule (B), root under light background (C) and dark background (D), and immature leaf (E), respectively, with the immature leaf of a WT plant as the control (F). *GUS* assay in immature leaf of T-DNA insertion *rid1* plant (G) and WT plant (H), respectively. (A–H, Scale bar, 20mm.) Confocal composite images of GFP and transmission channels show transverse sections: ≈2 cm above the SAM (I), across the top of the SAM (J), and the stem below the SAM (K) of *RID1::RID1::GFP* transgenic plant (I–K, Scale bar, 100 μm.)

***RID1* Regulates Genes in Flowering Pathways but Not in the Circadian Clock.** To further investigate the role of *RID1* in the transition to flowering, we performed RT-PCR analysis of *Hd1*, *Ehd1*, and *Hd3a*, all of which play important roles in rice flowering (5–7). It was shown that although expression of *Hd1* was detectable in mature as well as immature leaves in both WT and *rid1* plants, expression of *Ehd1* and *Hd3a* was detected only in the mature leaves of WT plants (Fig. S1).

We performed quantitative RT-PCR analysis (with the primers in Table S3) to assay the diurnal expression patterns of these genes together with *RID1* in the *rid1* mutant and WT plants under both SDs and LDs (Fig. 4). Transcript levels of *Ehd1* and *Hd3a* were largely reduced in the *rid1* mutants (Fig. 4A–D) under both SDs and LDs, suggesting that *Ehd1* and *Hd3a* are regulated by *RID1*. The transcript level of *Hd1* in the *rid1* mutant was lower than in WT, especially during the dark period, under both SDs and LDs (Fig. 4E and F), suggesting that expression of *Hd1* is partly regulated by *RID1*. The *RID1* transcript level was very low in the WT plant and did not seem to show diurnal or circadian patterns under either SDs or LDs (Fig. 4G and H). These results suggested that *RID1* transcription may not be controlled by circadian clocks.

It was recently reported that *Ghd7*, encoding a CCT domain protein, has a key role in photoperiod flowering by regulating the putative *Ehd1*–*Hd3a* pathway under LDs (17). We compared the expression patterns of *Ghd7* in the *rid1* mutant and WT plants under both SDs and LDs. The transcript level of *Ghd7* in the *rid1* mutant was lower than in WT, especially during the light period under both SDs and LDs (Fig. S3), suggesting that expression of *Ghd7* is partly regulated by *RID1*.

It has been shown that the expression of *FT*-like (*FTL*) genes around dawn can be attributed to *Hd1* and that *Ehd1* may cause the expression of *FTL* genes during daytime (6, 28). There are 13 *FTL* genes in the rice genome (29), six of which (*FTL1/FTL*, *FTL3/RFT1*,

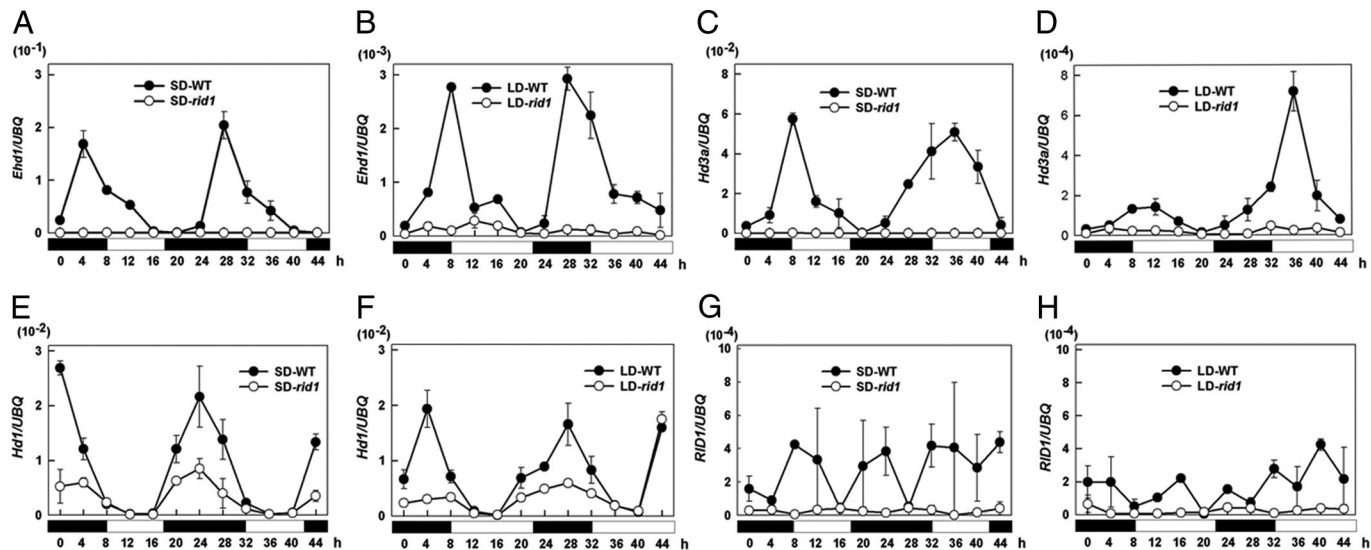


Fig. 4. Diurnal expression patterns of *RID1*, *Ehd1*, *Hd1*, and *Hd3a* in WT (filled circles) and *rid1* (open circles) plants under SDs (A, C, E, and G) and LDs (B, D, F, and H) by quantitative RT-PCR analysis. The expression levels are relative to the *UBQ* mRNA. The average values (means \pm SEM) are based on two separate RNA extractions each with at least three technical repeats. The open and filled bars at the bottom represent the light and dark periods, respectively.

FTL4, *FTL5*, *FTL6*, and *FTL11*), in addition to *Hd3a* (*FTL2*), showed expression 35 d after germination under SDs (30). We thus compared the expression of these six *FTL* genes in WT and *rid1* plants. Like *Hd3a*, expression of *RFT1* (*FTL3*) was dramatically reduced in the *rid1* mutant compared to WT under both LDs and SDs. *FTL1* and *FTL4* were slightly reduced under both LDs and SDs (Fig. S4A–F). Expression of *FTL5* was down-regulated in *rid1* only under LDs, whereas *FTL6* was down-regulated only under SDs (Fig. S4H and I). In contrast, the transcript level of *FTL11* in the *rid1* mutant was slightly higher than that in WT under both SDs and LDs (Fig. S4K and L). Thus, all of the six *FTL* genes are regulated, at least partly, by *RID1*.

It has also been reported that overexpression of *RCN1* and *RCN2*, the rice homologs of *TERMINAL FLOWER 1/CENTRO-RADIALIS*, delays the transition to reproductive phase in rice (10). We investigated the expression of these two genes. Their transcript levels were not obviously affected in the *rid1* mutant (Fig. S5), indicating that *RCN1* and *RCN2* are likely independent of *RID1* in regulating rice flowering.

We also investigated the effect of the *rid1* mutation on the circadian clock by examining the diurnal expression of *Cab1R*, a well characterized clock-controlled gene (31). The results showed that expression of *Cab1R* is not affected in the *rid1* mutant under either LDs or SDs (Fig. S6), suggesting that the circadian clock may not be affected by the *rid1* mutation.

Taken together, the mutation in *RID1* affected expression of the genes known to be involved in flowering. *RID1* seems to be independent of the circadian clock, as it neither showed a circadian rhythm in expression pattern nor affected the circadian clock.

Possible Targets of the *RID1* Protein. Kozaki *et al.* (24) found 16 oligonucleotides, with an 11-bp consensus motif TTTGTCG/CT/CT/aT/aT, that showed binding activity with the IDD protein *in vitro*. To identify possible targets of the *RID1* protein, we searched the rice genomic sequences for this motif and found positive hits in the putative promoter regions of 761 predicted genes. Among the genes surveyed in this work, only one positive hit, 5'-TTTGTCG-TAAT-3', was found in the promoter region of *Hd1*. Because *Hd1* mRNA was detected in all of the examined tissues, including the SAM and immature leaves (Fig. S1), it may have a partly overlapping expression domain with *RID1*.

To examine whether *Hd1* may be directly regulated by *RID1*, we

tested the binding activity of the IDD1 domain in *RID1* with this motif by using a one-hybrid yeast assay. The cotransformants could not grow on the SD/Leu⁻/Trp⁻/His⁻ medium with 30 mM 3-AT, whereas the transformed positive and negative controls showed the expected results in three independent repeats (data not shown). Detailed comparison of this motif with the published data (24) showed that the sequence of this motif is not exactly the same as those of any of the 16 motifs identified previously. These results indicated that the IDD domain in *RID1* could not bind to this putative motif, suggesting that none of these genes is a direct target of the IDD protein.

Discussion

***RID1* Acts as a Master Switch of Phase Transition.** Genetic analyses in *Arabidopsis spp.* have established multiple pathways that independently regulate flowering time, including autonomous, photoperiod, vernalization, and hormone signaling. Although a large number of flowering-regulation mutants have been identified, all of the mutants only showed quantitative effects by either accelerating or delaying flowering under certain environmental conditions, and none of the mutants alone could cause a never-flowering phenotype. Moreover, even the *co-2/jca-1/ga1-3* triple mutant, having simultaneous defects in photoperiod, autonomous, and GA-dependent flowering pathways and never flowering under both LDs and SDs, flowered after a 7-week vernalization treatment (32). Thus, none of the mutants identified in *Arabidopsis* corresponded to a gene that can singly act as the master switch for the phase transition to produce a never-flowering phenotype equivalent to the *rid1* mutant in rice identified in this study.

In rice, photoperiod is the most important environmental cue for signaling flowering, as vernalization is not required for flowering (6). Two independent photoperiod pathways, one involving *Hd1* and the other involving *Ehd1* that control heading date by regulating *Hd3a* (and/or *RFT1*) have been identified. Our analysis clearly demonstrated that the main flowering pathways previously identified in rice were blocked in the *rid1* mutant, such that expression of *Ehd1* and *Hd3a/RFT1* was completely repressed and expression of *Hd1* partly reduced. Although there may exist additional flowering pathway(s) in rice that have not been identified presently, the never-flowering phenotype of this mutant indicated that *RID1* acts as the master switch of phase transition and that loss of function of this regulator would result in failure of flowering.

gen). An LR recombination reaction between the attL and attR site was performed in between the entry clone and the destination vector, pGWB4 (39), which contains the GFP gene, to generate the construct *RID1::RID1::GFP*. The resulting construct was introduced into *Agrobacterium tumefaciens* EHA105 and then into the Japanese rice Zhonghua 11 by *Agrobacterium*-mediated transformation as previously described (25).

DNA Blot Hybridization. The probes for Southern blot hybridization were the PCR products amplified with T-primers (Table S2). The experimental procedures for Southern analysis followed methods described previously (40).

GFP and GUS Imaging. GFP and GUS images of transgenic rice plants were viewed under a stereo light microscope (LEICA MZFL III), and the photos were taken by using a digital camera (Nikon E5400). For preparing the transverse sections, the shoot apex of *RID1::RID1::GFP* transgenic plants was fixed into a hole of a potato block. Transverse sections were then made by hand-sectioning. The sections were analyzed by using a laser-scanning confocal microscope (LEICA TCS SP2). Fluorescence was excited with a 488-nm argon laser and emission images were collected in the 510–540 nm range.

Real Time Quantitative RT-PCR. For diurnal expression analysis, plants were grown for 28 d in the greenhouse under natural-day-length conditions. For SD samples, the plants were transferred to a growth chamber set for SDs (10 h light/14 h dark, 28°C), and entrained for 3 d. Then, penultimate and immature leaves were harvested every 4 h during a 48 h period from WT and *rid1* plants, respectively. For LD samples, plants were transferred to a growth chamber set for LDs (14 h light/10 h dark, 28°C) and entrained for 3 d. Samples were collected in the same way as SDs.

The TRIzol reagent (Invitrogen) was used according to the manufacturer's instructions to extract total RNAs from various tissues or organs of *rid1* and the WT plants. Relative quantification of gene expression was performed on an ABI PRISM 7500 PCR instrument (Applied Biosystems). A rice Ubiquitin gene was used as the endogenous control. The real time PCR primers for the *RID1*, *Ehd1*, *Hd3a*, *Hd1*, *Cab1R*, *RCN1*, *RCN2*, and *FTL* genes are listed in Table S3. Real-time PCR was performed in an optical 96-well plate that included *SYBR Premix EX Taq* and 0.5 μ l of Rox Reference Dye II (Takara), 1 μ l of the cDNA sample, and 0.2 μ M each gene-specific primer, in a final volume of 25 μ l. The reactions followed the following temperature profile: 95°C for 10 seconds, 50 cycles of 95°C for 5 seconds, and 60°C for 40 seconds. Disassociation curve analysis was performed as follows: 95°C for 15 seconds, 60°C for 20 seconds, and 95°C for 15 seconds. Data were collected by using the ABI PRISM 7500 sequence detection system following the instruction manual.

Yeast One-Hybrid Assay. Yeast one-hybrid assay was carried out by using a Clontech system. Triple tandem repeats of putative motif 5'-TTTGTGCGTAAT-3' from the *Hd1* promoter was inserted into pHis2. Putative DNA-binding domain of zinc finger Z2 and Z3 in IDD domain was amplified by using the primer pair *RID1-Rec2* (Table S2) and fused to the GAL4 activation domain in the vector pGAD7-Rec2. The two constructs were sequence-verified and cotransformed to the yeast strain Y187 for determination of the DNA-protein interactions.

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- Baurle I, Dean C (2006) The timing of developmental transitions in plants. *Cell* 125:655–664.
- Corbesier L, Coupland G (2006) The quest for florigen: A review of recent progress. *J Exp Bot* 57:3395–3403.
- Mouradov A, Cremer F, Coupland G (2002) Control of flowering time: Interacting pathways as a basis for diversity. *Plant Cell* 14:S111–S130.
- Parcy F (2005) Flowering: A time for integration. *Int J Dev Biol* 49:585–593.
- Yano M, et al. (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. *Plant Cell* 12:2473–2483.
- Doi K, et al. (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls. *FT-like* gene expression independently of *Hd1*. *Genes Dev* 18:926–936.
- Kojima S, et al. (2002) *Hd3a*, a rice ortholog of the *Arabidopsis* *FT* Gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. *Plant Cell Physiol* 43:1096–1105.
- Takahashi Y, Shomura A, Sasaki T, Yano M (2001) *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the α subunit of protein kinase CK2. *Proc Natl Acad Sci USA* 98:7922–7927.
- Sugano S, Andronis C, Ong MS, Green RM, Tobin EM (1999) The protein kinase CK2 is involved in regulation of circadian rhythms in *Arabidopsis*. *Proc Natl Acad Sci USA* 96:12362–12366.
- Nakagawa M, Shimamoto K, Kyozuka J (2002) Overexpression of *RCN1* and *RCN2*, rice *TERMINAL FLOWER 1/CENTRORADIALIS* homologs, confers delay of phase transition and altered panicle morphology in rice. *Plant J* 29:743–750.
- Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* 422:719–722.
- Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80:847–857.
- Suárez-López P, et al. (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410:1116–1120.
- Michaels SD, Amasino RM (1999) FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* 11:949–956.
- Izawa T, Takahashi Y, Yano M (2003) Comparative biology comes into bloom: Genomic and genetic comparison of flowering pathways in rice and *Arabidopsis*. *Curr Opin Plant Biol* 6:113–120.
- Kim SL, Lee S, Kim HJ, Nam HG, An G (2007) *OsMADS51* is a short-day flowering promoter that functions upstream of *Ehd1*, *OsMADS14*, and *Hd3a*. *Plant Physiol* 145:1484–1494.
- Xue WY, et al. (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat Genet* 40:761–767.
- Izawa T (2007) Adaptation of flowering-time by natural and artificial selection in *Arabidopsis* and rice. *J Exp Bot* 58:3091–3097.
- Colasanti J, Yuan Z, Sundaresan V (1998) The indeterminate gene encodes a zinc finger protein and regulates a leaf-generated signal required for the transition to flowering in maize. *Cell* 93:593–603.
- Englbrecht CC, Schoof H, Böhm S (2004) Conservation, diversification and expansion of C2H2 zinc finger proteins in the *Arabidopsis thaliana* genome. *BMC Genomics* 5:39.
- Agarwal P, et al. (2007) Genome-wide identification of C2H2 zinc-finger gene family in rice and their phylogeny and expression analysis. *Plant Mol Biol* 65:467–485.
- Colasanti J, et al. (2006) The maize INDETERMINATE1 flowering time regulator defines a highly conserved zinc finger protein family in higher plants. *BMC Genomics* 7:158.
- Wong A, Colasanti J (2007) Maize floral regulator protein INDETERMINATE1 is localized to developing leaves and is not altered by light or the sink/source transition. *J Exp Bot* 58:403–414.
- Kozaki A, Hake S, Colasanti J (2004) The maize ID1 flowering time regulator is a zinc finger protein with novel DNA binding properties. *Nucleic Acids Res* 32:1710–1720.
- Wu C, et al. (2003) Development of enhancer trap lines for functional analysis of the rice genome. *Plant J* 35:418–427.
- Zhang JW, et al. (2006) RMD: A rice mutant database for functional analysis of the rice genome. *Nucl Acids Res* 34:D745–D748.
- Zhang J, et al. (2007) Non-random distribution of T-DNA insertions at various levels of the genome hierarchy as revealed by analyzing 13804 T-DNA flanking sequences from an enhancer-trap mutant library. *Plant J* 49:947–959.
- Izawa T, et al. (2002) Phytochrome mediates the external light signal to repress *FT* orthologs in photoperiodic flowering of rice. *Genes Dev* 16:2006–2020.
- Chardon F, Damerval C (2005) Phylogenomic analysis of the PEBP gene family in cereals. *J Mol Evol* 61:579–590.
- Ishikawa R, et al. (2005) Suppression of the floral activator. *Hd3a* is the principal cause of the night break effect in rice. *Plant Cell* 17:3326–3336.
- Sugiyama N, Izawa T, Oikawa T, Shimamoto K (2001) Light regulation of circadian clock-controlled gene expression in rice. *Plant J* 26:607–615.
- Reeves PH, Coupland G (2001) Analysis of flowering time control in *Arabidopsis* by comparison of double and triple mutants. *Plant Physiol* 126:1085–1091.
- Tooke F, Ordidge M, Chirugwi T, Battay N (2005) Mechanisms and function of flower and inflorescence reversion. *J Exp Bot* 56:2587–2599.
- Muszynski MG, et al. (2006) *Delayed flowering1* encodes a basic leucine zipper protein that mediates floral inductive signals at the shoot apex in maize. *Plant Physiol* 142:1523–1536.
- Corbesier L, Coupland G (2005) Photoperiodic flowering of *Arabidopsis*: Integrating genetic and physiological approaches to characterization of the floral stimulus. *Plant Cell Environ* 28:54–66.
- Komiya R, Ikegami A, Tamaki S, Yokoi S, Shimamoto K (2008) *Hd3a* and *RFT1* are essential for flowering in rice. *Development* 135:767–774.
- Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) *Hd3a* protein is a mobile flowering signal in rice. *Science* 316:1033–1036.
- Corbesier L, et al. (2007) *FT* protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 316:1030–1033.
- Nakagawa T, et al. (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104:34–41.
- Liu KD, et al. (1997) A genome-wide analysis of wide compatibility in rice and the precise location of the *SS* locus in the molecular map. *Theor Appl Genet* 95:809–814.