

## Complex Regulatory Networks of Flowering Time in Rice

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### Abstract

Rice flowering is inhibited when days are long during Spring and early Summer. This phenomenon is mediated by several independent pathways. Several genes are preferentially expressed under such conditions, including Grain yield and heading date 7 (Ghd7), Heading date 1 (Hd1), Heading date 5 (Hd5), and Heading date 6 (Hd6). By contrast, *Oryza sativa* Constans-like 4 (OsCOL4) deters flowering regardless of day length. The AP2-like genes Supernumerary Bract (SNB) and *Oryza sativa* Indeterminate Spikelet1 (OsIDS1) constitutively inhibit flowering. However, as days become shorter in late Summer, flowering is induced. Although Hd1 protein functions as a repressor under long days, it becomes a promoter under short days (SD). Both OsMADS50 and OsDof12 induce flowering specifically under LD, while *Oryza sativa* Indeterminate 1 (OsId1) causes flowering regardless of day length. Levels of expression by the repressors decrease as plants mature. For example, Ghd7 transcripts are more abundant in young plants. Transcripts of SNB and OsIDS1 are degraded by miR172, which is induced in older plants. Most of the upstream signals are transferred to Early heading date 1 (Ehd1), an immediate upstream regulator of the florigen genes Heading date 3a (Hd3a) and Rice FT 1 (RFT1). However, some signals directly turn on the florigens that are transferred to the shoot apical meristem, where the reproductive transition occurs.

**Keywords:** Day length; Florigens; Flowering time; Regulatory genes

### Introduction

Plant yields are maximized and the exchange of genetic information among individuals is enhanced when flowering occurs at the optimal time. This phenomenon is modulated by environmental factors such as day length as well as by endogenous cues, e.g., developmental age. Rice (*Oryza sativa*) is a facultative short-day (SD) plant that flowers earlier under SD conditions. Photoreceptors and circadian clock genes play roles in modulating the expression of genes that regulate this timing. Flowering is inhibited during the early stage of development to allow sufficient vegetative growth. Various transcription factors (TFs) modulate flowering time by several independent pathways. Chromatin remodeling factors and microRNAs also control that activity. Signals are merged to Ehd1 or directly to florigens that induce flowering when an adequate amount has accumulated. Summaries of rice research have covered photoperiodic flowering [1-4] comparative biology [5-7], and the florigen mechanism [2]. Other reviews have focused on the relationships among photoperiod, epigenetic regulation [8] (Sun et al.), and temperature [9]. Here, we review integrations among various flowering pathways.

### Review

Several rice cultivars have been domesticated for cultivation in a range of growing regions. Flowering time, often defined by heading date, is one of the most important agronomic traits that can be adapted to a new environment to increase yields. Genes that influence flowering time have been identified through analyses of Quantitative Trait Loci (QTL) from cultivars growing in different geographical locations [10]. Genes that control this regulatory network have also been found by studying either naturally occurring mutants or those that are generated through T-DNA or Tos17 insertions [11-13].

Table 1 summarizes those genes and roles.

### Florigens

Heading date 3a (Hd3a) protein is a florigen in rice. Generated in the leaf phloem and transferred to the shoot apical meristem (SAM), this protein induces reproductive development when a plant reaches a certain growth stage [14]. Hd3a was initially identified by QTL

mapping that utilized a population derived from a cross between the photoperiod-sensitive cultivar 'Nipponbare' and the photoperiod-insensitive cultivar 'Kasalath' [15]. Through high-resolution mapping, the candidate gene was located within an approximately 20-kb region that includes four genes [16]. One of those is highly homologous to Arabidopsis Flowering Locus T (FT), which prompts flowering. A genomic DNA fragment containing the gene from 'Kasalath' causes early flowering when introduced to 'Nipponbare', indicating that the FT-homologous gene is Hd3a [17].

Among the 13 rice proteins homologous to FT, RFT1 also functions as a florigen [18,19]. RFT1 is adjacent to Hd3a, separated by 11.5 kb on Chromosome 6, suggesting that the two arose through tandem duplication [18-20]. The RFT1 amino acid sequence shares 91% identity with Hd3a [16,18,21,22] (Chardon and Damerval). Transgenic plants expressing RNAi of both Hd3a and RFT1 do not flower until 300 days after sowing (DAS) [23]. Overexpression of Hd3a and RFT1 causes flowering at the callus induction stage [17,24]. These results are evidence that Hd3a and RFT1 are essential for flowering in rice [25]. Overexpression of FTL1, another FT-like gene in rice, also induces early flowering at the callus stage [22].

Hd3a is preferentially functional under SD whereas RFT1 has an LD role [19,23,25]. During short photoperiods, flowering of Hd3a RNAi plants is delayed by approximately one month, whereas timing for RFT1 RNAi plants is similar to that for the wild type, or WT [19,23,25]. By contrast, under LD conditions, RFT1 RNAi plants bolt later while the flowering time for Hd3a RNAi plants is similar to the WT [19]. Although temporal expression of both Hd3a and RFT1

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Gene name	TIGR locus ID	Function	Note	Elucidation method	Pathways
<i>Hd3a</i>	LOC_Os06g06320	Florigen	Phosphatidylethanolamine-binding protein (PEBP)	QTL/Overexpression/RNAi	
<i>RFT1</i>	LOC_Os06g06300	Florigen	Phosphatidylethanolamine-binding protein (PEBP)	RNAi/QTL/Overexpression	
<i>OsMADS14</i>	LOC_Os03g54160	Floral transition	AP1 homolog	Overexpression	
<i>OsMADS15</i>	LOC_Os07g01820	Floral transition	AP1 homolog	Overexpression	
<i>Hd1/Se1</i>	LOC_Os06g16370	SD activator/LD repressor	CONSTANS-like (COL)	QTL/Tos17/T-DNA/Overexpression	<i>Hd3a</i>
<i>Ehd1</i>	LOC_Os10g32600	Signal integrator	B-type response regulator	QTL/RNAi/Overexpression	<i>Hd3a</i>
<i>Hd4/Ghd7/Lhd4</i>	LOC_Os07g15770	LD repressor	CONSTANS-like (COL)	QTL/Overexpression	<i>Ehd1</i>
<i>Hd5/DTH8/Ghd8/LHD1/Ds9</i>	LOC_Os08g07740	SD activator/LD repressor	HAP3 subunit	QTL/Ds	<i>Hd1,Ehd1</i>
<i>Hd6/CK2α</i>	LOC_Os03g55389	LD repressor	Caseine kinase 2α	QTL	<i>Hd1, Hd3a</i>
<i>Hd16/EL1/CKI</i>	LOC_Os03g57940	LD repressor	Caseine kinase I	QTL/T-DNA	<i>Ghd7</i>
<i>OsMADS50</i>	LOC_Os03g03100	LD activator	SOC1 homolog	T-DNA/Overexpression	<i>OsLFL1</i>
<i>OsMADS56</i>	LOC_Os10g39130	LD repressor	SOC1 homolog	Overexpression	<i>OsLFL1</i>
<i>OsDof12</i>	LOC_Os03g07360	LD activator	DNA-binding with one zinc finger protein	Overexpression	<i>Hd3a</i>
<i>OsMADS51</i>	LOC_Os01g69850	SD activator	Type I MADS-box protein	T-DNA/Overexpression	<i>OsGI,Ehd1</i>
<i>OsCO3</i>	LOC_Os09g06464	SD repressor	CONSTANS-like (COL)	Overexpression	<i>Hd3a</i>
<i>Ehd2/Osld1/RID1</i>	LOC_Os10g28330	Constitutive activator	C2H2-type zinc finger protein	QTL/T-DNA/RNAi	<i>Ehd1</i>
<i>Ehd4</i>	LOC_Os03g02160	Constitutive activator	CCCH-type zinc finger protein	QTL	<i>Ehd1</i>
<i>OsCOL4</i>	LOC_Os02g39710	Constitutive repressor	CONSTANS-like (COL)	T-DNA/Overexpression	<i>Ehd1</i>
<i>OsLF</i>	LOC_Os05g46370	Constitutive repressor	Atypical HLH protein	Activation tagging by T-DNA	<i>OsGI</i>
<i>SNB</i>	LOC_Os07g13170	Constitutive repressor	AP2-like	Overexpression	<i>miR172,Ehd1</i>
<i>OsiDS1</i>	LOC_Os03g60430	Constitutive repressor	AP2-like	Overexpression	<i>miR172,Ehd1</i>
<i>OsLFL1</i>	LOC_Os01g51610	Constitutive repressor	B3 domain transcription factor	Overexpression	<i>Ehd1</i>
<i>OsGI</i>	LOC_Os01g08700	Circadian rhythm/Flowering activator	GIGANTEA ortholog	γ-ray radiation/RNAi/T-DNA	
<i>Hd2/OsPRR37</i>	LOC_Os07g49460	Circadian rhythm/SD activator/LD repressor	Pseudo response regulator	QTL/T-DNA	<i>Hd3a</i>
<i>Hd3b/Hd17/Ef7/OsELF3</i>	LOC_Os06g05060	Circadian rhythm/LD activator	ELF3 homolog	QTL/T-DNA/RNAi	<i>Hd1,Ghd7,OsGI</i>
<i>OsEF3</i>	LOC_Os01g38530	Circadian rhythm/Flowering activator	ELF3 homolog	T-DNA	
<i>OsPhyA</i>	LOC_Os03g51030	SD activator/LD repressor	Phytochrome	Tos17/T-DNA	
<i>OsPhyB</i>	LOC_Os03g19590	Constitutive repressor	Phytochrome	γ-ray radiation/T-DNA	
<i>OsPhyC</i>	LOC_Os03g54084	LD repressor	Phytochrome	Tos17/T-DNA	
<i>SE5</i>	LOC_Os06g40080	SD activator/LD repressor	Heme oxygenase	γ-ray radiation	
<i>OsCRY2</i>	LOC_Os02g41550	Constitutive activator	Cryptochrome	antisense	
<i>OsVIL1/OsVIL2</i>	LOC_Os12g34850	Flowering activator	VIN3-like (PRC2 complex)	RNAi	<i>OsLF</i>
<i>OsVIL2/LC2/OsVIL3</i>	LOC_Os02g05840	Flowering activator	VIN3-like (PRC2 complex)	T-DNA	<i>OsLFL1</i>
<i>OsEMF2b</i>	LOC_Os04g08034	Flowering activator	Su(z)12-like (PRC2 coomplex)	T-DNA/RNAi/Overexpression	<i>OsLFL1</i>
<i>OsTrx1</i>	LOC_Os09g04890	LD activator	TrxG	T-DNA	<i>Ghd7</i>
<i>Ehd3</i>	LOC_Os08g01420	Flowering activator	Plant Homeodomain (PHD) finger motif	QTL	<i>Ghd7</i>
<i>SDG724/LVP1</i>	LOC_Os09g13740	LD activator	SET domain	Somaclonal variation by tissue culture	<i>OsMADS50</i>
<i>SDG711</i>	LOC_Os06g16390	LD repressor	SET domain	RNAi/Overexpression	
<i>SDG718</i>	LOC_Os03g19480	SD activator	SET domain	RNAi	

**Table 1:** Flowering regulators in rice.

peaks at 35 DAS under SD conditions, transcript levels are higher for the former [23]. Because RFT1 transcripts are more abundant at later developmental stages in Hd3a RNAi plants under SD [23], RFT1 appears to function as a florigen when Hd3a is suppressed. In

temperate regions, rice is grown under LD conditions, where RFT1 is preferentially functional as a florigen. An indica cultivar, ‘Nona Bokra’, flowers extremely late (about 200 DAS) in its natural habitat in Tsukuba, Japan (36°N) but bolts at about 60 DAS under SD conditions

[26] (Uga et al.). Overexpression of the RFT1 allele from 'Nipponbare' or 'Kasalath' induces extremely early flowering while overexpression of that allele from 'Nona Bokra' does not [27] possibly because an amino acid substitution of E to K at the 150th position of RFT1 causes a functional defect in that cultivar. In addition, polymorphisms in the RFT1 promoter region reduce this expression in 'Nona Bokra' [27].

Florigens are produced in leaves and transported to the SAM where they induce reproductive developments [14,19]. After their arrival, they bind to the 14-3-3 proteins GF14b and GF14c [28,29], and the complex interacts with OsFD1 [2]. Crystallographic analyses have also shown that these three proteins form a florigen activation complex [2] that is composed of two Hd3a molecules, the GF14c (14-3-3 protein) dimer, and two OsFD1s. That complex binds to the promoter of OsMADS15 to induce expression of the target gene [2]. Expression of OsMADS14 and OsMADS15 is decreased in the SAM of Hd3a RNAi or RFT1 RNAi plants [19,23,25]. Whereas overexpression of OsMADS14 causes extremely early flowering at the callus induction stage [30], flowering by GF14c overexpression (OX) plants is delayed approximately 20 d. The gf14c/GF14c heterozygous mutants bolt early, by about 14 d under SD [28]. These findings suggest that, although GF14c alone functions as an inhibitor, it becomes an activator when it forms that complex with Hd3a and OsFD1.

### Hd1 and Ehd1

Hd3a is controlled by Hd1, which binds to the Hd3a promoter [31]. Hd1 is a rice ortholog of Arabidopsis Constans (CO) [32]. First identified by QTL mapping of a cross between 'Nipponbare' and 'Kasalath' [15,32]. Hd1 is allelic to SE1 (Photoperiodic Sensitivity 1), a major QTL controlling photoperiodic sensitivity [33]. The se1 mutant has been induced by  $\gamma$ -ray irradiation of seeds from the japonica cultivar "Gimbozu". It flowers early under LD conditions [33]. Nonfunctional Hd1 causes late flowering under SD but early flowering under LD [32], indicating that Hd1 can be either a flowering activator or repressor, depending upon light conditions. This behavior is contradictory to that of Arabidopsis CO, which is a constitutive flowering activator under both SD and LD [34]. The hd1-1 mutants generated by Tos17 insertion also show flowering phenotypes similar to se1 [35]. Transcript levels of Hd3a and RFT1 are decreased in hd1 mutants under SD conditions, but increased under LD [28,35]. How Hd1 functions differently between SD and LD is unknown. Overexpression of Hd1 causes late-flowering, but the phenotype is not observed in the osphyB mutant background [36], thereby demonstrating that OsPhyB is necessary for the suppressive functioning of Hd1. As a member of the family of CO-like proteins, Hd1 contains conserved B-box zinc fingers and CCT domains [37,38].

Expression of Hd3a is also controlled by Ehd1, as first revealed by QTL mapping of a cross between 'Taichung 65' and *Oryza glaberrima* [21]. Introgression of the Ehd1 allele from *O. glaberrima* causes early flowering in 'Taichung 65' under both SD and LD [39]. Ehd1 protein is a member of the B-type response regulators, which have a receiver domain and a GARP domain [39]. The latter functions to bind DNA; an alteration of Gly in the domain to Arg greatly reduces this binding capacity in 'Taichung 65' [39]. In that cultivar, a single amino acid substitution in the GARP domain disrupts binding to the target DNA [39]. Ehd1 induces transcript levels for Hd3a, RFT1, OsMADS14, and OsMADS15 [39]. Ehd1 RNAi plants flower later, by 7 d under SD [40] and by 10 d under LD [41]. All of these observations indicate that Ehd1 is a flowering activator.

### LD-preferential regulatory elements

In temperate zones, rice is grown under LD conditions. When

plants are young, flowering is inhibited to allow for sufficient vegetative growth and tillering. After a certain period, a flowering signal is induced to initiate the formation of reproductive organs.

Grain yield and heading date 7 (Ghd7)/Late Heading Date 4 (Lhd4) is an LD-specific repressor identified through QTL mapping of plant height and grain yield traits [42,43]. Introgressing a functional allele of Ghd7 from 'Minghui 63' to 'Zhenshan 97' can cause pleiotropic phenotypes such as late flowering and increases in height, stem diameter, and yield [42,43]. Ghd7 encodes a CO-like protein containing the B-box and CCT domains [38,43]. This gene suppresses flowering by inhibiting the expression of Ehd1 [43]. Ghd7 is strongly expressed in the vascular bundle where Ehd1 is expressed, providing evidence that Ghd7 functions upstream of Ehd1 [43]. Ghd7 is phosphorylated by HEADING DATE 16 (Hd16), a casein kinase-1 protein [24]. It expression is very low under SD but significantly higher under LD [43,44]. Natural variations include Ghd7-1, Ghd7-2, Ghd7-3, Ghd7-0, and Ghd7-0a. Among them, Ghd7-0 and Ghd7-0a are nonfunctional alleles mainly observed in high-latitude cultivars that are grown during shorter seasons [43]. The rest of the alleles are functional and found in low-latitude cultivars [43] (Xue et al.). Therefore, these reports indicate that Ghd7 alleles have played an important role during the progression of rice domestication.

Heading date 5 (Hd5)/QTL for days to heading on chromosome 8 (DTH8)/Grain yield and heading date 8 (Ghd8)/Late Heading Date 1 (LHD1) encodes a putative HAP3 subunit of the CCAAT-box-binding TF [45]. The HAP proteins have three subunits – HAP2, HAP3, and HAP5 – that form trimeric complexes [46,47]. Introgression of the functional Hd5 allele causes late flowering under LD by suppressing expression of Ehd1, Hd3a, and RFT1 [45,48-50]. However, under SD conditions, expression by those genes is not affected by Hd5. Flowering inhibition is independent of activity by Ghd7 and Hd1 [45]. By contrast, the ds9 mutant, another allelic mutant for Hd5, shows an early-flowering phenotype under LD but a late-flowering phenotype under SD [51]. Those flowering patterns are similar to the phenotype of the hd1 mutant [52]. Because the HAP complex forms a trimetric complex with CO protein in Arabidopsis, Hd5 cooperates with Hd1 through protein-protein interactions. The former also controls plant architecture, e.g., height, yield, grain dry weight, and the number of tillers.

Heading date 6 (Hd6) encodes a CK2  $\alpha$ -subunit and was identified by crossing 'Nipponbare' and 'Kasalath' [15,53]. The Hd6 allele from 'Nipponbare' is nonfunctional due to an early stop in the coding region. Introgression of the functional Hd6 allele from 'Kasalath' to 'Nipponbare' results in late flowering [53]. Hd6 functions as a flowering repressor of Hd3a and RFT1 under LD [10]. Although Hd6 has a late-flowering phenotype when functional Hd1 allele is present, it does not phosphorylate Hd1 [10].

Heading date 16 (Hd16)/Early flowering 1 (EL1) is another flowering repressor [50,54]. This gene was identified from a cross between the japonica rice 'Nipponbare' and 'Koshihikari' [55] (Matsubara et al.). 'Koshihikari' flowers later than 'Nipponbare' under SD but earlier under LD [55]. Introgression of a deficient allele of Hd16 from 'Koshihikari' into 'Nipponbare' weakens photoperiodic sensitivity and increases expression of floral activators Ehd1, Hd3a, and RFT1 under LD [24]. Hd16 encodes a casein kinase I that phosphorylates the rice DELLA protein SLR1 [50] and Ghd7 protein [24,50,56]. Therefore, Hd16 might possibly act as a mediator between floral transition and other developmental processes such as gibberellin signaling and tillering.

OsMADS50 is an LD-specific promoter of flowering [57]. Mutation induced by T-DNA causes a flowering delay of more than one month under LD, but has no such effect under SD [57]. OsMADS50 is an ortholog of Suppressor of Overexpression of CO 1 (SOC1), a flowering activator in Arabidopsis [58]. OsMADS50 suppresses expression of OsLFL1 [41] which inhibits Ehd1 by binding to the promoter region [59,60]. Transcript levels of OsMADS50 increase continuously until five weeks after germination [41]. This expression pattern is opposite to that of Ghd7, supporting the idea that OsMADS50 is a floral inducer. OsMADS56, the most homologous to OsMADS50, functions antagonistically when it binds to OsMADS50 [41]. Plants that over-express OsMADS56 flower later, by more than a month, under LD because expression of Ehd1, Hd3a, and RFT1 is repressed [41]. Both OsMADS50 and OsMADS56 are MADS-box TFs that contain a highly conserved MADS-box domain and K domain. The MADS-box domains function in DNA binding and dimerization while the K domains are involved in dimerization. The C domain is the least-conserved in both length and sequence. In some cases, it either possesses transactivation capability or contributes to the formation of multimeric complexes among MADS proteins [61,62]. The C region is shorter in OsMADS56 than in OsMADS50 and their sequences are diverse, suggesting that the former inhibits the latter.

Overexpression of OsDof12 causes early flowering under LD but not under SD [63] (Li et al.). In OsDof12 OX plants, transcript levels of Hd3a and OsMADS14 are increased under LD while those of Hd1, OsMADS51, Ehd1, and OsGI are not changed [63]. These results suggest that OsDof12 induces flowering by a pathway that does not go through Ehd1.

### SD-preferential regulatory elements

In addition to the inducer Hd1, several regulatory elements control flowering time under SD. They include OsMADS51, a Type I MADS-box protein [40]. The *osmads51* mutant flowers approximately two weeks later under SD, but its timing is unaltered under LD. The OsMADS51 OX plants bolt early, by about 10 d, under SD because expression by Ehd1, Hd3a, and OsMADS14 is enhanced [40]. However, flowering time is not changed under LD. This implies that OsMADS51 induces flowering only under SD, probably because it generates a product that functions preferentially under such conditions. Alternatively, the protein either inhibits an SD-specific repressor or enhances an SD-specific inducer.

Another SD-specific suppressor, OsCO3, is a member of the CO-like proteins and is similar to HvCO3 in barley [38,64]. Transcript levels are high during the daytime and low at night [64]. OsCO3 OX plants show SD-preferential flowering that is delayed by approximately 40 d because Hd3a, FTL, and OsMADS14 are suppressed [65].

### Constitutive regulators

OsIndeterminate 1 (OsId1)/Early heading date 2 (Ehd2)/Rice Indeterminate 1 (RID1) is a constitutive activator. RNAi plants of OsId1 have significantly delayed flowering under both SD and LD [65] and knock-out mutants do not flower for more than 365 d [66,67]. This indicates that OsID1 is required for promoting a flowering signal regardless of light conditions. However, no research has shown that overexpression of OsId1 causes early flowering. Therefore, it is possible that OsId1 must form an activation complex with another protein. OsID1 regulates the expression of Ehd1 and its downstream genes Hd3a and RFT1. Transcription of other regulators is not affected in *osid1* mutants, suggesting that OsID1 may directly induce Ehd1 expression. OsID1 is the rice ortholog of maize Indeterminate 1 (Id1),

which encodes a TF with an indeterminate domain [65]. Because *id1* mutants show phenotypes of prolonged vegetative growth [68], it has been suggested that Id1 is involved in the synthesis of a florigen [69,70]. The presence of Ehd1 homologs in maize implies that the Id1–Ehd1 pathway is conserved among grass species.

Early heading date 4 (Ehd4) is another constitutive activator that encodes a CCCH-type zinc finger protein. The *ehd4* mutant was identified as a somaclonal variant in Kitaake [71]. The *ehd4* mutant flowered late under both SD and LD [71]. In the mutant, transcript levels of Ehd1, Hd3a and RFT1 are decreased, suggesting that Ehd4 prompts flowering time via Ehd1.

OsCOL4 functions as a constitutive flowering repressor [72]. The *oscol4* mutants flower earlier, by approximately two weeks under SD and approximately three weeks under LD because Ehd1 expression is enhanced. Overexpression of OsCOL4 delays flowering by approximately three weeks under SD and approximately six weeks under LD conditions when transcript levels of Ehd1 are suppressed [72]. Expression of OsCOL4 is decreased in *osphyB* mutants and flowering time is similar between *osphyB oscol4* double mutants and *osphyB* single mutants, indicating that OsCOL4 is positively controlled by OsPhyB [72]. Whereas OsPhyB is involved in the night break (NB) effect [35], OsCOL4 is NB-insensitive, demonstrating that the former controls flowering time through an alternative pathway.

OsLF encodes an atypical bHLH TF that is a constitutive flowering repressor. Overexpression of OsLF causes late flowering regardless of photoperiod length [73] Under SD, expression by OsGI and Hd1 is partially decreased in OX lines [73].

As a flowering activator, miR172 suppresses AP2-like genes in Arabidopsis [74]. Similarly, miR172a and miR172d induce flowering in rice [75]. Transcripts of the AP2-like genes Supernumerary Bract (SNB) and *Oryza sativa* Indeterminate Spikelet1 (OsIDS1) are targeted by miR172s. Their overexpression delays flowering by suppressing the expression of Ehd1 [75]. Phytochromes inhibit miR172, thereby increasing the expression of those AP2-like genes (Lee et al.). This indicates that the phytochrome–miR172–AP2–Ehd1 pathway also plays a role in controlling flowering time.

### Circadian rhythm

GIGANTEA (GI) controls CO, a positive regulator of FT in Arabidopsis [76]. The GI–CO–FT pathway is conserved in rice [31]. Knockout mutants and RNAi-suppressed plants of OsGI flower late under SD but early under LD, exhibiting reduced photoperiodic sensitivity [31,77] OsGI positively controls Hd1 expression, with the latter being reduced in OsGI RNAi plants but increased in OX plants [31]. Transcriptome analyses between WT and *osgi* mutants have revealed that numerous rhythmic genes are affected [77]. In particular, expression by Ehd1, Hd3a, and Ghd7 is diminished in those mutants [44]. That of OsMADS51 is also decreased in OsGI antisense plants [40], indicating that OsGI controls flowering time via several independent pathways. An *osgi* knockout mutant generated by T-DNA insertion flowers late by 36 d under SD and by 9 d under LD conditions [78]. This SD-preferential phenotype is similar to that of the mutant by gamma irradiation [79]. Under SD, transcript levels of floral activators such as Hd3a, RFT1, Ehd1, Hd1, OsMADS51 and OsId1 are decreased in the *osgi*, demonstrating that the delayed flowering is due to decreased expression by the flowering activators [78]. However under LD, transcript level of OsGI is reduced to a low level at later stage of development when the flowering signals are generated. Consequently,

effect of OsGI on controlling flowering time under LD conditions is minimal.

Heading date 2 (Hd2) was identified by QTL mapping using crossed progenies between 'Nipponbare' and 'Kasalath' [15]. Introgressing the Hd2 allele of 'Kasalath' to 'Nipponbare' causes late flowering under SD but early flowering under LD [32]. This is evidence that Hd2 is involved in photoperiodic sensitivity. Hd2 is closely linked to *Oryza sativa* Pseudo Response Regulator 37 (OsPRR37), which encodes a pseudo-response regulator that functions as a major component of the circadian clock [80-83]. In two early-flowering Hokkaido cultivars, 'Kitaake' and 'H143', OsPRR37 is defective [84,85].

QTL mapping has shown that Heading date 3b (Hd3b)/Heading date 17 (Hd17)/Early flowering 7 (Ef7)/*Oryza sativa* Early flowering 3 (OsELF3) is a flowering inducer [16]. Hd3a encodes a homolog of Arabidopsis, EARLY FLOWERING 3 (ELF3), which has crucial roles in maintaining circadian rhythms [16,86-90]. In Arabidopsis, ELF3 modulates re-setting of the circadian clock [91,92]. A mutation in that gene leads to an early and photoperiod-insensitive flowering phenotype in Arabidopsis [93]. Hd17, an allele of Hd3b, has also been identified by QTL mapping of a cross between 'Nipponbare' and 'Koshihikari' [94]. Introgression of the Hd17 allele from 'Koshihikari' to 'Nipponbare' causes late flowering because of enhanced expression by Ghd7. The ef7 mutant, another allelic mutant for Hd3b, was developed via  $\gamma$ -irradiation of seeds from the japonica cultivar 'Gimbozu' [88]. Double-mutant analyses suggest that Hd3b functions as a floral activator by suppressing Ghd7 and Hd1. Hd3b is preferentially expressed in the mesophyll cells of the leaf blade, and also weakly in floral organs such as the lemma, palea, stamen, and pistil [88]. Because Ghd7 is preferentially expressed in the vascular tissue [43] (Xue et al.), further study is needed to determine how the mesophyll preferentially-expressed Hd3b controls Ghd7. Tos17 and T-DNA insertions cause late flowering by enhancing Ghd7 [89,90]. Various circadian clock components, e.g., OsLHY, OsPRR1, -37, -73, -95, and OsGI are changed in the ef7 mutants [89,90]. Therefore, one might conclude that, similar to Arabidopsis ELF3, Hd3b is involved in circadian clock control. In the rice genome, OsEF3 is also highly homologous to ELF3. Mutations in the rice gene cause pleiotropic effects, including late flowering [95].

### Photoreception

Photoreception is one of the most important processes by which plants determine flowering time. In rice, day length is recognized by three phytochromes, OsPhyA, OsPhyB, and OsPhyC. Mutations in OsPhyB or OsPhyC result in early flowering, indicating that they function as flowering repressors [96]. OsPhyB suppresses flowering by activating OsCOL4 [35,72] and is also involved in the NB effect, as observed in SD plants [97,98]. Flowering in rice is inhibited by night breaks because of decreased Hd3a expression [35]. However, the NB effect is not seen in the osphyB mutant, and Hd3a transcript levels are not altered there [35].

The flowering time of osphyB osphyC double mutants is similar to that of osphyB single mutants, probably due to the post-translational process of OsPhyC in osphyB mutants, where the amount of protein is significantly lower [79]. In Arabidopsis, PhyC protein levels are also reduced in phyB mutants [99,100]. In addition, phyB and phyC mutants show early flowering phenotypes under SD, and flowering time is similar between phyB and the phyB phyC double mutants [100]. This phenomenon is also found in rice [96,100]. It has been reported that PhyB interacts with PhyC in Arabidopsis [101], suggesting that

OsPhyB and OsPhyC function as flowering repressors by forming a heterodimer, whereas OsPhyB or OsPhyC alone is unstable.

Although mutation of OsPhyA by itself does not affect flowering time, the double mutants osphyA osphyB and osphyA osphyC flower earlier under SD and later under LD when compared with osphyB or osphyC alone [96]. This demonstrates that OsPhyA cooperates with other phytochromes to modulate the expression of flowering regulators [3]. Ghd7 expression is increased in osphyB and osphyB osphyC mutants but decreased in osphyA osphyC and osphyA osphyB, indicating that OsPhyA positively controls Ghd7 expression while OsPhyB and OsPhyC have the opposite effect [79]. Interestingly, Ehd1 expression is not changed despite Ghd7 expression being elevated in the osphyB osphyC mutants. This suggests that OsPhyA controls flowering time through an alternative pathway [79]. In Arabidopsis, PhyA prompts floral induction by stabilizing CO protein whereas PhyB suppresses flowering via CO degradation [102]. In rice, Hd1 protein levels are similar between light and dark conditions in 35S::Hd1::myc plants [36]. This implies that, unlike CO in Arabidopsis, Hd1 protein is not degraded by light in rice.

SE5 encodes a heme oxygenase that is highly homologous to Arabidopsis HY1, which is involved in the biosynthesis of phytochrome chromophore [77,103] (Izawa et al.; Andrés et al.). The se5 mutants exhibit an extremely early flowering phenotype under both SD and LD [103,104] flowering at 44 d after germination, regardless of day length, because of elevated transcript levels of Ehd1 and Hd1 [104]. This phenotype is similar to that of osphyA osphyB mutants [3,77,35,103].

Flowering time is also controlled by a blue light receptor cryptochrome (CRY) in Arabidopsis [102,105-107]. The rice genome has three CRY genes, OsCRY1a, OsCRY1b, and OsCRY2 [108,109]. While OsCRY1a and OsCRY1b are responsible for regulating blue light-mediated de-etiolation [108,109] OsCRY2 functions in controlling flowering time [108,109] OsCRY2 antisense plants flower late under both SD and LD [108] indicating that OsCRY2 is a flowering activator. In Arabidopsis, CRY2 activates flowering by stabilizing CO protein [102]. However, the molecular mechanism for OsCRY2 is not yet known in rice.

In Arabidopsis, GI interacts with several blue light receptors, e.g., Zeitlupe (ZTL) and Flavin-Binding Kelch Repeat F-Box 1 (FKF1) [110,111]. The protein complexes are more stable under blue light conditions [110,111]. In rice, Ehd1 expression is prompted by blue light treatment, but this activation is diminished in osgi mutants, suggesting that OsGI is involved in blue light signaling [3].

### Epigenetic regulation

Expression by some flowering time genes is developmentally regulated. For example, transcripts of OsLFL1 are maintained at a high level for the first four weeks but gradually decrease as plants mature. By comparison, transcripts of OsVIL2 (*Oryza sativa* VIN3-like 2)/LC2 (Leaf inclination 2)/OsVIL3 (*Oryza sativa* VIN3-like 3) remain at a low level but increase rapidly as OsLFL1 expression declines. Whereas overexpression of OsLFL1 causes late flowering [59,60] knockout mutations of OsVIL2 delay flowering under both SD and LD [112]. However, a knockout mutation of LC2, an allelic mutation of OsVIL2, causes late flowering only under SD [54,113]. The difference in results reported by those two research groups may be related to the day length that was tested. Whereas experiments by the former group entailed a 14.5-h photoperiod, plants used by the latter group were exposed to 14 h of light.

OsVIL2 protein binds to histone H3 and OsLFL1 chromatin (Yang et al.). Histone H3 lysine 27 trimethyl (H3K27me3) levels of OsLFL1 chromatin are reduced in *osvil2* mutants (Yang et al.). This indicates that OsVIL2 suppresses expression of OsLFL1 by mediating the methylation of lysine 27 residue of histone H3. OsVIL2 binds to *O. sativa* Embryonic Flower 2b (OsEMF2b), a core component of the polycomb repressive complex 2 (PRC2) that represses the expression of target genes by condensing their chromatins [112]. The *osemf2b* mutants also show late flowering phenotypes [112,114]. *Emf2b* promotes flowering by directly suppressing the expression of *OsLFL1* [115]. OsVIL1-RNAi plants flower late under SD conditions [113]. OsVIL1 engages with OsVIL2, suggesting that the two cooperate as flowering activators through protein-protein interactions.

Transcription of *Ghd7* is epigenetically regulated by OsTrx1 (*Oryza sativa* Trithorax 1), which encodes a histone methyltransferase [75]. OsTrx1 belongs to a Trithorax group (TrxG) of proteins that enhance the expression of target genes by loosening their chromatins. The *ostrx1* mutants show significantly delayed flowering that is preferential under LD [75]. In those mutants, *Ghd7* transcripts are more abundant under LD [75].

OsTrx1 protein interacts with Early heading date 3 (Ehd3) protein in Plant Homeodomain (PHD) finger regions [75]. The *ehd3* mutant was identified as a late-flowering variant of M2 plants from a  $\gamma$ -ray-mutagenized line of *O. sativa* ssp. *japonica* 'Tohoku IL9' [116]. Those mutants flower 20 d later than the WT under SD and do not flower for more than 365 d under LD [116]. As with *ostrx1* mutants, *Ghd7* transcripts are increased in *ehd3* mutants, all evidence that OsTrx1 and Ehd3 cooperatively suppress *Ghd7*.

The rice genome has at least 37 SET domain group (SDG) proteins that may be involved in chromatin remodeling via histone demethylation [117]. For example, SDG724/Long Vegetative Phase 1 (LVP1), which has histone methyltransferase activity, preferentially functions as a flowering activator under LD [118]. In the *lvp1* mutant, expression by activators OsMADS50, Hd3a, RFT1, and Ehd1 is significantly decreased under LD [118]. These findings confirm that SDG724 serves as an LD-preferential activator by inducing OsMADS50. Both SDG711 and SDG718 encode the rice enhancer of *zeste* [E(z)], a subunit of PRC2 [119]. SDG711 is a flowering repressor under LD, as demonstrated by the late flowering phenotype of OX plants, whereas SDG711 RNAi plants display early flowering phenotypes under LD [119]. In the SDG711 OX plants, expression is reduced for Ehd1, Hd3a, RFT1, OsMADS14, OsMADS15, and OsLF while transcript levels of Hd1 are increased under LD [119]. By contrast, in the SDG711 RNAi plants, expression is preferentially increased for OsMADS14 and OsLF but decreased for Hd1 under LD [119]. Those results demonstrate that SDG711 functions as an LD-preferential flowering repressor by inhibiting OsLF. Furthermore, SDG718 RNAi plants flower late preferentially under SD, with expression being decreased for OsMADS14, RFT1, Hd3a, and Hd1 but increased for OsLF [119]. Therefore, SDG718 functions as an SD-preferential flowering activator by suppressing OsLF.

## Conclusions

In addition to the conserved GI-Hd1-Hd3a pathway present in all plant species, rice has several other pathways that control flowering time. Most of them are merged to Ehd1 or, occasionally, directly to florigens. The *Ghd7* pathway is the major route that functions under LD. Several upstream elements, e.g., phytochromes, GI, and chromatin remodeling factors, modulate *Ghd7* expression. The OsMADS50

pathway induces flowering preferentially under LD. By contrast, the OsMADS51 pathway enhances flowering specifically under SD while OsCO3 inhibits flowering only under SD. OsID1 is a constitutive activator that is essential for flowering. The constitutive repressor OsCOL4 is controlled by OsPhyB. Flowering is induced by the miR172 pathway through the degradation of mRNA from AP2 family genes. Although several regulatory genes that control flowering time have been identified, their relationships and the way in which their proteins function are not well understood and must still be addressed. Moreover, the regulatory mechanisms for plant responses to environmental stimuli such as temperature extremes, nutrient deficiencies, and various stresses require further exploration.

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