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Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation

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Photoperiodic control of flowering time consists of a complicated network that converges into the generation of a mobile flowering signal called florigen. Recent advances identifying the protein FT/Hd3a as the molecular nature responsible for florigen activity have focused current research on florigen genes as the important output of this complex signaling network. Rice is a model system for short-day plants and recent progress in elucidating the flowering network from rice and Arabidopsis, a long-day plant, provides an evolutionarily comparative view of the photoperiodic flowering pathway. This review summarizes photoperiodic flowering control in rice, including the interaction of complex layers of gene networks contributed from evolutionarily unique factors and the regulatory adaptation of conserved factors.

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Introduction

Many plant species have an ability to flower during seasons preferable for their reproduction, and this ability depends mainly on the precise measurement of seasonal changes in day length and temperature [1]. The day-length dependent, or photoperiodic, control of flowering allows these plant species to adapt to the growth conditions in variable latitudes, altitudes, and seasons or different cropping locations [2]. Flowering plants can be categorized into three groups according to their photoperiodic flowering response. Long-day plants (LDPs) and short-day plants (SDPs) flowers more rapidly when day length gets longer or shorter, respectively, but day-neutral plants are not affected by day length.

Photoperiodic flowering has long been considered as a systemic process, including day-length measurement in leaves, generation of a mobile flowering signal and its transport from leaves to the shoot apex, and perception of the signal at the shoot apical meristem to initiate floral evocation [1]. Recent molecular genetic work in Arabidopsis and rice identified the FLOWERING LOCUS T(FT)/Heading date 3a (Hd3a) protein as the molecular nature for this mobile flowering signal called florigen [3–5]: *FT/Hd3a* gene expression is specifically upregulated upon an inductive photoperiod in leaf phloem tissue, these proteins are detected at the shoot apex where no transcription or mRNA accumulation of these genes are observed, and the loss-of-function mutation or RNAi suppression of these genes causes photoperiod-insensitive late flowering [6•,7•,8•]. Photoperiodic information perceived in leaves is ultimately integrated into the level of florigen production, as we now understand it as the level of *FT/Hd3a* expression. Thus, current efforts to dissect the flowering gene network focus on how these genes interact to control *FT/Hd3a* expression. In this context, LDP and SDP express more FT/Hd3a during longer and shorter day lengths, respectively.

The molecular basis for control of flowering has been studied extensively using Arabidopsis, a LDP. These investigations provided a deep understanding of crucial regulatory steps such as epigenetic regulation of vernalization [9], autonomous or endogenous hormone regulation of flowering [10], and light and circadian clock interactions in photoperiodic response [11], all of which converge at the control of *FT* gene expression. On the contrary, rice is a facultative SDP that shows several fundamental differences in flowering response compared with LDP. First, the photoperiodic response is completely opposite in Arabidopsis and rice because LD promotes flowering in Arabidopsis but represses flowering in rice [12•]. Second, SDP, but not LDP, show the critical day-length response that a small addition of day length of about 30 min significantly delays flowering [13••]. Finally, SDP, but not LDP, show the night-break response where the light exposure for a short (about 10 min) period in the night suppresses flowering [14]. In addition, recent advances in flowering time research in rice have identified more a complex and unique flowering pathway involving the day-length dependent switching of expression of two florigen genes [15••] and different targets for the natural variation in flowering time control in rice compared with that in Arabidopsis [16•]. Here, we will summarize our current understanding of the rice flowering network that is contributed from evolutionarily conserved factors and uniquely acquired factors (Table 1) and discuss the

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Table 1

Genes involved in the photoperiodic flowering of rice

Rice gene	Arabidopsis homolog	Gene ID of rice gene	Note	Reference
OsGI	*GI*	*Os01g0182600*	Circadian clock related protein	[20]
Hd1	*CO*	*Os06g0275000*	B-box zinc-finger protein with CCT domain	[12•,19•,23]
Hd3a	*FT*	*Os06g0157700*	Similar to phosphatidylethanolamine-binding protein	[7•,45]
RFT1	*FT*	*Os06g0157500*	Similar to phosphatidylethanolamine-binding protein	[8•,15••,45]
RCN1	*TFL1*	*Os11g0152500*	Similar to phosphatidylethanolamine-binding protein	[54]
Ehd1	*None*	*Os10g0463400*	B-type response regulator	[13••,26]
Ehd2/OsID1/RID1	*None*	*Os10g0419200*	C2-H2 zinc-finger protein, maize *Indeterminate1* ortholog	[29]
SE5	*HY1*	*Os06g0603000*	Heme oxygenase involved in phytochrome chromophore formation	[55]
PHYB	*PHYB*	*Os03g0309200*	Phytochrome	[14]
ETR2	*ETR2, EIN4*	*Os04g0169100*	Ethylene receptor	[22]
Hd6	*CKA1, CKA2, At2g23070*	*Os03g0762000*	Casein kinase II alpha subunit	[37]
OsMADS50	*SOC1*	*Os03g0122600*	MADS box protein	[40]
OsMADS51	*None*	*Os01g0922800*	MADS box protein	[27]
OSMADS56	*SOC1*	*Os10g0536100*	MADS box protein	[39]
Ghd7	*None*	*Os07g0261200*	CCT domain protein	[13••,35••]
OsLFL1	*FUS3*	*Os01g0713600*	B3 domain transcription factor	[39]
RFL	*LFY*	*Os04g0598300*	FLORICAULA/LEAFY transcription factor	[44]
OsMADS14	*AP1, CAL, FUL*	*Os03g0752800*	MADS box protein	[50]

molecular mechanism of the above-mentioned differences.

Short-day promotion of Hd3a expression in rice

The evolutionarily conserved regulatory module for photoperiodic flowering consists of GIGANTEA(GI)-CONSTANS(CO)-FT signaling pathways, where the clock-associated protein GI upregulates expression of *CO*, encoding a B-box zinc finger transcription factor, and in turn CO activates expression of the florigen gene *FT*, encoding a small protein with homology to phosphatidylethanolamine-binding protein [3,4]. The GI-CO-FT pathway is active only during LD in Arabidopsis, because *CO* expression starts to increase at the end of light period during LD. Thus, a sufficient amount of CO protein can be accumulated to induce FT expression, by escaping from the ubiquitin-mediated degradation in darkness through the activity of the RING-finger ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1). In SD, CO expression starts to increase in darkness, thus translated protein is immediately degraded [17,18]. GI-CO-FT also plays a central role in rice, but regulatory modification of this conserved module completely reverted the photoperiodic response on florigen gene expression [12•,19•].

The rice counterpart of the GI-CO-FT pathway is composed of their orthologous proteins, OsGI-Heading date1 (Hd1)-Hd3a, that is active only in SD but is modified to change its activity during LD (Figure 1). *OsGI* was identified by the differential display approach, and subsequent functional analysis revealed it as an activator of Hd1 expression [12•,20]. Arabidopsis GI participates with the circadian clock components by contributing to the degradation of core clock oscillator protein [21].

OsGI expression shows a daily circadian oscillation with a peak at the end of the light period, implying upstream regulation by the circadian clock [20]. Recently, the ethylene receptor ETR2 was shown to be required for *OsGI* expression, but the precise mechanisms for ethylene signaling to control OsGI remain unclear [22].

The most important downstream component of OsGI is Hd1, which has a major impact on photoperiodic Hd3a induction. *Hd1* was first identified as the key flowering QTL between different rice subspecies, and a positional cloning approach revealed it to encode a single ortholog of Arabidopsis *CO*, which encodes the B-box zinc-finger protein with a C-terminal CCT (CONSTANS, CONSTANS-LIKE, and TIMING OF CAB EXPRESSION1) domain [23,24]. Under SD conditions, loss-of-function alleles of *Hd1* delay flowering and reduce *Hd3a* mRNA accumulation [14,19•,23]. Interestingly, *Hd1* expression peaks at midnight, whereas *Hd3a* expression peaks at the beginning of the light period, suggesting a timing mechanism to form such a peak phase difference [12•].

The precise molecular mechanism by which Hd1 upregulates *Hd3a* expression during SD remains unclear, but night break experiments suggest the direct involvement of phytochrome B in this process [14,25]. Night break is the phenomenon that a short light exposure during the dark period can significantly delay flowering of SDPs. Like other SDPs, rice clearly shows a night-break response and detailed gene expression analyses revealed

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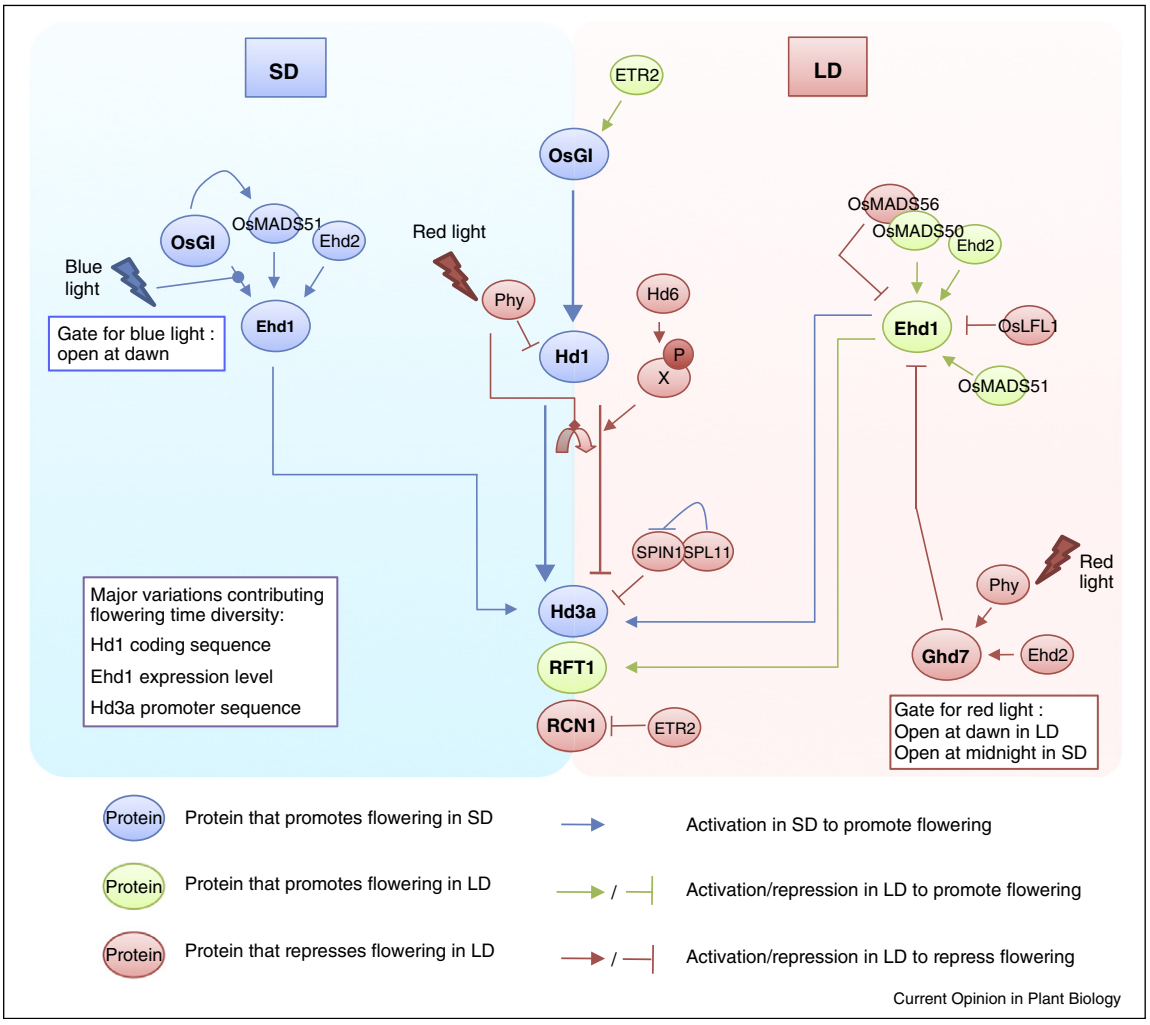
Figure 1



The molecular network for florigen gene regulation in rice.
(Left) In SD conditions, Hd1 protein acts as an activator of Hd3a florigen gene expression. Thus, the evolutionarily conserved OsGI-Hd1-Hd3a pathway promotes flowering. A B-type response regulator, Ehd1, also induces Hd3a expression in SD conditions. Ehd1 expression is activated by blue light illumination in the morning, and this timing is controlled by OsGI. Ehd2, a C2H2 zinc-finger protein orthologous to maize Indeterminate1, and OsMADS51 also activate Ehd1 in SD conditions.
(Right) In LD conditions, Hd1 function is converted into a repressor of Hd3a expression. This process is specifically evoked during LD by the coincidence of the clock-regulated Hd1 expression and phytochrome-mediated light signaling. Hd6 CK2alpha enhances the repressor activity. Ghd7, a CCT-motif protein, represses Ehd1 expression in LD conditions to delay flowering. Ghd7 expression is induced by phytochrome-mediated red light signaling in the morning of LD, but this timing shifts to midnight in SD conditions. Although LD is the suppressive condition for rice flowering, there is a LD-specific flowering promotion pathway in rice. OsMADS50 activates Ehd1 expression, and in turn Ehd1 activates RFT1 expression. RFT1 acts as the LD-specific florigen.
The natural variation of SD flowering in rice is well correlated with the variation in Hd3a expression level that is determined by a combination of Hd1 allelic variation, Hd3a promoter subtypes and Ehd1 expression level. Ghd7 also contributes to the variation in flowering time and growing locations.

that night break suppresses *Hd3a* at the transcriptional level, without any effect on *OsGI* and *Hd1*. Hd1 activity is thought to be severely attenuated by light illumination at midnight because the *Hd1* mutation reduced *Hd3a* expression in the absence of a night break treatment. Conversely, the *phyB* mutant maintained a higher level of *Hd3a* expression in the presence of a night break treatment. Thus, the night break signal supresses *Hd3a* expression via phyB and Hd1 activities [14,25].

Another important activator of *Hd3a* expression is Early heading date1 (Ehd1), a B-type response regulator protein [26]. Ehd1 can bind DNA through its GARP (maize GOLDEN2, the ARR B-class proteins from *Arabidopsis*, and *Chlamydomonas Psr1*) domain, and mutation of this domain or RNAi suppression of this gene decreased *Hd3a* expression under SD conditions [26,27]. Interestingly, *Ehd1* is an evolutionarily unique gene that does not have an ortholog in the Arabidopsis

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genome [26], and the photoperiodic regulation of *Ehd1* expression also involves a gene network distinct from Arabidopsis. *Ehd1* expression is promoted through the activities of *OsMADS51* [27] and *Ehd2/Rice Indeterminate1 (RID1)/Oryza sativa Indeterminate1 (OsID1)* that three research groups independently identified and hereafter will be simply referred to as *Ehd2* [28–30]. *Ehd2* is an ortholog of maize *indeterminate1* (*id1*), encoding the C2-H2 zinc-finger protein with a strong activity to promote flowering. However, several phenotypic aspects are different between maize *id1* and rice *ehd2*, for example, Ehd2 affects *FT-like* gene expression through *Ehd1* activation, whereas *id1* seems not to regulate maize *FT-like* genes [31].

OsGI strongly activates *Ehd1* expression mainly through two pathways, one is dependent on OsMADS51 whose expression is abolished in an *OsGI* antisense suppression line [27], and the other is dependent on GI-controlled blue light signaling that activates *Ehd1* expression at the beginning of the light period [13••]. The latter property is essential for forming the critical day-length response. Ehd1 is upregulated at the beginning of a light period, and this response utilizes the blue morning light as the crucial cue to start expression. Detailed physiological experiments revealed that the blue light sensitivity is specifically gated at and around the beginning of the light period when OsGI must determine the timing. Thus the *osgi* mutant abolishes blue-light induction of *Ehd1* at the beginning of the light period [13••]. The night break experiment and phytochrome mutant analysis also showed that *Ehd1* is an additional target of the phytochrome signaling, but since this issue is closely related to LD signaling it will be discussed later [13••,32].

Natural variation in rice flowering time

Accumulating evidence for the SD flowering pathway allowed us to explore the molecular nature of flowering time diversity in rice [16•]. Cultivated rice varieties show substantial diversity in flowering time under SD conditions, and comprehensive analyses by combining gene expression studies, sequence comparisons and transient expression assays revealed that early flowering during SD is well correlated with high expression of *Hd3a*. Moreover, this crucial variation in Hd3a expression is contributed mainly by the *Hd3a* promoter sequence, *Hd1* functional polymorphisms, and *Ehd1* expression level (Figure 1). These results share an important aspect in the natural variation of florigen genes because recent reports identified that the Arabidopsis *FT* promoter also has variations contributing to flowering time differences [33], but there is a striking contrast with the situation in Arabidopsis in which the major determinant of flowering time diversity was found at vernalization related loci [34]. The allelic variation of another flowering time gene in rice, *Ghd7* (for *Grain number, plant height, and heading date 7*), is also associated with the latitude of the cropping area in Asia [35••]. *Ghd7* is a repressor of *Ehd1* expression under LD (discussed below). Strong alleles of *Ghd7* tend to be found in the southern part of Asia, and weaker or non-functional alleles appear more frequently in the northern part. It is interesting that both *Ehd1* and *Ghd7* are unique genes in rice and have contributed to the natural variation in flowering time and growing areas.

Long-day suppression of Hd3a expression

During LD, rice flowering is delayed about 30 days and *Hd3a* expression under LD conditions is quite low to ensure the promotion of flowering [8•]. The central mechanism for *Hd3a* suppression comes from modification of the conserved OsGI-Hd1-Hd3a pathway where Hd1 activates *Hd3a* during SD, but its function is converted into a repressor to attenuate *Hd3a* expression during LD (Figure 1) [12•,19•]. This means that *hd1* mutant exhibited not only delayed flowering under SD, but also early flowering under LD. Phytochrome signaling is the most important modifier of the daylength-dependent conversion of Hd1 activity, because this conversion is not observed in a phytochrome-deficient mutant background such as *photoperiod sensitivity5 (se5)*, in which a homolog of the heme oxygenase gene essential for phytochrome chromophore maturation is mutated [19•]. In *se5*, Hd1 protein always acts as the activator of Hd3a independent of day length, suggesting that phytochrome signaling converts Hd1 into a repressor.

This finding is further supported by the direct manipulation of *OsGI* expression and the resulting change in *Hd1* and *Hd3a* expression levels [12•]. *Hd1* expression peaks at midnight under SD. Thus, Hd1 protein normally does not accumulate at a time when phytochrome signaling occurs. However, when *OsGI* is overexpressed, *Hd1* expression gets higher in the daytime, allowing Hd1 protein to interact with the phytochrome signaling pathway, resulting in the conversion of Hd1 to a repressor of *Hd3a* expression.

Hd1 repressor function can be enhanced by casein kinase 2 (CK2) activity that includes Hd6 protein as the CK2α subunit. *Hd6 CK2α* was first identified as a QTL that delays flowering, and interestingly, this effect appears in a LD-specific manner [36]. Extensive molecular genetic studies revealed that Hd6 CK2α clearly delays flowering and efficiently suppresses *Hd3a* expression only when *Hd1* is functional. However, Hd6 CK2α does not phosphorylate Hd1 protein directly, suggesting the presence of an unknown substrate that is expected to work with Hd1 [37].

Hd3a expression is upregulated by not only Hd1 but also Ehd1, and the latter is suppressed by Ghd7, which is a small protein with a CCT-domain [13••]. *Ghd7* is also unique to rice with no counterpart in Arabidopsis. Another key mechanism for photoperiodic *Hd3a* expression comes

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from the LD-specific upregulation of the strong repressor Ghd7. *Ghd7* expression is specifically upregulated during LD, and then Ghd7 activity strongly suppresses *Ehd1* expression and downstream *Hd3a* expression. The *ghd7* mutant always expresses higher levels of *Hd3a* in a day-length independent manner [13••]. The molecular mechanism for this LD-dependent *Ghd7* expression exemplifies the complex and unique aspects of the rice flowering network. *Ghd7* expression is induced through phytochrome signaling, and the sensitivity to red light is gated at the beginning of the light period during LD. The complex physiological experiments combining red-light illumination for *Ghd7* induction and blue-light illumination for *Ehd1* induction revealed that *Ghd7* requires a substantial duration to repress *Ehd1* expression, hence *Ghd7* in the morning suppresses *Ehd1* during the next morning under LD. Surprisingly, this timing of red light sensitivity shifts from morning in LD conditions to midnight during SD. If red light is exposed at midnight of a SD as the night break treatment, *Ghd7* is immediately upregulated to suppress *Ehd1* and subsequent *Hd3a* expression [13••].

Recent genetic studies provided more factors affecting *Hd3a* supression during LD. An example is the E3 ubiquitin ligase, Spotted leaf 11, that was formerly known as a negative regulator of disease resistance [38]. SPL11 protein controls *Hd3a* expression through physical interaction with SPL11 interacting protein1 (SPIN1), the STAR-domain protein with an RNA/DNA binding property, but whether this activity is essential for *Hd3a* repression is unclear. *OsMADS56* and *Oryza sativa LEC1 and FUSCA-LIKE1* (*OsLFL1*) attenuates *Ehd1* expression during LD, and OsLFL1 has been proposed to interact with *Ehd1* chromatin to downregulate *Ehd1* expression [39].

Long-day promotion of RFT1 expression

Rice is a facultative short-day plant and can finally flower during non-inductive LD conditions. One can assume that although *Hd3a* expression is quite low during LD, residual Hd3a activity eventually makes the plant flower. Although *Hd3a* RNAi suppression strongly delays flowering only during SD, *Hd3a* RNAi plants flower quite normally during LD [15••]. This observation indicates the presence of another key factor promoting flowering during non-inductive LD. Detailed analysis of flowering mutants revealed that this factor is *RICE FLOWERING LOCUS T1* (*RFT1*), the closest paralog of *Hd3a* [15••]. *RFT1* RNAi suppression showed a contrasting phenotype from *Hd3a* RNAi. *RFT1* RNAi suppression had no effect on flowering in SD conditions but impaired flowering specifically during LD. Consistent with this observation, *RFT1* expression increased during LD in leaf phloem tissue, and RFT1 protein was shown to move from leaves to the shoot apex by using a RFT1-GFP fusion protein. All these observations strongly indicate that RFT1 is the LD-specific florigen, and rice uses two florigen genes dependent on day length. In addition, when both Hd3a and RFT1 activities are suppressed, flowering is completely abolished, suggesting that flowering is fully dependent on florigen activity in rice [8•,15••].

RFT1 expression in LD conditions is induced through an evolutionarily unique pathway where *OsMADS50* and *Ehd1* play central roles. *OsMADS50* is a homolog of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) in Arabidopsis [40], which integrates various signaling inputs to promote flowering (Figure 1). Gene expression analysis using *osmads50* and *RFT1* RNAi plants indicate the presence of an LD-specific flowering pathway comprised of OsMADS50-Ehd1-RFT1, where *osmads50* abolishes *Ehd1* and *RFT1* expression and flowering in LD conditions, and *RFT1* RNAi delays flowering in LD conditions without affecting *OsMADS50* and *Ehd1* expression. This evolutionarily unique pathway provides rice plants the facultative short-day nature so that they can flower during non-inductive conditions.

SOC1 and LFY function in floral induction in rice and Arabidopsis

SOC1 and LFY in Arabidopsis are the most important integrators for flowering response and have long been studied for their function in meristems [41]. Arabidopsis *SOC1* strongly promotes flowering. *SOC1* expression increases in the apical meristem, and this upregulation requires FT activity [42]. *SOC1* mis-expression from the phloem-specific *SUC2* promoter can only weakly rescue the *soc1* mutation, suggesting that SOC1 acts mainly in the meristem and has limited activity in leaves [43]. By contrast, rice *OsMADS50*, a homolog of Arabidopsis *SOC1*, has a clearly different mode of function. *OsMADS50* acts in leaves upstream of *RFT1* [15••]. The *osmads50* mutation abolishes *Ehd1* and *RFT1* expression in leaves, causing a non-flowering phenotype during LD. *OsMADS50* expression is very low in the meristem, and its expression is unaffected by the floral transition. Thus, Arabidopsis SOC1 acts as the floral integrator in the meristem downstream of FT, but its rice homolog, OsMADS50, defines the LD-specific flowering pathway and acts as the upstream regulator of *RFT1* in leaves.

Another contrast between rice and Arabidopsis flowering can be found in LFY function. In Arabidopsis, the *lfy* mutation has no effect on the timing of flowering, measured by leaf number from germination to bolting, indicating a weak contribution to flowering [41]. By contrast, the rice LFY ortholog, RICE FLORICAULA/LEAFY (RFL), promotes flowering in rice. RNAi suppression of *RFL* strongly delays flowering, and *RFL* overexpression promotes flowering slightly in a certain rice cultivar [44]. Gene expression analysis of these transgenic plants suggests that RFL increases *OsMADS50* and *RFT1* expression in leaves, another difference from Arabidopsis LFY that functions specifically in the meristem.

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Table 2

Florigen interacting proteins

Protein	Gene name	Organism	Reference
bZIP-type transcription factor	FD (At4g35900)	Arabidopsis	[47]
	FDP (At2g17770)	Arabidopsis	[47]
	DLF1 (EF093789)	Maize	[49]
	SPGB (EF136919)	Tomato	[52]
14-3-3	GF14c (Os08g0430500)	Rice	[51]
	14-3-3/74 (AF079450)	Tomato	[52]
NIMA-like kinase	SPAK (AF079103)	Tomato	[52]

Molecular action of florigen in the floral transition of shoot apical meristems

Hd3a and FT belong to the PEBP (phosphatidylethanolamine-binding protein) family that is highly conserved among organisms from bacteria to humans [45]. PEBP is a small globular protein with a pocket structure for anion binding [46]. Several florigen interacting proteins have been identified (Table 2). In *Arabidopsis*, FT protein interacts with FD, a bZIP-type transcription factor, to activate *AP1*, a floral meristem identity gene [47,48]. No distinct domains for transcriptional regulation, such as acidic amino acid-rich or glutamine-rich domains, have been found in FT and Hd3a. Such a regulatory pathway as FT-FD in *Arabidopsis* could exist in rice, although the *FD* ortholog in rice remains to be identified [49]. RNAi plants of *Hd3a* or *RFT1* showed strong attenuation of *OsMADS14* and *OsMADS15* RNA, as well as a delay in flowering [8•,15••]. OsMADS14 and OsMADS15 belong to the AP1 subfamily of the plant MADS family [50]. The genetic role of *OsMADS14* and *OsMADS15* in the process of floral transition remains to be analyzed.

A rice 14-3-3 isoform, GF14c, was reported to interact with Hd3a [51]. The interaction of FT with a tomato 14-3-3 has also been reported [52], suggesting a universal role of 14-3-3 in the regulation of flowering by florigen. Although overexpression experiments suggest that 14-3-3 is involved in determinacy in tomato and regulation of flowering in rice, the molecular mechanism of how 14-3-3 modulates florigen activity remains to be studied. SPAK, a NIMA-like kinase of tomato, has also been reported to interact with FT [52], albeit its role in flowering control remains unknown.

Conclusions and perspectives

Recent progress has demonstrated that evolutionarily conserved genes and uniquely acquired genes are involved in the molecular network that regulates flowering in rice. These flowering time genes shape multiple aspects of rice flowering, including the LD-specific promotion of flowering and natural variations in flowering time. These new data provoke further interesting issues, such as the evolutionary processes responsible for the photoperiodic flowering pathway in rice, the precise molecular nature of the modification on the conserved OsGI-Hd1-Hd3a module by day length, and the mechanism of transport and function of Hd3a/RFT1. Unlike Arabidopsis, the flowering of rice completely depends on a pair of florigen genes [8•]. Thus, rice provides a unique system to study florigen function. In addition, the florigen genes or the regulators of their expression are linked to crop productivity [35••,53]. Therefore, these flowering genes should become interesting targets for the future improvement of rice production.

Disclosure statement

The authors declare no conflicts of interest.

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This work shows that Hd3a protein moves from leaf to shoot apex in rice and promotes flowering. *Hd3a* is transcribed in the leaf phloem tissue, its mRNA is only present in the leaf whereas Hd3a protein is accumulated in the shoot apex.

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