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doi:10.1093/aob/mcu032, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

REVIEW: PART OF A SPECIAL ISSUE ON FLOWER DEVELOPMENT

Molecular control of seasonal flowering in rice, arabidopsis and temperate cereals

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Received: 1 November 2013 Returned for revision: 11 December 2013 Accepted: 4 February 2014

• *Background* Rice (*Oryza sativa*) and *Arabidopsis thaliana* have been widely used as model systems to understand how plants control flowering time in response to photoperiod and cold exposure. Extensive research has resulted in the isolation of several regulatory genes involved in flowering and for them to be organized into a molecular network responsive to environmental cues. When plants are exposed to favourable conditions, the network activates expression of florigenic proteins that are transported to the shoot apical meristem where they drive developmental reprogramming of a population of meristematic cells. Several regulatory factors are evolutionarily conserved between rice and arabidopsis. However, other pathways have evolved independently and confer specific characteristics to flowering responses.
• *Scope* This review summarizes recent knowledge on the molecular mechanisms regulating daylength perception and flowering time control in arabidopsis and rice. Similarities and differences are discussed between the regulatory networks of the two species and they are compared with the regulatory networks of temperate cereals, which are evolutionarily more similar to rice but have evolved in regions where exposure to low temperatures is crucial to confer competence to flower. Finally, the role of flowering time genes in expansion of rice cultivation to Northern latitudes is discussed.
• *Conclusions* Understanding the mechanisms involved in photoperiodic flowering and comparing the regulatory networks of dicots and monocots has revealed how plants respond to environmental cues and adapt to seasonal changes. The molecular architecture of such regulation shows striking similarities across diverse species. However, integration of specific pathways on a basal scheme is essential for adaptation to different environments. Artificial manipulation of flowering time by means of natural genetic resources is essential for expanding the cultivation of cereals across different environments.

Key words: *Oryza sativa*, rice, *Arabidopsis thaliana*, cereals, photoperiodic flowering, vernalization, florigen, flower development.

INTRODUCTION

Floral initiation is a major physiological change that sets the switch from vegetative to reproductive development in most plant species. The transition from a vegetative (production of stem and leaves) to a reproductive stage (production of inflorescences and flowers) determines the time of flowering (or heading date in cereals) and is one of the most important developmental switches in the life cycle of plants. To maximize reproductive success and guarantee sufficient seed production for propagation of the species, flowering time should be tightly regulated through the integration of environmental inputs (daylength, temperature, light quality, water and nutrient availability) with endogenous cues (juvenility, stage of development). Depending on their requirement for daylength, plants can be classified into three categories. Long-day (LD) plants flower when the photoperiod exceeds a critical daylength, short-day (SD) plants flower when the photoperiod is shorter than a critical daylength and day-neutral plants flower regardless of daylength. The critical daylength for floral induction is specific to each species but often varies between accessions of the same species. In many plant species, flowering can also be stimulated by exposure to low non-freezing temperatures for several weeks. This process, known as vernalization, occurs in temperate zones during winter and prepares the plant to switch to reproductive growth only after the cold season, when temperatures become favourable again. Molecular genetic studies on model plants such as arabidopsis and rice (*Oryza sativa*) have allowed identification of genes controlling responses to environmental inputs and many of those have been shown to be conserved between the two model species, despite 150 million years of divergent evolution (Chaw *et al.*, 2004). However, during evolution, other pathways have evolved and several factors have been recruited to novel functions, increasing the diversity of flowering behaviours and adapting the species to grow across broader areas. Although arabidopsis and rice have been crucial to establish the basal architecture of floral regulatory networks, studies conducted on other species, including temperate cereals (e.g. wheat and barley) greatly contributed to our understanding of the molecular mechanisms controlling flowering.

In this review we will focus on rice as a model for SD plants and discuss the pathways involved in daylength measurement and flowering, mainly in comparison with arabidopsis. We will discuss similarities of the core regulatory pathways controlling photoperiodic flowering but will also address the pathways that have evolved specifically in rice, which are not present in arabidopsis. Finally, we will contrast regulatory networks active in temperate cereals with those of arabidopsis and rice. We will take into account the spatial separation of functions in leaves and at the shoot apex.

Besides its importance as a model system, rice is a crop and staple food for most parts of the world. It was first domesticated in Southern China and has evolved to adapt to a range of geographical regions over time. Currently, rice is cultivated over a wide range of latitudes from 55°N to 36°S (Khush, 1997). This has been possible through the diversification of flowering time. In fact, flowering of rice is accelerated under SD conditions; however, artificial selection has led to successful cultivation even under LD conditions. Some varieties of rice with weak or no photoperiod sensitivity flower very early at Northern latitudes, particularly in Europe, Northern China and Japan. We will discuss the genetic factors that have allowed such expansion and that represent an increasing list of molecular tools that plant breeders can use to expand further rice cultivation worldwide.

THE PHOTOPERIODIC PATHWAY IN ARABIDOPSIS AND RICE

Facultative LD species, such as arabidopsis, accelerate flowering as days become longer, and several genes have been isolated that control responses to photoperiodic cues (Andrés and Coupland, 2012). The corresponding mutants flower later when exposed to long inductive days, typical of spring or early summer, but do not affect flowering time when plants are grown under SDs (Turck *et al.*, 2008). Central to the photoperiod pathway in arabidopsis is *CONSTANS* (*CO*), a zinc finger transcription factor that integrates environmental and endogenous information to trigger flowering at the appropriate time of the year. In the vascular tissue of leaves, the CO protein directly activates expression of *FLOWERING LOCUS T* (*FT*), which encodes a florigenic protein promoting flowering (An *et al.*, 2004; Tiwari *et al.*, 2010). Expression of *CO* is regulated by the circadian clock that sets its rhythmic cycling to reach a peak at the end of the day under LDs. Cycling of *CO* mRNA depends on the activity of a protein complex formed by GIGANTEA (GI) and FLAVIN BINDING, KELCH REPEAT, F-BOX 1 (FKF1), a protein containing an F-box and blue light photoreceptor domains. The interaction between GI and FKF1 requires light, and the presence of GI protein is necessary to confer stability to the FKF1 protein (Sawa *et al.*, 2008; Fornara *et al.*, 2009). Upon interaction with GI, FKF1 targets a group of DOF transcription factors, collectively known as *CYCLING DOF FACTOR* (*CDF*) genes, for degradation (Fornara *et al.*, 2009). CDF proteins directly bind to the promoters of *CO* and *FT*, to prevent their expression when plants are exposed to short photoperiods (Imaizumi *et al.*, 2005; Y. H. Song *et al.*, 2012). Degradation of the CDFs occurs at the DNA of target loci and results in de-repression of *CO* and *FT*, allowing flowering to occur (Fig. 1). Therefore, genotypes in which the activity of the *CDF* genes is strongly reduced are insensitive to daylength, and flower early under any photoperiodic condition (Fornara *et al.*, 2009; Gómez-Ariza and Fornara, 2012).

CONSTANS is also tightly regulated at the post-transcriptional level. Despite its transcription being high during the night under both SDs and LDs, the protein does not accumulate in the dark because it is quickly directed to the proteasome through the activity of an E3 ubiquitin ligase encoded by *CONSTITUTIVE PHOTOMORPHOGENIC 1* (*COP1*) (Jang *et al.*, 2008; Liu *et al.*, 2008). Other proteins, including phytochromes and ubiquitin ligases, influence the stability of CO protein at different times of day, to ensure it accumulates only when the day is sufficiently long (Valverde *et al.*, 2004; Pineiro *et al.*, 2012; Y. H. Song *et al.*, 2012).

Regulation of photoperiodic flowering through *CO* has features of the external coincidence model of photoperiodism, originally proposed by Pittendrigh and Minis (1964). According to this model, an endogenous oscillator sets the rhythmic phase of expression of target molecules. Coincidence of a particular phase of expression with an external factor, such as light, triggers a developmental response. Several steps of the photoperiodic cascade of arabidopsis represented in Fig. 1 require light at the appropriate time of a circadian cycle to induce flowering. The GI–FKF1 regulatory complex is expressed and stabilized only when the peak of mRNA expression of the two genes coincides with light under long daylengths. This allows the corresponding proteins to interact and the complex to be stabilized. Similarly, CO protein accumulation takes place only at the end of an LD, in the presence of light that stabilizes it. Only under these conditions can the CO protein accumulate, activate *FT* expression and induce flowering. Under SDs, GI and FKF1 proteins do not interact, preventing *CO* mRNA from increasing at the end of the day. Therefore, light is necessary at the appropriate time of day to activate the pathway. In agreement with this model, in mutants in which the diurnal waveform of *CO* mRNA is also displaced towards the light phase under SDs, or in which the protein is allowed to accumulate because of mutations in genes controlling its post-transcriptional stability, *FT* expression and flowering are activated regardless of daylength (Yanovsky and Kay, 2002; Valverde *et al.*, 2004; Jang *et al.*, 2008; Fornara *et al.*, 2009). This mechanism incorporates endogenous and environmental information to synchronize flowering with seasons characterized by LDs.

Rice shares a similar photoperiodic pathway, mediating daylength responses. *OsGI* and *Heading Date 1* (*Hd1*) have been cloned and shown to encode homologues of arabidopsis *GI* and *CO*, respectively (Yano *et al.*, 2000; Hayama *et al.*, 2003). Several homologues of *FT* are encoded in the rice genome and at least three of them can promote flowering when overexpressed, i.e. *Hd3a*, *RICE FLOWERING LOCUS T 1* (*RFT1*) and *FT-like 1* (*FTL1*) (Izawa *et al.*, 2002; Kojima *et al.*, 2002; Ogiso-Tanaka *et al.*, 2013).

Similar to arabidopsis, control of *Hd1* expression is crucial to confer a photoperiodic response (Brambilla and Fornara, 2013). *Hd1* was originally identified as a major quantitative trait locus (QTL) and was later cloned by map-based approaches (Yano *et al.*, 2000). *Hd1* is highly homologous to *CO* and the Hd1 protein is thought to be involved in DNA binding. However, direct interaction of Hd1 with the *Hd3a* promoter has not been reported. Additionally, whereas *CO* promotes flowering under LDs, *Hd1* promotes flowering under SDs but represses flowering under LDs. The bi-functionality of *Hd1* became clear through the analysis of plants carrying *hd1* loss-of-function alleles that cause late flowering under SDs and early flowering under LDs (Yano *et al.*, 2000; Izawa *et al.*, 2002). The effect on flowering is correlated with *Hd3a* mRNA levels that are reduced in *hd1* mutants grown under SDs and increased when *hd1* mutants are grown under LDs (Izawa *et al.*, 2002). Therefore, *Hd1* has opposite effects on *Hd3a* expression and flowering that switch depending on daylength.

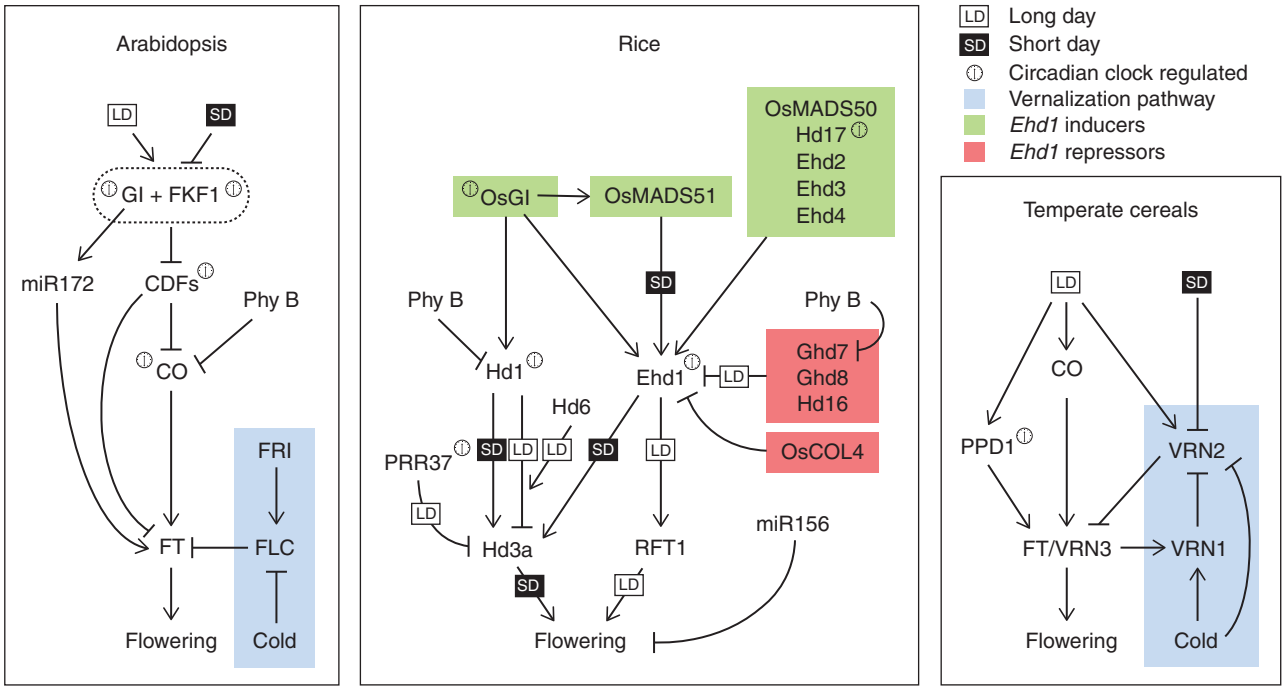

FIG. 1. Simplified regulatory networks controlling florigen production in leaves of different plant species. Networks of arabidopsis (left panel), rice (central panel) and temperate cereals (right panel) are compared. Small white and black boxes indicate regulatory connections occurring primarily under LDs and SDs, respectively. Arrows indicate transcriptional activation, whereas flat-ended arrows indicate transcriptional repression. The blue boxes in both the arabidopsis and the temperate cereal models include genes involved in vernalization responses. The green and red boxes in the rice model include positive and negative regulators of *Ehd1* expression, respectively. A clock symbol close to a gene indicates that its transcription is controlled by the circadian clock.

OsGI is a positive regulator of *Hd1* expression, under both SDs and LDs. In arabidopsis, overexpression of *GI* induces *CO* transcription and rapid flowering under any daylength. Also in rice, overexpression of *OsGI* in transgenic plants triggers higher *Hd1* expression. However, because of the bi-functional features of *Hd1*, higher levels of OsGI and Hd1 lead to decreased *Hd3a* transcription and inhibition of flowering under both SDs and LDs (Hayama *et al.*, 2003). The same inhibitory effect was observed when the *Hd1* gene itself was overexpressed under SDs, abolishing its diurnal cycling and causing high levels of transcripts also to accumulate during the light phase. These data suggested that exposure of Hd1 protein to light converted it to a repressor of flowering, even if the length of the light phase was below the critical threshold necessary for flowering (Ishikawa *et al.*, 2011). The molecular mechanisms responsible for switching Hd1 function are currently unknown. In arabidopsis, CO acts as floral activator only and, therefore, what is crucial in this species is the shape of CO protein accumulation during the day, which needs to be tightly controlled in order to reach a maximum at dusk under LDs. Conversely, accumulation of Hd1 protein occurs during both the light and dark phases, suggesting that a different mechanism is responsible for the bi-functional activity of Hd1 (Ishikawa *et al.*, 2011).

OsGI transcription is controlled by the circadian clock and shows a rhythmic expression pattern which peaks at the end of the light period, similarly to arabidopsis (Hayama *et al.*, 2003). Mutations in *osgi* affect a large set of rice genes, besides *Hd1*, and about 75 % of rice transcripts show altered levels in the mutant, during a diurnal time course (Izawa *et al.*, 2011). Many of these genes encode proteins probably involved in circadian clock function, at least based on homology with known arabidopsis clock regulators, and *OsGI* can affect expression of *LATE ELONGATED HYPOCOTYL* (*LHY*), and of several *PSEUDO RESPONSE REGULATOR* (*PRR*) genes, including *PRR1*, *PRR59* and *PRR95*. Interestingly, *Hd2*/*PRR37* expression was not affected in the *osgi* mutant, possibly indicating independent control of heading date by these factors (Fig. 1). However, how OsGI protein activates expression of *Hd1* has not been clarified yet. A homologue of *FKF1* exists in rice and shows a diurnal expression pattern identical to that described in arabidopsis (Murakami *et al.*, 2007; Higgins *et al.*, 2010). DOF transcription factors are also present in the rice genome, and one of them, *OsDOF12*, is implicated in photoperiodic flowering (Li *et al.*, 2008, 2009). Transcription of *OsDOF12* is high in leaves and diurnally regulated with a trough during the night (Izawa *et al.*, 2011). Transgenic rice overexpressing *OsDOF12* flowers early compared with wild-type plants under LD, but not SD conditions, showing higher *Hd3a* expression compared with non-transgenic controls (Li *et al.*, 2009). However, no altered levels of *Hd1* mRNA were reported, suggesting a different genetic route for *OsDOF12* action on flowering (Li *et al.*, 2009).

Based on these observations, it was possible to conclude that the external coincidence model can be applied to rice, albeit with modifications from the scheme proposed for an LD plant (Hayama *et al.*, 2003). Short-day plants measure the duration of the dark phase, during which expression of florigenic proteins starts. Hd1 is likely to act as a sensor of night length, being converted to a floral activator when exposed to darkness. Expression of *Hd1* peaks during the night phase and, when this is sufficiently

long, can promote enough Hd3a protein expression and initiate flowering. In this modified model, the coincidence of darkness with peak expression of *Hd1* is crucial to trigger a developmental response.

THE *EHD1–GHD7* PATHWAY IS UNIQUE TO RICE

Flowering in rice does not strictly require the *OsGI–Hd1–Hd3a* signalling pathway, and activation of florigens can be achieved through independent mechanisms. Natural variation between rice species and cultivars allowed cloning of *EARLY HEADING DATE 1* (*Ehd1*) which encodes a B-type response regulator that does not have orthologues in arabidopsis and defines a unique floral activation pathway in rice (Doi *et al.*, 2004). *Ehd1* promotes flowering particularly under SDs, in parallel to *Hd1*, but can also promote flowering under LDs, when *Hd1* acts as a repressor (Doi *et al.*, 2004). The Ehd1 protein consists of a receiver domain at its N-terminus and a GARP DNA-binding motif (Riechmann *et al.*, 2000). It induces flowering under SDs and LDs by upregulating *Hd3a* or *RFT1* independently of *Hd1*, demonstrating the potential of *Ehd1* and *Hd1* to act redundantly on separate pathways (Doi *et al.*, 2004).

Regulation of *Ehd1* is crucial for correct flowering time in rice, and a large group of activators and repressors has been cloned and shown to modulate its expression (Fig. 1). Repressors of *Ehd1* have a central role in the photoperiodic network and, among them, *Grain number, plant height and heading date 7* (*Ghd7*) is particularly important to shape *Ehd1* diurnal and seasonal transcription. *Ghd7* encodes a CCT domain protein and is expressed at higher levels under LDs, correlating with limited expression and activity of *Ehd1* under non-inductive photoperiods (Xue *et al.*, 2008). Besides its effect on flowering, *Ghd7* controls other traits in rice including grain number and plant height, indicating pleiotropic roles for the protein in other processes (Xue *et al.*, 2008).

Analysis of *Ghd7* and *Ehd1* expression in response to light has defined a novel coincidence mechanism and a double gating system that sets critical daylength recognition for *Hd3a* expression under specific photoperiods (Itoh *et al.*, 2010). Rice plants exposed to photoperiods shorter than 13·5 h induce *Hd3a* and *Ehd1* expression, while reducing *Ghd7* expression. Such interplay is achieved by a double regulatory mechanism dependent on *OsGI* and phytochromes. Induction of *Ehd1* expression in the morning requires functional *OsGI*, which sets a gate (a sensitive phase set by the circadian clock in response to light) around dawn, which is independent of daylength, because it occurs at the same time under both LDs and SDs. The gate is sensitive to blue light in the morning and, when open, *Ehd1* expression increases and *Hd3a* is activated. OsGI protein accumulation reaches its trough at dawn and therefore its effect on the gate is likely to be indirect. Under LDs, *Ghd7* expression is high and its inducibility is gated at the same time as the *OsGI* and blue light-dependent gate. Under these conditions, induction of *Ghd7* in the morning is sufficient to repress *Ehd1* transcription and delay flowering. However, as daylength decreases under a critical threshold, maximum inducibility of *Ghd7* is gated during the night, resulting in reduced expression the following morning and de-repression of *Ehd1* (Itoh *et al.*, 2010). Expression of *Ghd7* requires functional phytochromes and it is abolished in the *PHOTOPERIOD INSENSITIVITY 5* (*SE5*) mutant, which encodes a haem oxygenase very similar to *LONG HYPOCOTYL 1* (*HY1*) of arabidopsis and is impaired in phytochrome chromophore biosynthesis (Izawa *et al.*, 2000). Plants in which *SE5* is mutated are insensitive to photoperiod and flower early under both SDs and LDs (Izawa *et al.*, 2000). Expression of *Ghd7* is also strongly reduced in plants where a long night is interrupted by a short red light pulse (a 'night break') that converts phytochromes to the inactive form. These data suggested that functional phytochromes are required for correct expression of *Ghd7* and floral repression, and that red light signals are integrated in the photoperiodic flowering network through *Ghd7*. Rice has three phytochrome genes (PhyA–PhyC) but *se5* single mutants lack all functional forms. Therefore, single and double phytochrome mutants have been useful tools to understand how correct *Ghd7* expression is determined (Osugi *et al.*, 2011). The results suggest that phytochromes are not required to set the light-sensitive phase for *Ghd7* expression. However, PhyA homodimers and PhyB–PhyC heterodimers are independently sufficient to trigger *Ghd7* transcription, whereas PhyB can repress it. The action of phytochromes on *Ghd7* expression is therefore more complex than previously anticipated by the analysis of *SE5*.

Neither such a double gating mechanism nor the existence of homologues of *Ehd1* and *Ghd7* has been observed in arabidopsis. Expression of *FT*, as opposed to *Hd3a* expression, showed no critical daylength threshold in mathematical modelling experiments based on biological data (Salazar *et al.*, 2009). Additionally, the flowering time of arabidopsis accessions grown under several SD and LD photoperiods indicated that most ecotypes could discriminate variations of 2 h under several SDs and LDs, and no critical photoperiod could be determined (Giakountis *et al.*, 2010). Despite the fact that broad genetic variation could also be expected among rice varieties, these data point to a crucial difference in the way photoperiod is perceived in an LD and SD plant to promote flowering, probably reflecting the different adaptation to their environment.

Regulators of Ghd7

The double gating mechanism based on the interplay between *Ehd1* and *Ghd7* is crucial for daylength measurement, and proper regulation of *Ghd7* expression and activity sets the sensitivity of the measure. Consistent with *Ghd7* being central to the pathway, extensive natural genetic variation was reported at the *Ghd7* locus and at loci regulating its activity. Such variation has allowed the cloning of a number of additional QTLs involved in flowering.

Map-based cloning of *Heading date 16* (*Hd16*) revealed that this gene encodes a caseine kinase-I protein (Hori *et al.*, 2013; Kwon *et al.*, 2014). The Hd16 protein directly interacts with and phosphorylates Ghd7, thus converting it to an active repressor of flowering. Natural allelic variants of *Hd16* showing reduced functionality de-repress expression of *Ehd1* and the florigens, leading to accelerated flowering particularly under LD conditions. Natural variation at the *Ehd3* locus allowed cloning of a repressor of *Ghd7*. Ehd3 encodes a nuclear protein with two PHD-finger motifs, and Matsubara *et al.* (2011) demonstrated that it is a repressor of *Ghd7* and activator of *Ehd1* expression particularly under LDs. The two functions can be genetically separated, indicating that activation of *Ehd1* by

Ehd3 can also be achieved independently of *Ghd7* repression (Matsubara *et al.*, 2011).

Finally, cloning of *Hd17* revealed that it encodes a homologue of *EARLY FLOWERING 3* (*ELF3*) which represses *Ghd7* expression under SDs and LDs, resulting in increased levels of *Ehd1*. The effect on flowering is probably indirect and due to the influence of *OsELF3* on circadian clock function (Saito *et al.*, 2012; Zhao *et al.*, 2012; Y. Yang *et al.*, 2013).

Activators of Ehd1 *expression*

Several genes have been cloned whose activity eventually converges on *Ehd1* transcriptional regulation.

Early heading date 2 (*Ehd2*) encodes a putative transcription factor with zinc finger motifs, which is an orthologue to *INDETERMINATE1* (*ID1*) of maize (Matsubara *et al.*, 2008*a*). Similarly to *ID1*, *Ehd2* is expressed mainly in leaves (Colasanti *et al.*, 2006). Plants mutated in *Ehd2* prevent upregulation of *Ehd1*, show delayed flowering under SDs and cannot flower under LDs, indicating that *Ehd2* is a fundamental gene for the LD promotion of flowering (Matsubara *et al.*, 2008*a*; Wu *et al.*, 2008).

In addition to *Ehd2*, *OsMADS51*, a type I MADS-box transcription factor, promotes flowering upstream of *Ehd1* under SD conditions (Kim *et al.*, 2007). Plants in which expression of *OsMADS51* is artificially increased or decreased display altered heading dates that can be observed only under SD, but not under LD conditions. Expression of *OsMADS51* depends on *OsGI*, and Kim *et al.* (2007) demonstrated that reduction of *OsGI* transcription by RNA interference (RNAi) is correlated with lower expression of *OsMADS51* and *Ehd1*. This study showed how an *OsGI*-dependent signalling cascade can activate *Hd3a* under SDs, independently of *Hd1*, through *Ehd1* and *OsMADS51*.

The *Ehd4* locus encodes a nuclear-localized CCCH-type zinc finger protein unique to the *Oryza* genus (Gao *et al.*, 2013). Loss-of-function mutants flower late under any condition, but particularly under LDs. Delayed flowering is associated with low levels of *Ehd1* and the florigens, but not of *Hd1*, indicating that *Ehd4* specifically targets the *Ehd1* pathway (Fig. 1).

Repressors of Ehd1 *expression*

Several repressors of *Ehd1* expression have been cloned that can function in a range of photoperiods. *Hd5*/*DAYS TO HEADING 8* (*DTH8*)/*Ghd8* (from here on *Ghd8*) encodes a putative *HEME ACTIVATOR PROTEIN 3* (*HAP3*) subunit of the CCAAT-box-binding transcription factor complex. It acts as floral repressor under LD conditions and delays flowering by downregulating the expression of *Ehd1*, *Hd3a* and *RFT1* (Wei *et al.*, 2010). Under SDs, *Ghd8* was reported to induce expression of these floral regulators, promoting flowering and showing some degree of bi-functionality, similarly to *Hd1* (Yan *et al.*, 2011). Expression of *Ghd8* is not influenced by *Ghd7* and *Hd1*, two major LD repressors, indicating a distinct genetic pathway for flowering time control (Wei *et al.*, 2010). However, in arabidopsis, HAP3 and HAP5 proteins have been shown to interact physically with CO protein, forming a CCAAT-box-binding complex directly controlling *FT* expression (Wenkel *et al.*, 2006; Cai *et al.*, 2007; Kumimoto *et al.*, 2010). If such a mechanism were operating in rice, HAP/Ghd8 proteins would act in the same genetic pathway as Hd1 to control *Hd3a* expression, perhaps directly. It will be interesting to determine whether Ghd8 and Hd1 proteins can physically interact to control expression of *Ehd1* or the florigens and influence photoperiodic flowering responses. Genetic data indicate that when overexpressed in arabidopsis, *Ghd8* triggers early flowering under LDs, and causes no alteration of flowering time under SDs, similarly to overexpression of other HAP subunits of arabidopsis, providing evidences that the function of this class of proteins is conserved between monocots and dicots (Kumimoto *et al.*, 2010; Yan *et al.*, 2011).

OsLFL1 (*Oryza sativa LEC2 and FUSCA3 Like 1*) is a B3 transcription factor that can delay flowering upon overexpression, by repressing *Ehd1* and its downstream targets (Peng *et al.*, 2008). Repression of *Ehd1* mediated by *OsLFL1* is probably direct, as demonstrated by chromatin immunoprecipitation and gel shift assays (Peng *et al.*, 2007). Binding of OsLFL1 protein is mediated by RY motifs present in the promoter region of *Ehd1*. Such motifs can also mediate transactivation of a reporter gene in yeast when OsLFL1 protein is expressed from an effector plasmid. *OsLFL1* is the only direct regulator of *Ehd1* reported to date. Its transcriptional control is mediated by chromatin modifications that require *O. sativa VERNALIZATION INSENSITIVE LIKE 2* (*OsVIL2*) and *O. sativa EMBRYONIC FLOWER 2b* (*OsEMF2b*), the former encoding a PHD finger histone-binding protein, and the latter encoding a component of Polycomb Repressor Complex 2 (PRC2) (J. Yang *et al.*, 2013). A protein complex containing OsVIL2 and OsEMF2b can associate with the *OsLFL1* promoter and enrich histones with H3K27me3 marks, leading to silencing of the locus. Consistently, *osvil2* and *osemf2b* mutants are late flowering and show decreased expression of *Ehd1* and the florigens (J. Yang *et al.*, 2013). These mechanisms highlight the importance of epigenetic regulation of gene expression to fine-tune environmental responses. The fundamental nature of these processes also accounts for its occurrence across divergent plant groups (Sung *et al.*, 2006; Oliver *et al.*, 2009; J. Yang *et al.*, 2013).

OsCO-Like 4 (*OsCOL4*) is a member of the *CONSTANS-LIKE* (*COL*) family in rice. It is a constitutive flowering repressor that functions under both SD and LD conditions (Lee *et al.*, 2010). The *OsCOL4* mutant plants showed early flowering under both SDs and LDs, while the overexpressing transgenic lines showed a late flowering phenotype (Lee *et al.*, 2010). The expression of *Ehd1* and *Hd3a* was higher in *OsCOL4* mutants, suggesting that it functions upstream of these floral regulators. Neither the overexpressors nor the mutant plants had altered transcription levels of *Hd1* or *OsGI*, indicating that *OsCOL4* is specific to the *Ehd1* pathway.

DIFFERENTIAL REGULATION OF FLORIGEN EXPRESSION UNDER LONG AND SHORT DAYS

Plants exposed to inductive daylengths activate expression of florigenic proteins in the vasculature of leaves. In arabidopsis, FT and TWIN SISTER OF FT (TSF) proteins are expressed in the phloem of leaves and act as mobile, long-distance signals to trigger developmental reprogramming at the shoot apical meristem (SAM) (An *et al.*, 2004; Yamaguchi *et al.*, 2005; Corbesier *et al.*, 2007; Jaeger and Wigge, 2007; Mathieu *et al.*,

2007). *Hd3* and *RFT1* encode two rice florigens and, similarly to their arabidopsis orthologues, are expressed in the rice phloem and move to the SAM (Tamaki *et al.*, 2007; Komiya *et al.*, 2009). Plants suppressing both *Hd3a* and *RFT1* mRNA expression by RNAi did not flower after 300 d under SDs. RNAi suppression of *Hd3a* only delayed flowering under SDs, whereas suppression of *RFT1* expression delayed flowering under LDs but not under SDs, indicating that the two florigens have distinct effects on floral promotion depending on the photoperiod. Additionally, *RFT1* is redundant to *Hd3a* under SDs (Komiya *et al.*, 2008, 2009). Critical daylength recognition does not affect *RFT1* expression as severely as *Hd3a* expression, and *RFT1* is essentially less influenced by LD repression as compared with *Hd3a* (Itoh *et al.*, 2010). These data have contributed to build a model by which *RFT1* and *Hd3a* encode LD- and SD-specific florigens, respectively. The diverse impact of these two florigens on photoperiodic flowering raises interesting observations, as they are very similar in structure and located physically close to each other (11·5 kb), suggesting a common origin through tandem duplication, but also indicating the existence of distinct mechanisms of transcriptional regulation (Komiya *et al.*, 2008). Accumulating evidence indicates that changes in the chromatin state can influence florigen expression, in both rice and arabidopsis, and possibly cause differential regulation of florigens in rice (Komiya *et al.*, 2008; Adrian *et al.*, 2010; Gu *et al.*, 2013). Periodic deacetylation of histones at the *FT* locus in arabidopsis are associated with transcriptional repression of *FT*. Components of a histone deacetylase complex (HDAC) associate with the *FT* locus to limit its expression at the end of the day (Gu *et al.*, 2013). Plants mutated in such components show enrichment of acetylated histones at *FT*, increase *FT* expression and cause earlier flowering compared with wild-type controls. Interestingly, in such mutants, *FT* induction still requires functional *CO*, suggesting that chromatin modifications leading to de-repression of *FT* still need the presence of a transcriptional activator. Although acetylation of histones at the *FT* locus has been associated with increased transcription of *FT*, these chromatin modifications were reported to be a consequence of *FT* activation, rather than its cause, suggesting that the timing of these changes needs to be carefully monitored in order to reach a conclusion about a causal relationship (Adrian *et al.*, 2010). In rice, accumulation of *RFT1* mRNA in *Hd3a* RNAi-suppressed plants was associated with increased H3K9 acetylation at the *RFT1* locus, indicating a possible relationship between histone modifications and transcriptional activity at florigenic loci (Komiya *et al.*, 2008). Whether such modifications also involve the *Hd3a* locus and precede transcriptional activation remains to be determined. A mutant defective in a histone methyltransferase, *SDG724*, demonstrated delayed flowering and reduced levels of *Hd3a* and *RFT1* (Sun *et al.*, 2012). Interestingly, the *OsMADS50* and *RFT1* loci, but not the *Hd3a* locus, showed enrichment of H3K36me2/me3 chromatin marks, associated with transcriptionally active chromatin. These data indicate differential regulation of the LD flowering pathway by histone methylation and provide an example of how *RFT1* and *Hd3a* could be differentially controlled by the dynamics of chromatin states.

Recent cloning of *Hd2* showed that the gene underlying the QTL is encoded by *OsPRR37*, a homologue of *PRR7* of arabidopsis and *PPD1* of wheat and barley (Koo *et al.*, 2013). Flowering of plants carrying loss-of-function alleles of *OsPRR37* is accelerated under any daylength, but is particularly enhanced under LDs. Under such conditions, *OsPRR37* suppresses *Hd3a* but not *RFT1* expression, indicating differential sensitivity of the florigens to the presence of this floral regulator. The molecular mechanisms that allow *OsPRR37* to discriminate between *Hd3a* and *RFT1* are unclear, but provide another layer of control that fine-tunes photoperiodic responses (Fig. 1).

A GENE NETWORK AT THE SHOOT APICAL MERISTEM INTEGRATES ENVIRONMENTAL CUES

In previous sections, we have described how daylength affects flowering and how light duration is monitored through a regulatory network. The products of such a network are florigenic proteins which are highly expressed in response to inductive photoperiods and encode long-distance transmissible signals. Florigens have been isolated from several plant species and shown to control floral induction. In arabidopsis and rice, FT, Hd3a and RFT1 proteins are produced in leaves and transported to the SAM upon perception of the appropriate photoperiods, initiating panicle development (Corbesier *et al.*, 2007; Tamaki *et al.*, 2007). A complex network of regulatory proteins controls perception of florigenic signals at the apex and drives downstream developmental events.

Proteins that interact with Hd3a at the shoot apical meristem

Florigens cannot directly function as transcriptional regulators in meristematic cells and thus interaction with other transcription factors is essential for their functioning. In several plant species, basic leucine zipper (bZIP) transcription factors have been described as FT-interacting proteins required for florigen activity at the apical meristem (Abe *et al.*, 2005; Wigge *et al.*, 2005; Muszynski *et al.*, 2006; Li and Dubcovsky 2008; Taoka *et al.*, 2011; Dong *et al.*, 2012). Arabidopsis *FD* and rice *OsFD1* encode bZIP transcription factors required for florigen function. According to current models, the heterodimer formed by FT and FD in arabidopsis is a molecular hub integrating environmental cues and spatial information at the apical meristem (Abe *et al.*, 2005; Wigge *et al.*, 2005; Jaeger *et al.*, 2013). The rice homologue of FD, OsFD1, interacts with Hd3a and the dimer fulfils similar roles to the FT–FD unit. Direct interaction between OsFD1 and Hd3a could not be demonstrated, but contact between the two proteins was shown to be mediated by 14-3-3 proteins, now considered to be receptors of florigens (Taoka *et al.*, 2011, 2013). Initial studies performed in different species and aimed at identifying interactors of florigens were performed in yeast that contains proteins probably mediating the interaction between FD homologues and florigens (Pnueli *et al.*, 2001; Abe *et al.*, 2005; Wigge *et al.*, 2005; Li and Dubcovsky, 2008). The use of yeast as a heterologous system to test protein–protein interactions might have thus hidden the nature of the florigen receptor complex (or FAC, florigen activation complex). Structural and *in vivo* analyses in rice have demonstrated that the FAC unit is actually a heterohexamer formed by two molecules each of Hd3a, OsFD1 and a 14-3-3 protein that bridges the interaction between the florigen and the bZIP transcription factor. A similar structure for the FAC might apply to other plant species (Fig. 2). Mutagenesis of key

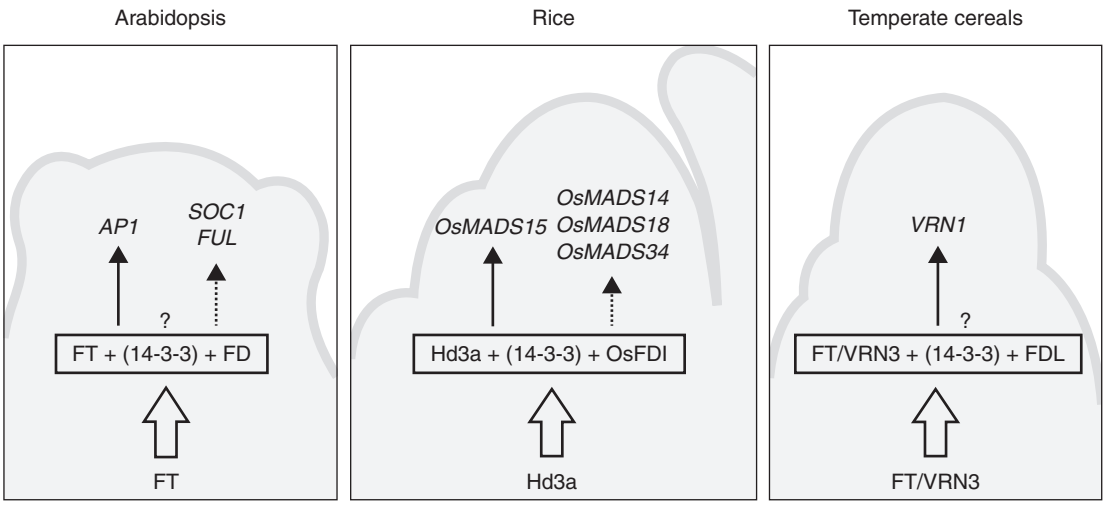

FIG. 2. Molecular responses of the shoot apical meristem to florigenic proteins. Interaction of florigen with *FD*-like genes is required to promote expression of MADS-box transcription factors, one of the first molecular events occurring upon floral transition. The requirement for 14-3-3 proteins has not been demonstrated in arabidopsis and temperate cereals, and the linker protein is therefore indicated with a question mark on the top. Arrows indicate direct transcriptional activation. Dashed arrows indicate indirect transcriptional activation.

residues at the interaction surface between Hd3a and 14-3-3 and between 14-3-3 and OsFD1 has uncovered several features of the complex and contributed to build the current model of florigen action at the SAM (Taoka *et al.*, 2013). Florigen is first received by a 14-3-3 protein in the cytosol of a meristematic cell, from which it is translocated to the nucleus where it binds OsFD1. Phosphorylation of OsFD1 is required for binding to 14-3-3 receptors, and to activate target genes (Taoka *et al.*, 2011). The presence of the bZIP transcription factor is a prerequisite for contacting DNA at the promoters of target genes, because florigens or 14-3-3 proteins have no DNA binding property. The full extent of potential targets of the FAC is currently unknown; however, it is becoming clear that assembly of the FAC is to a certain extent combinatorial, and bZIPs homologous to *OsFD1* can replace it to generate complexes controlling processes other than flowering (Tsuji *et al.*, 2013). The OsFD2 transcription factor is one such example of a bZIP protein capable of forming a FAC, but controlling leaf development rather than floral transition. Plant architecture is altered when *OsFD2* is overexpressed, but not when a mutated version unable to bind to 14-3-3 receptors is overexpressed. Since 14-3-3 proteins are ubiquitously expressed and florigens are detected in the entire meristem, bZIP proteins are probably restricting different FAC complexes to different cell types. The broad extent of florigen–bZIP interactions and the potential role of FACs in rice development are still to be fully explored. Additionally, the role of RFT1 in FAC formation has not been addressed yet, which might suggest additional combinatorial possibilities. These aspects are opening up novel possibilities for dissecting the full range of florigen functions in rice.

Molecular events occurring at the shoot apical meristem in response to photoperiodic induction

Inductive photoperiods trigger florigen expression and movement to the apical meristem, affecting the regulation of genes that are involved in inflorescence formation. In arabidopsis apices the FT–FD complex is recruited to the promoter of *APETALA1* (*AP1*), encoding a MADS-box transcription factor necessary for flower development, and triggers its activation (Wigge *et al.*, 2005). Early events occurring during the floral transition also include upregulation of other related MADS-box transcription factors, including *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FRUITFULL* (*FUL*), which are required for the promotion of flowering by *FT* (Fig. 2). In rice protoplasts, co-expression of Hd3a and OsFD1 proteins is required to induce expression of *OsMADS15*, a homologue of *AP1* (Taoka *et al.*, 2011). Mutagenized variants of Hd3a unable to bind to 14-3-3 proteins cannot activate *OsMADS15*, and an RNAi mutant simultaneously silencing four isoforms of 14-3-3 proteins also fails to induce *OsMADS15* to the levels observed in wild-type plants (Taoka *et al.*, 2011). A homologue of *SOC1*, encoded by *OsMADS50*, has been isolated in rice and shown to be required for flowering (Lee *et al.*, 2004). It is unclear if *OsMADS50* participates in the network regulating flowering at the SAM, but its transcription can be detected at the apex, suggesting that it shares features of its arabidopsis homologue (Kobayashi *et al.*, 2012). Other MADS-box genes, including *OsMADS14*, *OsMADS18* and *OsMADS34*, are upregulated at the apex in response to reproductive transition and are necessary for correct inflorescence development (Kobayashi *et al.*, 2012). These data indicate the existence of a conserved mechanism for floral induction at the apical meristem of plants, whereby florigens, interacting with FD-like transcription factors, activate expression of a set of MADS-box genes at the early stages of inflorescence development (Fig. 2). Further regulatory layers are connected with this basic developmental plan to fine-tune and stabilize floral transition from environmental noise (Fornara *et al.*, 2009; Jaeger *et al.*, 2013). Additionally, partial or complete redundancy between FD-like or MADS-box genes probably contributes to co-ordinate and stabilize inflorescence meristem specification downstream of florigenic signals (Kobayashi *et al.*, 2012; Torti and Fornara, 2012; Jaeger *et al.*, 2013).

In arabidopsis, the vegetative to reproductive phase change is largely controlled by microRNAs, including *miR156* and *miR172*. Expression of *miR156* decreases as plants age, and this pattern is complementary to that shown by *miR172*, whose

expression increases during development. Overexpression of *miR156* delays the floral transition in arabidopsis by limiting expression of *SQUAMOSA PROMOTER BINDING LIKE* (*SPL*) transcription factors (Wu and Poethig, 2006). Conversely, *miR172* accelerates flowering, by promoting *FT* expression in leaves and by targerting six related members of the *APETALA2* (AP2) clade, that repress flowering (Jung *et al.*, 2007; Yant *et al.*, 2010). The transcriptional dynamics of *miR172* depend on some of the SPLs targeted by *miR156*, generating a molecular loop that allows progression of phase change as plants age (Wang *et al.*, 2009; Wu *et al.*, 2009). Heading date in rice is only partially dependent on these microRNAs. Rice plants overexpressing *miR172* showed altered flower development, including homeotic convertions of floral organs and loss of determinacy (Zhu *et al.*, 2009). However, no altered heading date could be observed, unlike arabidopsis plants overexpressing *mir172* that showed extremely early flowering (Yant *et al.*, 2010). Overexpression of rice *miR156* influenced heading date, delaying flowering by several days and was associated with a strong increase in tiller number and dwarfism (Xie *et al.*, 2006). Whether the levels of *mir156* and *mir172* are reciprocally regulated in rice remains to be established.

SEASONAL FLOWERING RESPONSES IN TEMPERATE CEREALS

Flowering time pathways share a high degree of conservation between monocots and dicots. However, homologous genes could also be recruited to different functions during evolution, and novel pathways could evolve in specific lineages, to allow adaptation of species to different environments.

Most plants adapted to temperate climates, including arabidopsis and temperate cereals such as wheat (*Triticum* spp.) and barley (*Hordeum vulgare*), require prolonged exposure to cold temperatures before flowering. This process is known as vernalization. Since rice was domesticated in tropical regions it does not show vernalization responses. However, this is a crucial adaptation for species and varieties adapted to higher latitudes, because it prevents flowering when temperature is unfavourable, thus protecting the delicate inflorescence meristem from cold damage. Arabidopsis has been used extensively to understand the genetic and molecular bases of vernalization responses, and two genes, *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*), play a major role in preventing flowering before cold exposure (Ream *et al.*, 2012). Plants experiencing low temperatures repress *FLC* expression and maintain its repression also when returned to warm temperatures. Stable downregulation of *FLC* expression is associated with epigenetic silencing of *FLC* chromatin that is converted from active to inactive (J. Song *et al.*, 2012). Until recently it was believed that no homologue of *FLC* existed in monocots, but a recent report suggested that this is not the case. Ruelens *et al.* (2013) showed that tandem arrangements of MADS-box genes, including *FLC*, are evolutionarily conserved across Angiosperms, and *FLC* homologues can also be traced in monocot genomes (Ruelens *et al.*, 2013). These studies also suggest that *OsMADS51* and *OsMADS37* are the closest homologues of *FLC* in rice. Interestingly, *OsMADS51* has been shown to control heading date as an activator of *Ehd1* expression, indicating an opposite function in regulation of florigenic proteins to that performed by *FLC* in arabidopsis (Kim *et al.*, 2007).

In temperate cereals, vernalization responses are controlled by *VERNALIZATION* (*VRN*) loci (Ream *et al.*, 2012). In wheat and barley, broad genetic variation in the vernalization responses has been reported, and many varieties are known to have strict or no vernalization requirement. Varieties that need to be exposed to cold are planted before winter and flower only during the subsequent spring, whereas vernalization-insensitive accessions can be planted after winter. Such variation has been instrumental in isolating genes controlling the vernalization process and in establishing regulatory connections between vernalization genes (Fig. 1) (Yan *et al.*, 2003, 2004; Trevaskis *et al.*, 2003; Karsai *et al.*, 2005; Hemming *et al.*, 2008; Ream *et al.*, 2012). Temperate cereal varieties showing vernalization requirements express *VRN2* at high levels before vernalization. The *VRN2* locus encodes a CCT-domain protein showing sequence similarity to *Ghd7* of rice, and acts as a potent floral repressor that has to be downregulated during floral transition. Mutations in *VRN2* cause insensitivity to vernalization and confer a spring habit (Yan *et al.*, 2004). Exposure to low temperatures increases expression of *VRN1*, a floral promoter homologue of *FUL* and *AP1* of arabidopsis, and reduces expression of *VRN2* (Trevaskis *et al.*, 2006). Dominant allelic variants of *VRN1* carrying mutations in its regulatory regions express *VRN1* independently of exposure to low temperatures, and confer a spring growth habit (Loukoianov *et al.*, 2005). High levels of *VRN1* expression are associated with repression of *VRN2* transcription, which supported the idea that *VRN1* acts as repressor of *VRN2*. However, by loss-of-function *vrn1* mutants, it became clear that *VRN1* induction is not necessary to initiate repression of *VRN2* during vernalization, but is required to maintain its repression after exposure to cold (Chen and Dubcovsky, 2012). These data indicate that cold signals co-ordinately repress *VRN2* and activate *VRN1* expression during vernalization, whereas after vernalization *VRN1* maintains the repressed state at the *VRN2* locus (Fig. 1).

In barley, transcriptional dynamics of *VRN1* mRNA are probably caused by changes in the chromatin state of the *VRN1* locus, in which cold promotes an active chromatin state that is later maintained after plants are exposed to warm temperatures (Oliver *et al.*, 2009). Changes in the chromatin state were not observed at the *VRN2* locus, suggesting that *VRN1* is the primary target of chromatin remodelling complexes during vernalization (Oliver *et al.*, 2009).

As *VRN2* levels decrease, the vernalization requirement is satisfied and if plants are exposed to LDs, *VRN3*, a homologue of *Hd3a* and *FT*, is transcribed and moves to the apical meristem where it promotes flowering during spring (Yan *et al.*, 2006; Hemming *et al.*, 2008). The molecular mechanisms through which *VRN3* promotes flowering at the apex are conserved (Fig. 2). In wheat, VRN3/TaFT protein can interact with TaFDL transcriptional regulators, homologues of *FD* and *OsFD1*, and promote expression of *VRN1* (Li and Dubcovsky, 2008). Expression of *VRN1* is directly controlled by the TaFT–TaFDL heterodimer as at least one TaFDL protein can bind the promoter of *VRN1*. Whether 14-3-3 proteins mediate the interaction between TaFT and TaFDL is currently unclear (Fig. 2).

As in arabidopsis, temperate cereals flower earlier if exposed to LDs, whereas flowering is delayed under SDs. Photoperiodic

control of flowering becomes relevant only after the vernalization requirement has been satisfied and plants become competent to respond to daylength. The major gene controlling photoperiod sensitivity in both wheat and barley is *PHOTOPERIOD 1* (*Ppd1*), encoding a pseudoresponse regulator similar to *OsPRR37* of rice and *PRR7* of arabidopsis (Turner *et al.*, 2005; Beales *et al.*, 2007). Photoperiod-insensitive wheat plants carry *Ppd-D1a*, a semi-dominant allele that bears a 2 kb deletion in its upstream regulatory region and induces early flowering under both LDs and SDs (Beales *et al.*, 2007). The *Ppd-D1a* deletion causes increased expression of *Ppd-D1a* also during the night under SDs, when transcription of the wild-type gene is normally repressed. Misexpression induces *TaFT* constitutive activation and flowering, irrespective of daylength. In barley, the recessive *ppd-H1* loss-of-function allele cannot induce high expression of *HvFT* when plants are exposed to LD conditions. This results in delayed flowering and is advantageous for spring-sown varieties that can prolong vegetative growth, producing more biomass and eventually seeds (Turner *et al.*, 2005).

Interestingly, the effect of homologues of *OsPRR37* on flowering depends on the species. In LD plants such as arabidopsis and temperate cereals, functional alleles promote flowering through transcriptional induction of florigens; conversely, in SD species such as rice and sorghum, PRR genes repress expression and flowering of florigens under LDs (Murphy *et al.*, 2011; Koo *et al.*, 2013). The molecular mechanisms underlying PRR function will provide important clues to understand how information on daylength is elaborated and the causes FT activation.

Homologues of *CO* and *Hd1* have been cloned from temperate cereals; however, they seem not to be crucial to confer a photoperiodic response (Nemoto *et al.*, 2003; Shimada *et al.*, 2009). Overexpression of *HvCO1* in a *ppd-H1*-deficient accession accelerates flowering under both LD and SD conditions, indicating that *HvCO* is independent of the *PRR* pathway. However, plants overexpressing HvCO retained responsiveness to photoperiod, flowering later under SDs and indicating the existence of additional factors with major effects on flowering. Indeed, allelic variation at *Ppd-H1* was shown to be a major determinant of *HvFT1* expression and flowering time, and acted independently of *HvCO1* (Campoli *et al.*, 2012). Similar conclusions were suggested from studies in wheat (Shaw *et al.*, 2012) and point to a model whereby florigen expression is the convergence point of independent pathways with limited cross-talk (Fig. 1).

In temperate cereals the responses to daylength and vernalization are integrated. After a period of growth under LDs, exposure to SDs accelerates flowering in wheat, and can largely substitute for vernalization treatments (Dubcovsky *et al.*, 2006). Flowering is due to downregulation of *VRN2* expression under SDs, an effect also observed in barley and *Brachypodium* (Trevaskis *et al.*, 2006; Ream *et al.*, 2012). Thus, *VRN2* is a convergence point integrating photoperiodic and temperature information, and its correct expression is key for flowering at the most appropriate time of year.

RICE ADAPTATION TO LONG-DAY CONDITIONS INVOLVED ALLELIC CHANGES AT HEADING DATE LOCI

Rice domestication started about 10 000–13 000 years ago in the surroundings of the Pearl River in Southern China (Huang *et al.*, 2012). During this process, the founder ecotypes, probably belonging to the *O. rufipogon* progenitor, split into the five groups of cultivated rice known to date. Over the centuries, the area of rice cultivation expanded, first within tropical and subtropical Asia and then to other regions of the world, reaching temperate areas at higher latitudes. The success of rice adaptation depended on the acquisition of cold tolerance traits and the loss of photoperiod sensitivity. In temperate areas, seasonal variations in temperatures limit the period of rice cultivation from late spring to early autumn. Thus, rice flowering occurs during summer days, which are warm but long, and varieties adapted to temperate climates show reduced sensitivity to changes in daylength, flowering under conditions normally non-inductive.

Several genetic studies have been carried out in order to identify the molecular mechanisms that allow rice to flower at high latitudes. Five major QTLs controlling heading date in response to photoperiod were identified and described in detail in previous sections (Yamamoto *et al.*, 1998, 2000). All genes underlying these major QTLs have been cloned and, interestingly, four of them (*Hd1*, *Hd2*/*PRR37*, *Hd4*/*Ghd7* and *Hd5*/*DTH8*/*Ghd8*) were demonstrated to be repressors of flowering under LD conditions, whereas *Hd3a* encodes the major florigen normally targeted by the *Hd1*–*Hd4* repressors (Yano *et al.*, 2000; Kojima *et al.*, 2002; Xue *et al.*, 2008; Wei *et al.*, 2010; Yan *et al.*, 2011; Fujino *et al.*, 2012; Koo *et al.*, 2013). This genetic architecture probably reflects the tropical origin of the species, and indicates that floral repression is a default state that needs to be overcome in order for flowering to occur. However, it also provides the substrate for artificial selection of varieties better adapted to regions where daylength is not permissive.

Polymorphisms at loci encoding florigens exist and can partly account for flowering diversity. In particular, variations at the *Hd3a* promoter regions contribute to diversification of flowering time of a rice core collection (Takahashi *et al.*, 2009). A recent study has demonstrated how single nucleotide polymorphisms (SNPs) in the regulatory genomic region and an amino acid substitution in the protein sequence of *RFT1* provide flowering time divergence under LD conditions (Ogiso-Tanaka *et al.*, 2013). Rice varieties growing under natural LD conditions (where daylength is longer than 13 h and latitude over 23·6 °N) use both the *RFT1*- and *Hd3a*-dependent pathways to promote flowering, whereas rice varieties growing at southern latitudes mostly use the *Hd3a* pathway (Fig. 3A) (Ogiso-Tanaka *et al.*, 2013). However, since florigens are highly conserved across rice varieties and species, flowering diversification has mainly resulted from the regulation of florigen expression levels that are highly correlated with flowering time.

Repressors (or suppressors) of flowering play a crucial role in reducing florigen gene expression under LD conditions, leading to a strong delay in heading date. Non-functional alleles of repressors (or suppressors) of LD-dependent flowering have been associated with loss of sensitivity to photoperiod. Loss-of-function alleles of such genes cause an increase in florigen gene expression to promote flowering under LDs. Thus, defective alleles of repressors can be used by breeders to introduce variations in flowering time in rice varieties that grow under LD conditions. These alleles have been useful tools to introduce tropical varieties into temperate areas and to increase the northern limit of rice cultivation.

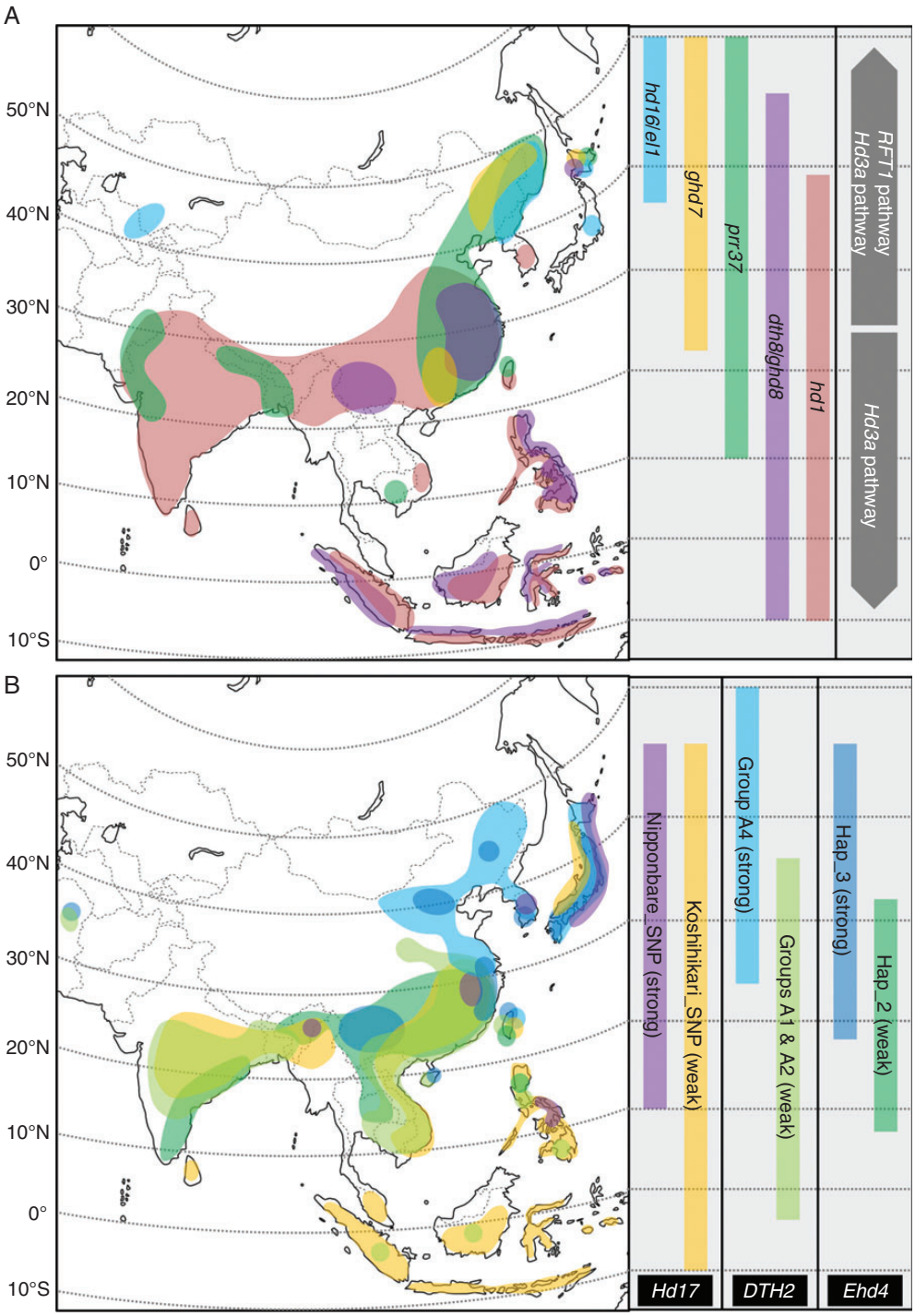

FIG. 3. Distribution of alleles influencing heading date in rice. The maps show the distribution of loss-of-function alleles of floral repressors, including *hd16*, *hd1*, *prr37*, *ghd7* and *dth8*/*ghd8* (A) or allelic variants of floral promoters associated with weak or strong activity, which include *Hd17*, *DTH2* and *Ehd4* (B). The colour of the distribution matches that of the corresponding gene, which is indicated on the right-hand side of the map. The length of the coloured bars on the right covers the latitudinal range across which varieties bearing the allelic variant are grown. Grey arrows (represented only in A) indicate the requirement for *Hd3a* expression at southern latitudes and for *Hd3a* and *RFT1* expression at higher latitudes. The maps are based on data reported in Xue *et al.* (2008), Takahashi *et al.* (2009), Wei *et al.* (2010), Fujino *et al.* (2012), Matsubara *et al.* (2012), Gao *et al.* (2013), Koo *et al.* (2013), Kwon *et al.* (2013) and Wu *et al.* (2013).

Non-functional alleles at *Hd1*, *PRR37*, *Ghd7* and *Ghd8* loci are generated by SNPs, insertions or deletions, leading to dramatic changes in florigen expression and heading dates. Special attention is required for *Hd1*, which acts as a repressor under LDs but as an inducer under SDs. A high occurrence of natural polymorphisms in *Hd1* has been correlated with variation in flowering time and *Hd3a* mRNA levels (Takahashi *et al.*, 2009). Rice cultivars with functional *Hd1* alleles showed

higher *Hd3a* expression levels and earlier flowering times under SD conditions, whereas those with non-functional *Hd1* alleles showed lower *Hd3a* expression levels and later flowering times (Takahashi *et al.*, 2009). Since the presence of non-functional alleles of *Hd1* influences flowering in opposite ways depending on daylength, rice varieties carrying natural *hd1* mutants have been found in a wide range of latitudes (Fig. 3A). Varieties bearing non-functional *Hd1* alleles grown under SDs will delay flowering time, which could be important to elongate the vegetative phase in order to increase grain production. The effect of non-functional *Hd1* alleles under SDs can be reinforced by the presence of non-functional *Ehd1* alleles, as observed in some Taiwanese rice varieties (Doi *et al.*, 2004). Conversely, non-functional *Hd1* alleles in varieties grown under LDs will anticipate heading, contributing to cultivation at high latitudes (Izawa, 2007). A recent study has revealed that *Hd2*/*PRR37* downregulates *Hd3a* expression under LD conditions and has demonstrated that natural variation at *PRR37* in many Asian rice cultivars has contributed to the expansion of rice cultivation to temperate areas, similar to previous reports in sorghum (Murphy *et al.*, 2011; Koo *et al.*, 2013). Non-functional *PRR37* alleles (Fig. 3A) were detected in a wide range of latitudes, including the northern limit of rice cultivation (Koo *et al.*, 2013). Genetic analysis revealed that the effect of *PRR37* on heading date is additive to that of *Ghd7*, and rice varieties carrying non-functional *PRR37* and *Ghd7* showed extremely early flowering under LDs (Koo *et al.*, 2013). *Ghd7* has been previously described as a key component in the adaptation of rice to northern latitudes because it downregulates the expression of *Ehd1* and, consequently, that of *Hd3a* and *RFT1* under LDs. Natural *ghd7* mutants (Fig. 3A) were found in early flowering rice varieties grown in central and southern China and in varieties from the Heilongjiang Province of North-eastern China, the latter being characterized by cool summers and a short growing season (Xue *et al.*, 2008). *Japonica* cultivars with both *Ghd7* and *PRR37* mutations were also found at high-latitude regions of North-eastern Asia, including Northern Japan. This suggests that naturally occurring mutations in *PRR37* and *Ghd7* play an important role in rice adaptation from low to high latitudes (Koo *et al.*, 2013). However, *Ghd7* acts on a separate genetic pathway to that of *PRR37* (Xue *et al.*, 2008; Fujino *et al.*, 2012; Koo *et al.*, 2013). This might indicate that pyramiding of non-functional alleles in cultivated varieties has probably allowed further expansion of the cultivation area, and artificial construction of early flowering genotypes has been particularly successful when independent repressor pathways were targeted (Ebana *et al.*, 2011). Polymorphisms in the *DTH8/Ghd8* sequence that create non-functional alleles have been related to loss of photoperiod sensitivity. Natural *ghd8* mutants were found in several provinces of China, the Philippines, Indonesia and Northern Japan (Wei *et al.*, 2010; Fujino *et al.*, 2012) (Fig. 3A). The mutant allele has been used in breeding programmes outside of Japan, its country of origin, and spread to Europe, where it probably conferred an agronomic advantage over functional alleles (Wei *et al.*, 2010; Fujino *et al.*, 2012). *Ghd8* expression does not affect *Ghd7* or *Hd1* expression, suggesting that multiple targeting of repressor pathways has the potential to accelerate flowering strongly. Combinations of defective alleles generate stronger phenotypes, as demonstrated with *prr37 ghd7* cultivars under LDs (Koo *et al.*, 2013), suggesting that accumulation of additional *Hd* mutant alleles could contribute to further reduction of photoperiodic sensitivity and crop cycle.

In addition to these major loci, polymorphisms in the DNA sequence of other alleles have also contributed to the northern adaptation of rice. From additional QTL analyses, *Hd6*, a minor heading date allele, was detected (Yamamoto *et al.*, 2000). *Hd6* enhances the repressive activity of *Hd1* and is defective in some *japonica* cultivars (Takahashi *et al.*, 2001; Ogiso *et al.*, 2010; Ebana *et al.*, 2011). This reduces (but does not abolish) *Hd1*-mediated repression under LDs, further contributing to diversification of flowering time. Matsubara *et al.* (2008*b*) identified new QTLs related to photoperiodic flowering. Among them, recent studies have shown how naturally occurring variants of *EL1/Hd16* alleles in *japonica* cultivars influence *Ghd7* activity (Matsubara *et al.*, 2012; Hori *et al.*, 2013; Kwon *et al.*, 2014). *Hd16* acts as a suppressor of LD-dependent flowering by phosphorylating Ghd7 (Hori *et al.*, 2013). Cultivars carrying non-functional *EL1/Hd16* variants (Fig. 3A) are closely associated with high latitudes, whereas the cultivars carrying functional *EL1/Hd16* variants are randomly distributed independently of latitude (Kwon *et al.*, 2014).

Natural variation at loci encoding floral activators has recently been shown to have an important role in adaptation to northern latitudes. In contrast to all the genes described above, allelic variants of *Hd17*, encoding an OsELF3-like protein, *DTH2*, which encodes a *CONSTANS*-like protein, and *Ehd4* do not create loss-of-function alleles but rather genetic variants showing a gradient of activity (Matsubara *et al.*, 2012; Gao *et al.*, 2013; Wu *et al.*, 2013). Genetic studies demonstrated that the Nipponbare_SNP of *Hd17*, allele 4 (A4) of *DTH2* and haplotype 3 of *Ehd4* have been fixed during the domestication of rice at high latitudes (Fig. 3B). These showed a stronger effect as floral promoters under natural LD conditions in comparison with other alleles (Matsubara *et al.*, 2012; Gao *et al.*, 2013; Wu *et al.*, 2013).

CONCLUSIONS AND PERSPECTIVES

Decades of research on flowering control have greatly expanded our understanding of the molecular mechanisms that initiate and drive reproductive phase transitions in different species. Molecular control networks are becoming increasingly complex as novel genes and regulatory mechanisms are described. Research that takes advantage of arabidopsis as a model organism often leads the way and opens up the possibility of exploring the function of orthologues from other species. However, arabidopsis is not representative of all plant species, and several examples discussed in this review indicate that several monocot-specific (or even *Oryza*-specific) genes do not have functional equivalents in dicots (Doi *et al.*, 2004; Yan *et al.*, 2004; Xue *et al.*, 2008; Matsubara *et al.*, 2011; Wang *et al.*, 2013; Wu *et al.*, 2013). Exploring genomes by DNA sequencing, QTL and association mapping, transcriptome profiling or mutant screens will keep providing new exciting insights into the way genes allow plants to interface with the environment. Mining genetic variation in crop species and their progenitors, and coupling it with the enormous potential of next-generation sequencing, will reach the dual objective of identifying novel regulators, perhaps difficult to pinpoint with other tools, and to exploit diversity to accelerate breeding programmes (Huang *et al.*, 2011; Zhao *et al.*, 2011).

ACKNOWLEDGEMENTS

This work was supported by an ERC Starting Grant (#260963) to F.F.

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