Supporting Information

Wu *et al.* 10.1073/pnas.0806019105

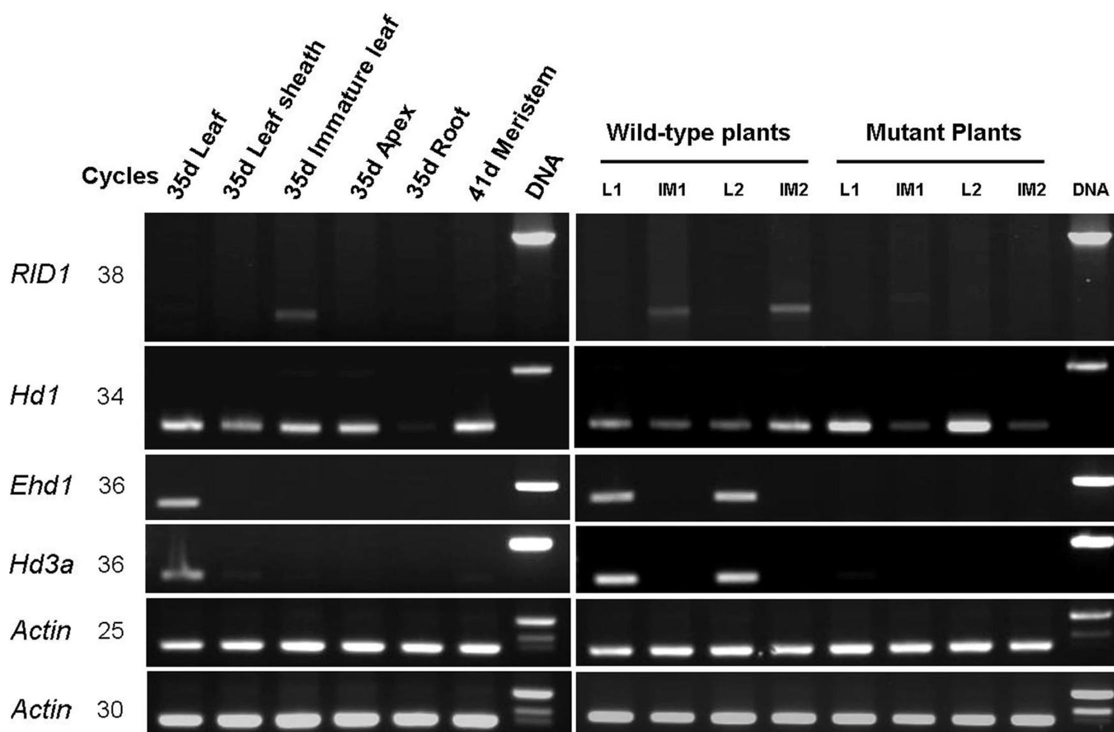

Fig. S1. RT-PCR analysis of the expression patterns of *RID1*, *Hd1*, *Ehd1*, *Hd3a* genes in various tissues in WT and *rid1* plants. *Actin* was used as a control for mRNA levels. L and IM indicate mature leaves and immature leaves, respectively. In this analysis, the TRIzol reagent (Invitrogen) was used according the manufacturer's instructions to extract total RNAs. Total RNA was treated with amplification-grade DNase I (Invitrogen) for 15 min to degrade any contaminating residual genomic DNA. The synthesis of the cDNA first strand was carried out with SuperScriptII reverse transcriptase (Invitrogen) according the manufacturer's instructions. Approximately 1/20 of the first-strand cDNA generated from 1 μg of total RNA was used as a template for PCR in a reaction volume of 20 μl with the TaqDNA polymerase (Promaga). The PCR was performed with specific primers listed in Table S2 in an ABI 9700 thermocycler (Applied Biosystems) with the following cycling profile: 94°C for 5 min; 28–38 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 min; and 72°C for 10 min. Ten microliters of the PCR product was separated in a 1.0% agarose gel and stained with ethidium bromide for visualization.

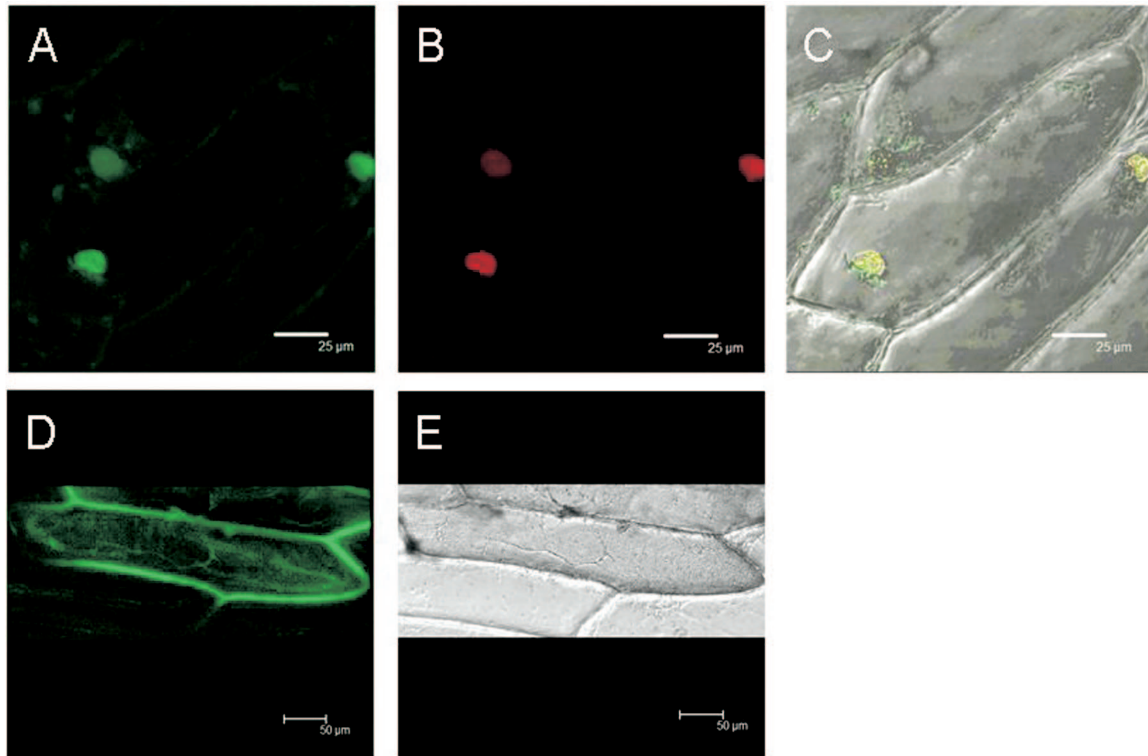

Fig. S2. Nuclear localization of the ΔRID1-GFP protein revealed by bombardment of onion epidermal cells. (*A*) GFP signal in the nuclei. (*B*) The same cells stained with DAPI. (*C*) Their merged image. (Scale bar, 25μm.) (*D*) Onion epidermal cells bombarded with the construct having GFP alone as the control and showing florescence in the cytosol and plasma membrane. (*E*) The same cells in transmission image. (Scale bar, 50μm.) For performing this assay, the N-terminal fragment of *RID1* coding region was amplified by PCR, using the primer pair RID1NLS (Table S1), and inserted into the BamHI-KpnI site of Ubiquitin-GFP-NOS plasmid (1) in frame with the C terminus of a RID1 peptide. Bombardment of onion epidermal cells was performed as described (1). The expression of the fusion protein of RID1 and GFP in the onion epidermal cells was observed by using a laser scanning confocal microscope (LEICA TCS SP2) 36 h after bombardment.

1. Huang LM, *et al.* (2007) Down-regulation of a *SILENT INFORMATION REGULATOR2*-related histone deacetylase gene, *OsSRT1*, induces DNA fragmentation and cell death in rice. *Plant Physiol* 144:1508–1519.

Fig. S3. The expression patterns of *Ghd7* under SDs (*A*) and LDs (*B*) in WT (filled circles) and *rid1* (open circles) plants. The open and filled bars at the bottom indicate the light and dark periods, respectively. The transcript level was quantified relative to the *UBQ* mRNA level. The average values (means ± SEM) were obtained from at least three real-time RT-PCR assays with two separate RNA extractions.

Fig. S4. The circadian expression patterns of six *FTL* genes under SDs (*A*, *C*, *E*, *G*, *I*, and *K*) and LDs (*B*, *D*, *F*, *H*, *J*, and *L*) in WT (filled circles) and *rid1* (open circles) plants. The expression levels are relative to the *UBQ* mRNA. The average values (means ± SEM) are based on two separate RNA extractions, each with at least three technical repeats. The open and solid bars at the bottom indicate the light and dark periods, respectively.

Fig. S5. The expression patterns of *RCN1* and *RCN2* under SDs (*A* and *C*) and LDs (*B* and *D*) showing no obvious difference between WT (filled circles) and *rid1* (open circles) plants. The open and filled bars at bottom represent the light and dark periods, respectively. The transcripts were quantified relative to the *UBQ* mRNA level. The average values (means ± SEM) were obtained from at least three real-time RT-PCR assays with two separate RNA extractions.

Fig. S6. The expression patterns of *Cab1R* under SDs (*A*) and LDs (*B*) in WT (filled circles) and *rid1* (open circles) plants. The open and filled bars at the bottom indicate the light and dark periods, respectively. The transcript level was quantified relative to the *UBQ* mRNA level. The average values (means ± SEM) were obtained from at least three real-time RT-PCR assays with two separate RNA extractions.

Table S1. Primers for RT-PCR analysis

Primer name	Sequence (5′–3′)	Amplified size	
		Genome, bp	cDNA, bp
OsActinL	CAATCGTGAGAAGATGACCC	644	395
OsActinR	GTCCATCAGGAAGCTCGTAGC		
RID1-RT1L	TCGAGCTATTGTCGTCGTTG	2,172	647
RID1-RT1R	GGAAGAGGGTGTACGTGTGC		
Hd3a-L	TCAGGGTTTTTTGCAAGATCGATGG	620	325
Hd3a-R	ACGCTGCAGTAGTACCAGGAATATC		
Hd1-L	GTTTGCAGAGAAGGAAGGGAGCGAGTG	1,038	401
Hd1-R	GGTCGTGCCTCTGCATACGCCTTTCTTG		
Ehd1-L	CGAAAGCAAATGCAAGATCA	498	349
Ehd1-R	TGGCAACTTGCTCTCTTGTC		

Table S2. Primers for genotyping analysis and vector construction

Primer name	Sequence (5′–3′)
P1	AAGGACGACTGTGGATTGAT
P2	TCTTCTTCCTGTTCTTGCTCT
P3	AATCCAGATCCCCCGAATTA
T-primer-F	GGCATCGGTAAACATCTGCT
T-primer-R	GCCTCAAGAAGCTCAAGTGC
RG-F-F	CACCCCTTATGTGTGCATGCTCTCT
RG-F-R	GAAGTTGTGGCTCCACGT
RID1NLS-F	AAAGGATCCTTCTTGATCCCGGTCAGGTC
RID1NLS-R	AAAGGTACCTCGTATGGTGGTGGGTAGCC
RID1-Rec2-F	AAAGAATTC CACAACCTCCCCTGGAAGCTC
RID1-Rec2-R	AAAGGATCC CGCAGTCGCAGCGGTACTCG

Table S3. Primers for real-time RT-PCR analysis

Primer name	Sequence (5′–3′)	Amplified size, bp
Ubq-qRT-F	AACCAGCTGAGGCCCAAGA	77
Ubq-qRT-R	ACGATTGATTTAACCAGTCCATGA	
RID1-qRT-F	CGACGACAATAGCTCGATCGC	97
RID1-qRT-R	GTGCATGGTCACGGAGCCTT	
Hd1-qRT-F	TCAGCAACAGCATATCTTTCTCATCA	80
Hd1-qRT-R	TCTGGAATTTGGCATATCTATCACC	
Ehd1-qRT-F	GGATGCAAGGAAATCATGGA	121
Ehd1-qRT-R	AATCCCATCGGAAATCTTGG	
Hd3a-qRT-F	CTTCAACACCAAGGACTTCGC	150
Hd3a-qRT-R	TAGTGAGCATGCAGCAGATCG	
Cab1R-qRT-F	GGACCGTAGCTTAGCAGTGGTTAA	73
Cab1R-qRT-R:	CCAACCCAAAGACAACGAACTC	
FTL1-qRT-F	TCGGAACGATTTGAAATGGTAA	95
FTL1-qRT-R	ATCGGTGGGAGCATTTATGTAACTA	
FTL3-qRT-F	TGACCTAGATTCAAAGTCTAATCCTT	92
FTL3-qRT-R	TGCCGGCCATGTCAAATTAATAAC	
FTL4-qRT-F	AGCTAGCCCTTCCACCTTATTGA	142
FTL4-qRT-R	TTGCAGCTGACTTTATGAAAGGAT	
FTL5-qRT-F	TCCTGAAAAAAATACCAATTGTAGCTAACT	93
FTL5-qRT-R	ATTTGATAAATGGGTCCAAGATATCC	
FTL6-qRT-F	TGAGCTCAAACCGTCACAGGT	96
FTL6-qRT-R	TCAGGATCCACCATCACCAGA	
FTL11-qRT-F	CAGCTCTCACAGTCTCAAAACACAT	100
FTL11-qRT-R	AGAGGCACTGAGTTTGAAAATTGG	
RCN1-qRT-F	GGACGCGAGGTCATAAGCTA	97
RCN1-qRT-R	TACAGTTTGCCTGCGCTTCTG	
RCN2-qRT-F	TGACTGACCCTGATGTGCCA	112
RCN2-qRT-R	ACCACCTCCCTCCCAAAAGA	
Ghd7-qRT-F	AGGTGCTACGAGAAGCAAATCC	60
Ghd7-qRT-R	GGGCCTCATCTCGGCATAG	