Review

Structure and function of florigen and the receptor complex

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In the 1930s, the flowering hormone, florigen, was proposed to be synthesized in leaves under inductive day length and transported to the shoot apex, where it induces flowering. More recently, generated genetic and biochemical data suggest that florigen is a protein encoded by the gene, *FLOWERING LOCUS T* (*FT*). A rice (*Oryza sativa*) FT homolog, Hd3a, interacts with the rice FD homolog, OsFD1, via a 14-3-3 protein. Formation of this tri-protein complex is essential for flowering promotion by Hd3a in rice. In addition, the multifunctionality of FT homologs, other than for flowering promotion, is an emerging concept. Here we review the structural and biochemical features of the florigen protein complex and discuss the molecular basis for the multifunctionality of FT proteins.

Day length control of flowering and florigen

Most plants continue to grow and produce leaves in the vegetative phase after germination. However, when environmental conditions change, plants change their growth to a reproductive phase and produce flowers and seeds for propagation. Such a developmental phase transition occurs at the shoot apical meristem (SAM), a pluripotent stem cell population on the tip of the stem [1,2]. Photoperiodic flowering is the flowering response to day length change, one of the most critical environmental stimuli for floral induction. W.W. Garner and H.A. Allard discovered that a tobacco (*Nicotiana tabacum*) variety, Maryland Mammoth, produced flowers only under short day conditions and never under long day conditions [3]. They concluded that day length is a critical factor for the induction of flowering. Plants can be broadly classified into three categories, based on their day length requirement for flowering. Short day plants induce flowering under short day conditions, whereas long day plants do so under long day conditions. Short and long days mean shorter or longer than the critical day length intrinsic to the respective plant species. Day neutral plants produce flowers irrespective of the environmental day length. For example, *Arabidopsis thaliana* is a long day plant and rice (*Oryza sativa*) a short day plant.

In 1936, Chailakhyan, a Russian plant physiologist, discovered that changes in day length for floral induction are recognized by leaves. Considering other physiological evidence, he proposed a flowering hormone, florigen that is produced in leaves after the stimulus of inductive day length, and then transported to the SAM for floral evocation [4]. Grafting experiments clearly showed that florigen is a universal signal for flowering, because the flowering stimulus is graft-transmissible from induced stock to a non-induced scion and between different plant species, as well as for plants with different photoperiodic requirements [5,6]. Although the molecular nature of florigen was an enigma for a long time, it is now broadly accepted that the major component of florigen is a protein encoded by the *FT* gene or its homologs [7–9]. The *FT* gene was first identified as the responsible gene in a late flowering mutant of *Arabidopsis* (*Arabidopsis thaliana*) [10–12]. Phloem specific expression of *FT* under the inductive day length [13,14], the requirement for intercellular trafficking of FT from companion cells [15,16], the existence of FT homologs in phloem exudate [17,18], and the long distance trafficking of FT and the rice homolog Hd3a to the SAM [19,20] strongly support the idea that the FT protein is the long-sought florigen. The virtual absence of *FT* RNA in graft receptor [21], phloem exudate [18] and around the SAM [20], the tissue specific knockdown of *FT* mRNA [16], and the synonymous substitutions of *FT* mRNA [22] strongly support the idea that *FT* RNA is not a major component of florigen responsible for the mobile flowering signal. Recently, *FT* RNA of *Arabidopsis* was reported to move over a long distance and contribute to floral induction [23,24]. It will be important to examine whether the *FT* RNA from other plant species is also translocated a long distance and how the translocated *FT* RNA contributes to the regulation of flowering *in vivo*.

This review summarizes the structural and biochemical aspects of the FT protein as a major protein component of florigen and the significance of the protein complex in the regulation of flowering. The expression of *FT* is regulated by various environmental cues: photoperiod, vernalization, ambient temperature, and gibberellin. For a more complete discussion of the regulation of *FT*, please refer to other recent reviews [25–32].

Structure of florigen (FT protein)

FT is a phosphatidylethanolamine-binding protein (PEBP) family member whose crystal structure is similar to that of mammalian PEBP [33]. PEBPs have an anion-binding pocket composed of highly conserved residues. FT and its homologs, Hd3a and TFL1, have the anion-binding pocket (Figure 1a). Anions, phosphate groups, and phospholipids

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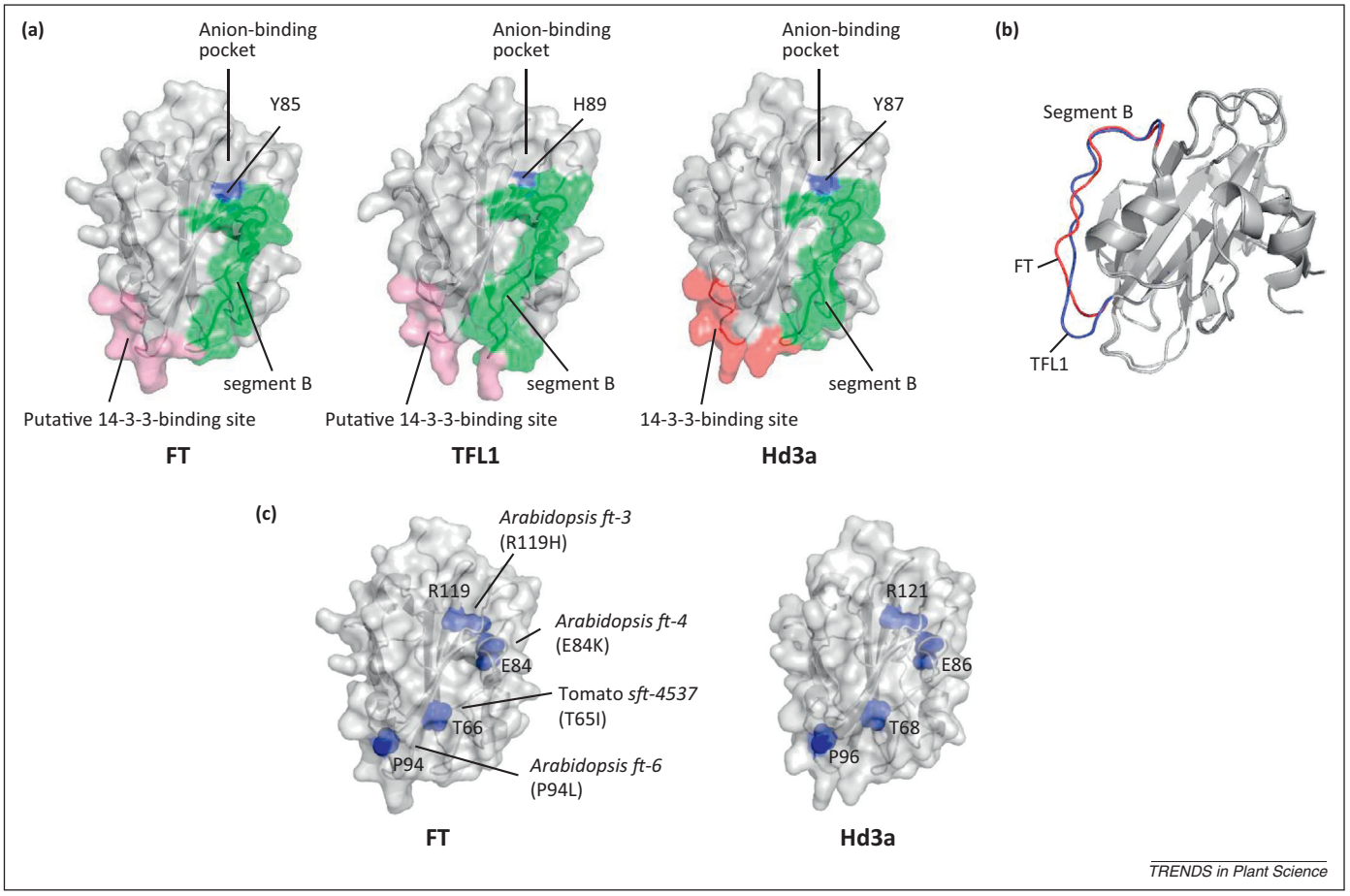

Figure 1. Structure of florigen (FT protein). **(a)** Structure of FT, TFL1, and Hd3a. All three have putative anion-binding pockets and segment B loops (green). Tyrosine or histidine residues characteristic of the FT or TFL1 family, respectively, are located in the vicinity of the pocket (blue). The 14-3-3-binding site of Hd3a is shown in red. The corresponding regions of FT and TFL1 are shown in pink. **(b)** Merged images of FT and TFL1. Note that the loop region encoded by segment B (red FT, blue TFL1) is highly divergent. **(c)** Location of missense mutations in *ft* mutant alleles. Location of missense mutations of *ft* alleles in *Arabidopsis* [11] and tomato [21] were mapped on the FT structure (left). The corresponding residues are mapped on Hd3a (right).

bind directly to mammalian PEBP in the vicinity of this pocket [33–36], whereas no such binding has been observed for FT or other plant PEBPs [33,37]. The Y120 residue within the anion-binding pocket is conserved among mammalian PEBPs, but is replaced with hydrophobic amino acids in plant PEBPs. This sequence difference may indicate that Y120, located at the bottom of the anion-binding pocket, is important for binding to anions, phosphate groups, and phospholipids. Two regions of FT that are critical for flowering promotion, segment B and Y85 (Y87 in Hd3a, H89 in TFL1) [33,38], are mapped on the structures (Figure 1a). The structure of segment B is significantly different between FT and TFL1 (Figure 1b) [33], but the structures of the anion-binding pockets, including Y85, are similar (Figure 1a, b). Three *Arabidopsis* and one tomato (*Solanum lycopersicum*) *FT* missense loss-of-function mutations, resulting in four amino acid substitutions have been reported [11,21]. These mutated positions are also mapped on the structure (Figure 1c). Most amino acids are not exposed to the surface and, thus, the mutation may collapse the 3D structure.

Interactors of FT and their functions

To understand the molecular function of FT, searches for FT-interacting proteins were conducted mainly by yeast two-hybrid (Y2H) screening (Table 1). Many bZIP transcription factors from several plant species have been identified as FT-interacting proteins. *FD* was isolated from the *fd* mutant of *Arabidopsis* [39,40], a late flowering mutant [10]. *FD* is expressed mainly in the shoot apex [39]. The bZIP protein encoded by *FD* interacts with FT *in vitro* and *in vivo* [39]. FT–FD interaction is essential for FD function because mutations of the interaction site in FD failed to complement the *fd* mutant [39]. Maize (*Zea mays*) *DLF1* was isolated from the *delayed flowering1* mutant of maize, also a late flowering mutant [41]. The bZIP protein encoded by *DLF1* interacts with ZCN8, a maize FT, in yeast [42,43]. Rice OsFD1 was identified as a rice homolog of DLF1 and the interaction with Hd3a was confirmed in yeast and *in planta* [44]. Coexpression of OsFD1 with Hd3a in protoplasts can upregulate *OsMADS15*, a floral meristem identity gene, whereas the mutant OsFD1, which is defective in Hd3a interaction, cannot [44]. As a common feature among these FT-interacting bZIP factors, 14-3-3-binding sites are found at their C termini (Table 1).

14-3-3 proteins have also been identified as FT-interacting proteins. A tomato 14-3-3 isoform, 14-3-3/74, was identified by Y2H interaction with FT and SP, a tomato TFL1 homolog [45]. Overexpression of *14-3-3* in tomato compensated for the loss of function of the *SP* gene [45]. In

Table 1. List of FT-interacting proteins

Gene name	Plant species	Gene ID	Protein	Detection of FT interaction	14-3-3-binding motif[a]	14-3-3 binding[b]	Refs
FD	*Arabidopsis*	At4g35900	bZIP	Y2H, pull-down, BiFC	Mode I (RSS**TAP**)	ND	[39,40]
FDP	*Arabidopsis*	At2g17770	bZIP	Y2H	Mode I (RSS**TAP**)	ND	[39,40]
SPGP	Tomato	EF136919	bZIP	Y2H	Mode I (RTS**TAP**)	Yes	[41]
DLF1	Maize	NM_001112492	bZIP	Y2H	Mode I (RMP**SAP**)	ND	[42]
OsFD1[c]	Rice	Os09g0540800	bZIP	Y2H, BiFC	Mode I (RVL**SAP**)	Yes	[43]
TaFDL2	Wheat (*Triticum aestivum*)	EU307112	bZIP	Y2H	Mode II (RRTS**SAP**)	ND	[85]
TaFDL6	Wheat	EU307114	bZIP	Y2H	No	ND	[85]
TaFDL13	Wheat	EU307115	bZIP	Y2H	No	ND	[85]
MdTCP2a	Apple (*Malus* × *domestica*)	AB531019	TCP	Y2H	No	ND	[86]
MdTCP2b	Apple	AB531020	TCP	Y2H	No	ND	[86]
MdTCP4a	Apple	AB531021	TCP	Y2H	No	ND	[86]
MdTCP4b	Apple	AB531022	TCP	Y2H	No	ND	[86]
SPAK	Tomato	AF079103	NIMA-like kinase	Y2H	Mode I (RRA**SLP**)	Yes	[41]
MdVOZ1a	Apple	AB531023	Zn-finger	Y2H	Mode II like (RKQR**SLP**)	ND	[86]
OsBIP116b	Rice	Os07g0185432	TPX2-like	Y2H	Mode II like (RTQCSPK**SAP**)	Yes	[43]
OsKANADI1	Rice	Os02g0696900	Myb	Y2H	Mode I (RLS**SAP**)	Yes	[43]
OsGF14b, OsGF14c, OsGF14e, OsGF14f	Rice	Os04g0462500 Os08g0430500 Os02g0580300 Os03g0710800	14-3-3 protein	Y2H, pull-down, BiFC, FRET-FLIM, NMR	No (dimerization with 14-3-3 protein)	Yes	[43,46]
14-3-3/74	Tomato	AF079450	14-3-3 protein	Y2H	No (dimerization with 14-3-3 protein)	Yes	[41]
FTIP1	*Arabidopsis*	At5g06850	C2 domain	Y2H, pull-down, PLA	No	ND	[47]

[a]Consensus sequence for 14-3-3 binding; mode I (R/K-X-X-pS/T-X-P), mode II (R/K-X-X-X-pS/T-X-P), and mode III (pS/T-X_{1-2}-COOH). The SAP motif is represented in bold.

[b]ND, not determined.

[c]An indirect interaction with Hd3a has been confirmed.

rice, eight different isoforms of 14-3-3 proteins are found in the sequenced genome, and four of them have been reported to interact with Hd3a [44,46]. Simultaneous knock-down of the four 14-3-3 isoforms compromised the upregulation of *OsMADS15* by coexpression of *Hd3a* and *OsFD1* in protoplasts [44].

FT-INTERACTING PROTEIN1 (FTIP1) was also identified from *Arabidopsis* by Y2H screening [47]. FTIP1 is a C2 domain-containing protein that is localized on the endoplasmic reticulum (ER) of companion cells. The *ftip1* mutant flowers late under long day conditions and has a defect in FT transport to the shoot apex [47], indicating an essential role of FTIP1 in the long distance trafficking of FT.

So far, most of the FT-interacting proteins have been identified by Y2H screening (Table 1). Therefore, their interaction with FT could be mediated by yeast endogenous proteins. Data suggest that OsFD1–Hd3a interaction in yeast is mediated by a yeast 14-3-3 protein [44]. No direct interaction between OsFD1 and Hd3a was detected by an NMR titration assay, isothermal titration calorimetry, or a pull-down assay with highly purified proteins, whereas direct interaction between Hd3a and 14-3-3 protein was observed by the same assays [44].

Structure of the florigen activation complex (FAC): 14-3-3 protein as a receptor for florigen

As shown in Figure 2, the crystal structure of the Hd3a complex has been determined as a heterohexamer composed of two Hd3a, two 14-3-3 proteins, and two OsFD1 molecules [44]. Two Hd3a monomers bind the C-terminal regions of dimeric 14-3-3 proteins to form a thick and deep W-shaped structure. The phosphorylated C terminus of OsFD1 binds to positively charged pockets formed within two corners of the inner base of the W-shape. In this structure, the binding sites in 14-3-3 proteins for Hd3a are separated from those for OsFD1, and no direct interaction is observed between Hd3a and OsFD1 (Figure 2a). This feature is consistent with the experimental results showing that there are direct interactions between Hd3a and 14-3-3 protein, and 14-3-3 protein and OsFD1 by NMR titration assays, isothermal titration calorimetry assays, and pull-down assays, but direct interaction between Hd3a and OsFD1 is not observed at all. Therefore, 14-3-3 protein forms a stable complex with Hd3a and OsFD1 simultaneously and mediates the indirect interaction between Hd3a and OsFD1.

The common aspects of 14-3-3 protein complex containing the phosphorylated peptide are as follows: (i) the structure of the 14-3-3 protein dimer is rigid, (ii) multiple 14-3-3-binding motifs are used simultaneously, and (iii) 14-3-3-binding motifs are present inside a disordered region [48]. These aspects are also found in the FAC structure containing phosphorylated OsFD1. Notably, the Hd3a-binding site in 14-3-3 protein is a novel site and far from the canonical phosphoserine-binding site, but close to the Cdc25C-binding site, which has been predicted by the mutation study of human 14-3-3σ [49]. The Hd3a-binding surface of 14-3-3 protein is approximately 1300 Å. One of

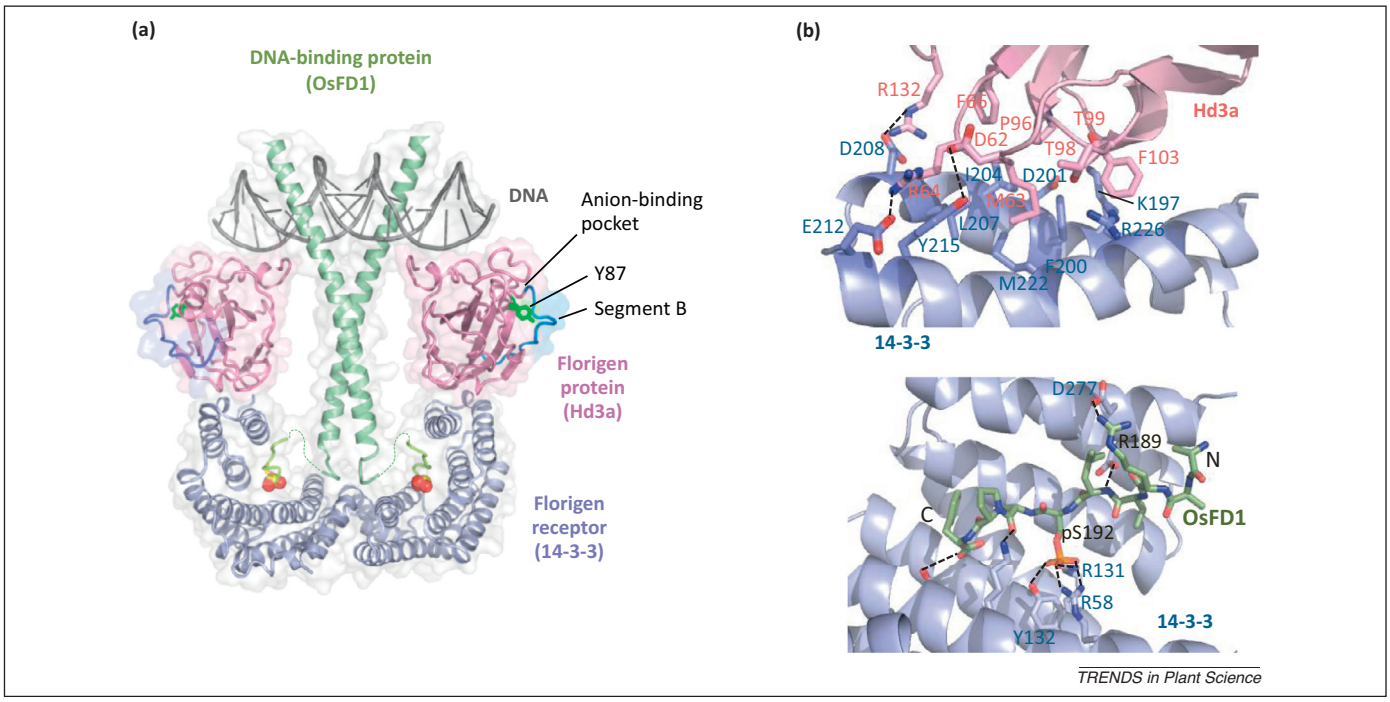

Figure 2. Structure of the florigen activation complex (FAC). **(a)** A modeled structure of the FAC–DNA complex. Crystal structure of FAC is composed of Hd3a, 14-3-3 protein, and the C-terminal region of OsFD1 peptide. Note that both segment B (blue) and the anion-binding pocket including Y87 (green) are exposed on the FAC surface, making it possible to interact with other factors (e.g., transcriptional coactivators). **(b)** The interaction surface of Hd3a–14-3-3 (left) and 14-3-3–OsFD1 (right). Note that Hd3a indirectly interacts with OsFD1 via the 14-3-3 protein. Reproduced, with permission, from [44].

the missense mutations in the *ft* alleles (*ft-6*) in *Arabidopsis* [11] is located on the surface (Figure 2b, left).

A model of the FAC–DNA complex is shown in Figure 2a, using the FAC structure as well as the structures of complexes between the mouse CREB bZIP region (residues 285–339) and C-box DNA [50]. The spatial alignment between 14-3-3 protein and bZIP is inferred by comparison with the structure of a complex between tobacco 14-3-3 protein and H^+-ATPase [51]. Two regions of FT that are critical for flowering, Y85 (Y87 in Hd3a) and segment B [33,38], are exposed on the surface of the modeled FAC–DNA structure and do not overlap with the 14-3-3-binding sites of Hd3a. The 14-3-3- and DNA-binding activities of an Hd3a mutant, Y87H, were both confirmed experimentally as being normal. These results indicate that neither region contributes to the structure of the FAC or to its interaction with DNA. Moreover, P77L and R121H mutations of Hd3a, located in DPDXP (Asp-Pro-Asp-X-Pro) and GIHR (Gly-Ile-His-Arg) motifs, respectively, did not affect the interactions with 14-3-3 protein [44]. Given that these motifs contribute to the conformation of the putative ligand-binding site [33,37], which is highly conserved in PEBP family members including FT and Hd3a, this result suggests that the Hd3a ligand-binding site is not involved in 14-3-3 binding. These data support the idea that other factors, such as coactivators, corepressors, general transcription factors, and/or other interacting molecules, may further interact with the FAC on an area of the Hd3a surface encompassing Y87, segment B, and the ligand-binding pocket.

On the surface of the 14-3-3 protein that binds to the phosphorylated OsFD1 fragment, electron density was determined for the seven C-terminal residues [R-V-L-(pS)-A-P-F] of OsFD1, and all of them were found to interact with 14-3-3 protein (Figure 2b, right). Overall features of the interaction between 14-3-3 protein and the phosphorylated peptide are similar to those of the canonical phosphoserine-binding site. Therefore, the Hd3a–14-3-3 complex may also interact with other bZIP transcription factors possessing the (S/T)-X-P motif (Table 1), to which 14-3-3 protein binds in the FAC. In fact, most of the FT-interacting bZIP factors contain a (S/T)-X-P motif at their C terminus (Table 1). For the FT-interacting proteins with no apparent (S/T)-X-P motif, however, it remains to be determined how they interact with FT protein.

Molecular mechanism of FAC formation *in vivo*

The mechanism of complex formation of Hd3a–14-3-3–OsFD1 has been studied extensively. In rice protoplasts, Hd3a is localized in both, the cytoplasm and the nucleus; GF14b, an isoform of rice 14-3-3 protein, localizes mainly in the cytoplasm and weakly in the nucleus; and OsFD1 is localized in the nucleus [44]. Identical subcellular distributions of *Arabidopsis* FT and FD have been observed in the SAM of *Arabidopsis* [39]. That is, the subcellular localization of each FAC component overlaps not completely, but partially. Extensive interaction analyses by the bimolecular fluorescence complementation (BiFC) technique [52] showed Hd3a–GF14b interaction in the cytoplasm, and GF14b–OsFD1 and Hd3a–OsFD1 interactions in the nucleus [44]. The BiFC interaction between *Arabidopsis* FT and FD in the nucleus has also been reported [39,53]. When OsFD1 is coexpressed, however, the Hd3a–GF14b complex is concentrated in the nucleus. Taken together, these results strongly favor a model in which FT protein transported from leaves to the SAM is first

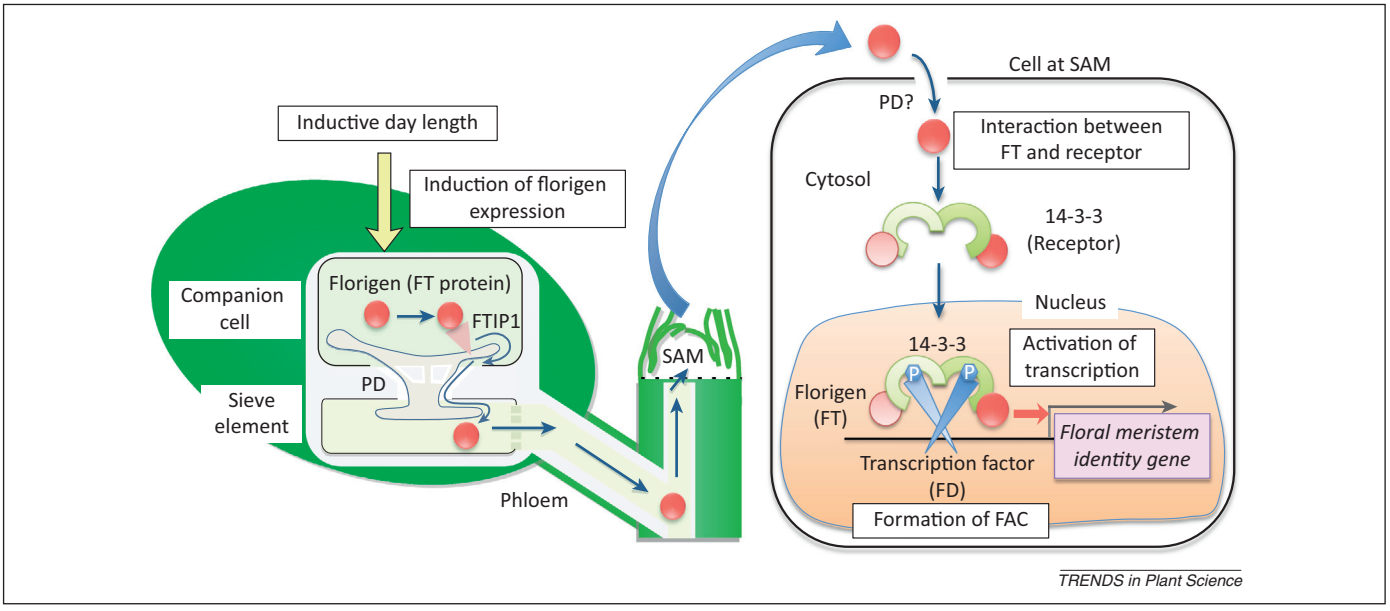

Figure 3. Model for the regulation of photoperiodic flowering by florigen. Under inductive day lengths, florigen (FT protein) is produced in the companion cells of vascular tissue. FT interacts with FT-INTERACTING PROTEIN1 (FTIP1) on the endoplasmic reticulum (ER) and is translocated into sieve elements. FT moves to the shoot apical meristem (SAM) through the phloem. In cells of the SAM, FT interacts with 14-3-3 protein in the cytoplasm, and then the FT–14-3-3 complex enters the nucleus to interact with FD. The resultant FT–14-3-3–FD complex (florigen activation complex, FAC) binds to the promoter regions of floral meristem identity genes (e.g., *AP1*), thereby activating their gene expression to promote flowering. Reproduced, with permission, from [44].

received by a 14-3-3 protein in the cytoplasm, and then the FT–14-3-3 complex is translocated to the nucleus, where it forms a larger protein complex with FD (Figure 3). Formation of the Hd3a–GF14b–OsFD1 tripartite protein complex in the nucleus has been confirmed by BiFC-based fluorescence resonance energy transfer (FRET)–fluorescence lifetime imaging microscopy (FLIM) analysis [44,54]. It is possible that FT protein makes complex with 14-3-3 in the companion cells of leaves, because the expression of *14-3-3* genes is not restricted in the SAM and fairly ubiquitous across plant tissues [55]. It is thought that FT protein also plays a role in leaves, because several *MADS* genes for flowering, including *OsMADS15*, are upregulated in the leaves of *Arabidopsis* and rice in a florigen gene-dependent manner [56,57]. However, it remains to be studied how the florigen protein produced in the leaves is allocated for long distance trafficking.

The FAC formation is thought to be essential for flowering promotion by FT, because the 14-3-3 interaction-defective mutant of Hd3a can neither promote flowering nor activate *OsMADS15* [44]. Phosphorylation of FD is thought to be essential for FAC formation and flowering promotion by FT because substitution of serine to alanine in the SAP motif of OsFD1 abolished 14-3-3 binding and *OsMADS15* activation in protoplasts [44]. This is consistent with the finding that the corresponding substitution of *Arabidopsis* FD impaired FT–FD interaction and failed to rescue the *fd* mutant [39]. Interestingly, overexpression of *OsFD1 S192E* (a phosphomimic substitution in the SAP motif) significantly promoted flowering [44], suggesting the possibility that phosphorylation of FD is a critical regulatory step for flowering. The SAP motif is relatively similar to the consensus motif for phosphorylation by Ca^{2+}-dependent protein kinase (CDPK) [39,58,59]. Identification of an FD kinase would reveal such a regulatory step.

The 14-3-3 proteins have also been identified as interaction partners with transcriptional regulators for the responses to plant hormones, such as brassinosteroid (BR), ABA, and gibberellic acid [60]. For instance, the nuclear accumulation of BZR1, a transcription factor for BR signal transduction in *Arabidopsis*, is negatively regulated by 14-3-3 binding [61,62]. It has not been reported that FT can modulate other plant hormone signaling pathways by 14-3-3 binding. What determines the binding specificity of FT to the FD–14-3-3 complex is an important question that needs to be resolved to understand the function of FT protein.

Florigen as a transcriptional regulator

Evidence supporting the idea that FT participates in transcriptional regulation is accumulating. First, FT/Hd3a interacts with FD/OsFD1 in the nucleus and the interaction is essential for the promotion of flowering [39,44]. Second, FT fused with a strong transcriptional activation domain, VP16, boosted flowering promotion by FT [40], whereas FT fusion with SRDX, a potent transcriptional repression domain [63], suppressed flowering [53]. However, well-known types of transcriptional activation domains such as acidic regions [64] have not been found in FT.

Pioneering work has been conducted to elucidate the molecular basis of the flowering promotion activity of FT by structural comparisons between FT and TFL1 [33,38]. *TFL1* is the responsible gene for *terminal flower 1* of *Arabidopsis*, a mutant with phenotypes of determinate growth and early flowering [65]. Overexpression of *TFL1* delays flowering [38,66]. Contrary to its genetic function, *TFL1* encodes a protein with close similarity to FT [11,12,65]. Modulation of *FT*/*TFL1* expression leads to the hypothesis that a balance between FT and TFL1 activity regulates flowering [11,12,67]. Based on the structural differences

between FT and TFL1, mutation analysis of FT and TFL1 was performed and several important residues and regions of FT for flowering promotion were identified [33,38]. The Y85 residue in FT is highly conserved among FT subfamily members and is located close to the putative anion binding pocket of PEBP. The corresponding residue of FT Y85 in TFL1 is substituted to histidine, and the histidine residue is highly conserved among TFL1 subfamily members [38]. The flowering promotion activity of FT is completely abolished by Y85H substitution [38,44]. Another difference between FT and TFL1 is the structure of the surface-exposed loop region [33]. The segment B region is highly conserved among members of the FT subfamily, whereas the corresponding regions in the TFL1 subfamily members are highly divergent among plant species [33]. A chimeric FT in which the segment B region of FT is substituted to that of TFL1 can repress flowering [33], showing that the segment B region confers the functional specificity of FT. Considering this evidence with the fact that neither Y85 nor segment B region is involved in FAC formation (Figure 2) [44], a model was proposed that the putative anion-binding pocket and/or the segment B region of FT recruits a transcriptional coactivator to activate the downstream target genes [44,53], for example, *AP1* or the homologs for flowering promotion [68–71]. Identification of the molecule that binds to florigen protein and mediates the flowering stimulus from the FAC to basal transcriptional machinery is important to understand the molecular mechanism of flowering.

Versatility of FT homologs: beyond flowering promotion

Day length affects a diverse set of traits in plants [72,73]. Although florigen was first proposed as a flowering hormone, later florigen was also proposed to be a potato (*Solanum tuberosum*) tuber-inducing hormone, tuberigen [74]. The tuberization stimulus is first produced in the leaves under short day conditions and is graft-transmissible; tobacco scions under long day conditions can induce flowering on its own and tuberization of the grafted potato stocks. Like florigen-induced flowering, the short day-induced tuberization is controlled by a photoperiod-dependent pathway [75,76]. Recently, *StSP6A*, a potato *FT* homolog, was shown to control tuberization [77]. Overexpression of the rice florigen gene, *Hd3a*, induced *StSP6A* expression and promoted tuberization as well as flowering in potato plants [77], supporting the idea that florigen functions as tuberigen. FT homologs are reportedly involved in short day-induced growth cessation and bud set in *Populus* [78–80], in flowering repression in sugar beet [81], in the control of leaf morphology and plant architecture of perennial tomato [67] and maize [82], in stomatal control in *Arabidopsis* [83], and in fruit yield of tomato [84]. In the case of *Populus*, *PtFT1* is predominantly expressed during late winter and *PtFT2* is expressed during the period of vegetative growth [80]. Reproductive onset is postulated to be determined by *PtFT1*, whereas vegetative growth and inhibition of bud set are promoted by *PtFT2* [80]. In the case of sugar beet, *BvFT2* is essential for flowering, whereas *BvFT1* represses flowering and its downregulation is crucial for the vernalization response [81]. Although the molecular mechanism of how FT controls stomata remains unknown, a balance between FT and TFL1 plays important roles in growth control and fruit yield of tomato [67,84].

Interestingly, the segment B region of potato StSP6A has three amino acid differences from the consensus sequence (LGRQTVYAPGWRQN) of FT subfamily members [9,77]. Such a variation in segment B might be important for the functional differentiation of the FT subfamily. Populus *PtFT1* and sugar beet *BvFT2* encode FT proteins with a consensus segment B, whereas *PtFT2* and *BvFT1* have one or three amino acid substitutions in segment B, respectively [78–81]. A swapping experiment demonstrated that the three amino acid substitution in BvFT1 is the major cause of their antagonistic functions [81]. It should be stressed that the critical residues for 14-3-3 binding are conserved among these FT homologs mentioned above. Therefore, an FAC-like complex could be formed to regulate these developmental and physiological programs.

Concluding remarks and future perspectives

The functional and structural analysis of FT and its protein complex revealed distinct functional regions in FT; the 14-3-3-binding region, a putative anion-binding pocket, and the segment B region. The 14-3-3-binding region is essential for FAC formation, target gene activation, and flowering. Both the putative anion-binding pocket and the segment B region are not involved in FAC formation but are important for floral promotion. The identification of binding factors that directly interact with these two regions is important for understanding the molecular mechanism of flowering promotion by florigen. The FT–14-3-3 module alone could bind to and modulate a variety of proteins with phosphorylated 14-3-3 recognition consensus sequences, because the pSer/pThr recognition groove of the 14-3-3 protein is not occupied by FT. Molecular determinants for the specificity of FT protein complex formation and, if it exists, identification of the factors that interact with the FT–14-3-3 module will contribute to revealing the molecular basis of the multifunctionality of FT proteins. Manipulation of the components in these FT protein complexes, by amino acid substitution of the active sites, or the contact residues, or by application of small chemicals that affect complex formation, will contribute to success in controlling flowering time and the growth habit of crops.

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References

1 Steeves, T.A. and Sussex, I.M., eds (1989) *Patterns in Plant Development* (2nd edn), Cambridge University Press
2 Poethig, R.S. (2003) Phase change and the regulation of developmental timing in plants. *Science* 301, 334–336
3 Garner, W.W. and Allard, H.A. (1922) Photoperiodism, the response of the plant to relative length of day and night. *Science* 55, 582–583
4 Chailakhyan, M. Kh. (1936) New facts in support of the hormonal theory of plant development. *Dokl. Akad. Nauk SSSR (C.R. Acad. Sci. USSR)* 4, 79–83 (in Russian)

5 Zeevaart, J.A.D. (1976) Physiology of flower formation. *Annu. Rev. Plant Physiol.* 27, 321–348
6 Zeevaart, J.A.D. (2008) Leaf-produced floral signals. *Curr. Opin. Plant Biol.* 11, 541–547
7 Kobayashi, Y. and Weigel, D. (2007) Move on up, it's time for change – mobile signals controlling photoperiod-dependent flowering. *Genes Dev.* 21, 2371–2384
8 Turck, F. *et al.* (2007) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. *Annu. Rev. Plant Biol.* 59, 573–594
9 Pin, P.A. and Nilsson, O. (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant Cell Environ.* 35, 1742–1755
10 Koornneef, M. *et al.* (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229, 57–66
11 Kobayashi, Y. *et al.* (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 286, 1960–1962
12 Kardailsky, I. *et al.* (1999) Activation tagging of the floral inducer *FT*. *Science* 286, 1962–1965
13 Takada, S. and Goto, K. (2003) TERMINAL FLOWER2, an *Arabidopsis* homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of *FLOWERING LOCUS T* by *CONSTANS* in the vascular tissues of leaves to regulate flowering time. *Plant Cell* 15, 2856–2865
14 An, H. *et al.* (2004) *CONSTANS* acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* 131, 3615–3626
15 Jaeger, K.E. and Wigge, P.A. (2007) FT protein acts as a long-range signal in *Arabidopsis*. *Curr. Biol.* 17, 1050–1054
16 Mathieu, J. *et al.* (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis*. *Curr. Biol.* 17, 1055–1060
17 Giavalisco, P. *et al.* (2006) Towards the proteome of *Brassica napus* phloem sap. *Proteomics* 6, 896–909
18 Lin, M.K. *et al.* (2007) FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. *Plant Cell* 19, 1488–1506
19 Corbesier, L. *et al.* (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 316, 1030–1033
20 Tamaki, S. *et al.* (2007) Hd3a protein is a mobile flowering signal in rice. *Science* 316, 1033–1036
21 Lifschitz, E. *et al.* (2006) The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6398–6403
22 Notaguchi, M. *et al.* (2008) Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering. *Plant Cell Physiol.* 49, 1645–1658
23 Li, C. *et al.* (2011) Mobile *FT* mRNA contributes to the systemic florigen signaling in floral induction. *Sci. Rep.* 1, 73
24 Lu, K.J. *et al.* (2012) Long-distance movement of *Arabidopsis FLOWERING LOCUS T* RNA participates in systemic floral regulation. *RNA Biol.* 9, 653–662
25 Amasino, R. (2010) Seasonal and developmental timing of flowering. *Plant J.* 61, 1001–1013
26 Imaizumi, T. (2010) *Arabidopsis* circadian clock and photoperiodism: time to think about location. *Curr. Opin. Plant Biol.* 13, 83–89
27 Srikanth, A. and Schmid, M. (2011) Regulation of flowering time: all roads lead to Rome. *Cell. Mol. Life Sci.* 68, 2013–2037
28 Tsuji, H. *et al.* (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. *Curr. Opin. Plant Biol.* 14, 45–52
29 Andrés, F. and Coupland, G. (2012) The genetic basis of flowering responses to seasonal cues. *Nat. Rev. Genet.* 13, 627–639
30 He, Y. (2012) Chromatin regulation of flowering. *Trends Plant Sci.* 17, 556–562
31 Kumar, S.V. *et al.* (2012) Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature* 484, 242–245
32 Mutasa-Göttgens, E. and Hedden, P. (2009) Gibberellin as a factor in floral regulatory network. *J. Exp. Bot.* 60, 1979–1989
33 Ahn, J.H. *et al.* (2006) A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. *EMBO J.* 25, 605–614
34 Banfield, M.J. *et al.* (1998) Function from structure? The crystal structure of human phosphatidylethanolamine-binding protein suggests a role in membrane signal transduction. *Structure* 6, 1245–1254
35 Serre, L. *et al.* (1998) Crystal structure of the phosphatidylethanolamine-binding protein from bovine brain: a novel structural class of phospholipid-binding proteins. *Structure* 6, 1255–1265
36 Granovsky, A.E. and Rosner, M.R. (2008) Raf kinase inhibitory protein: a signal transduction modulator and metastasis suppressor. *Cell Res.* 18, 452–457
37 Banfield, M.J. and Brady, R.L. (2000) The structure of Antirrhinum centroradialis protein (CEN) suggests a role as a kinase regulator. *J. Mol. Biol.* 297, 1159–1170
38 Hanzawa, Y. *et al.* (2005) A single amino acid converts a repressor to an activator of flowering. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7748–7753
39 Abe, M. *et al.* (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309, 1052–1056
40 Wigge, P.A. *et al.* (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* 309, 1056–1059
41 Muszynski, M.G. *et al.* (2006) *delayed flowering1* encodes a basic leucine zipper protein that mediates floral inductive signals at the shoot apex in maize. *Plant Physiol.* 142, 1523–1536
42 Danilevskaya, O.N. *et al.* (2008) A genomic and expression compendium of the expanded PEBP gene family from maize. *Plant Physiol.* 146, 250–264
43 Meng, X. *et al.* (2011) The FT-like *ZCN8* gene functions as a floral activator and is involved in photoperiod sensitivity in maize. *Plant Cell* 23, 942–960
44 Taoka, K-i. *et al.* (2011) 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* 476, 332–335
45 Pnueli, L. *et al.* (2001) Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. *Plant Cell* 13, 2687–2702
46 Purwestri, Y.A. *et al.* (2009) The 14-3-3 protein GF14c acts as a negative regulator of flowering in rice by interacting with the florigen Hd3a. *Plant Cell Physiol.* 50, 429–438
47 Liu, L. *et al.* (2012) FTIP1 is an essential regulator required for florigen transport. *PLoS Biol.* 10, e1001313
48 Obsil, T. and Obsilova, V. (2011) Structural basis of 14-3-3 protein functions. *Semin. Cell Dev. Biol.* 22, 663–672
49 Wilker, E.W. *et al.* (2005) A structural basis for 14-3-3sigma functional specificity. *J. Biol. Chem.* 280, 18891–18898
50 Schumacher, M.A. *et al.* (2000) The structure of a CREB bZIP.somatostatin CRE complex reveals the basis for selective dimerization and divalent cation-enhanced DNA binding. *J. Biol. Chem.* 275, 35242–35247
51 Ottmann, C. *et al.* (2007) Structure of a 14-3-3 coordinated hexamer of the plant plasma membrane H^+-ATPase by combining X-ray crystallography and electron cryomicroscopy. *Mol. Cell* 25, 427–440
52 Hu, C.D. *et al.* (2002) Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol. Cell* 9, 789–798
53 Hanano, S. and Goto, K. (2011) *Arabidopsis* TERMINAL FLOWER1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression. *Plant Cell* 23, 3172–3184
54 Shyu, Y.J. *et al.* (2008) Visualization of AP-1-NF-κb ternary complexes in living cells by using a BiFC-based FRET. *Proc. Natl. Acad. Sci. U.S.A.* 105, 151–156
55 Paul, A.L. *et al.* (2012) 14-3-3 phosphoprotein interaction networks – does isoform diversity present functional interaction specification? *Front. Plant Sci.* 3, 190
56 Komiya, R. *et al.* (2008) Hd3a and RFT1 are essential for flowering in rice. *Development* 135, 767–774
57 Teper-Bamnolker, P. and Samach, A. (2005) The flowering integrator FT regulates SEPALLATA3 and FRUITFULL accumulation in *Arabidopsis* leaves. *Plant Cell* 17, 2661–2675
58 Huang, J.Z. and Huber, S.C. (2001) Phosphorylation of synthetic peptides by a CDPK and plant SNF1-related protein kinase. Influence of proline and basic amino acid residues at selected positions. *Plant Cell Physiol.* 42, 1079–1087

59 Harper, J.F. *et al.* (2004) Decoding Ca^{2+} signals through plant protein kinases. *Annu. Rev. Plant Biol.* 55, 263–288
60 Jaspert, N. *et al.* (2011) *Arabidopsis* 14-3-3 proteins: fascinating and less fascinating aspects. *Front. Plant Sci.* 2, 96
61 Gampala, S.S. *et al.* (2007) An essential role for 14-3-3 proteins in brassinosteroid signal transduction in *Arabidopsis*. *Dev. Cell* 13, 177–189
62 Ryu, H. *et al.* (2007) Nucleocytoplasmic shuttling of BZR1 mediated by phosphorylation is essential in *Arabidopsis* brassinosteroid signaling. *Plant Cell* 19, 2749–2762
63 Mitsuda, N. *et al.* (2011) CRES-T, an effective gene silencing system utilizing chimeric repressors. *Methods Mol. Biol.* 754, 87–105
64 Gill, G. and Ptashne, M. (1987) Mutants of GAL4 protein altered in an activation function. *Cell* 51, 121–126
65 Bradley, D. *et al.* (1997) Inflorescence commitment and architecture in *Arabidopsis*. *Science* 275, 80–83
66 Ratcliffe, O.J. *et al.* (1998) A common mechanism controls the life cycle and architecture of plants. *Development* 125, 1609–1615
67 Shalit, A. *et al.* (2009) The flowering hormone florigen functions as a general systemic regulator of growth and termination. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8392–8397
68 Weigel, D. and Nilsson, O. (1995) A developmental switch sufficient for flower initiation in diverse plants. *Nature* 377, 495–500
69 Mandel, M.A. and Yanofsky, M.F. (1995) A gene triggering flower formation in *Arabidopsis*. *Nature* 377, 522–524
70 Ferrándiz, C. *et al.* (2000) Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. *Development* 127, 725–734
71 Kobayashi, K. *et al.* (2012) Inflorescence meristem identity in rice is specified by overlapping functions of three *AP1*/*FUL*-like MADS box genes and *PAP2*, a *SEPALLATA* MADS box gene. *Plant Cell* 24, 1848–1859
72 Thomas, B. and Vince-Prue, D., eds (1996) Photoperiodic control of development. In *Photoperiodism in Plants* (2nd edn), pp. 259–354, Academic Press
73 Langercrantz, U. (2009) At the end of the day: a common molecular mechanism for photoperiod responses in plants? *J. Exp. Bot.* 60, 2501–2515
74 Chailakhyan, M. Kh. *et al.* (1981) Photoperiodism and tuber formation in grafting of tobacco onto potato. *Dokl. Akad. Nauk SSSR* 257, 1276–1280
75 Rodríguez-Falcón, M. *et al.* (2006) Seasonal control of tuberization in potato: conserved elements with the flowering response. *Annu. Rev. Plant Biol.* 57, 151–180
76 Abelenda, J.A. *et al.* (2011) From the model to the crop: genes controlling tuber formation in potato. *Curr. Opin. Biotechnol.* 22, 287–292
77 Navarro, C. *et al.* (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. *Nature* 478, 119–122
78 Böhlenius, H. *et al.* (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* 312, 1040–1043
79 Hsu, C.Y. *et al.* (2006) Poplar *FT2* shortens the juvenile phase and promotes seasonal flowering. *Plant Cell* 18, 1846–1861
80 Hsu, C.Y. *et al.* (2011) *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10756–10761
81 Pin, P.A. *et al.* (2010) An antagonistic pair of *FT* homologs mediates the control of flowering time in sugar beet. *Science* 330, 1397–1400
82 Danilevskaya, O.N. *et al.* (2011) Beyond flowering time. *Plant Signal Behav.* 6, 1267–1270
83 Kinoshita, T. *et al.* (2011) *FLOWERING LOCUS T* regulates stomatal opening. *Curr. Biol.* 21, 1232–1238
84 Krieger, U. *et al.* (2010) The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. *Nat. Genet.* 42, 459–463
85 Li, C. and Dubcovsky, J. (2008) Wheat FT protein regulates VRN1 transcription through interactions with FDL2. *Plant J.* 55, 543–554
86 Mimida, N. *et al.* (2012) Apple FLOWERING LOCUS T proteins interact with transcription factors implicated in cell growth and organ development. *Tree Physiol.* 31, 555–566