A Pair of Related Genes with Antagonistic Roles in Mediating Flowering Signals

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Flowering in *Arabidopsis* is promoted via several interacting pathways. A photoperiod-dependent pathway relays signals from photoreceptors to a transcription factor gene, *CONSTANS* (*CO*), which activates downstream meristem identity genes such as *LEAFY* (*LFY*). *FT*, together with *LFY*, promotes flowering and is positively regulated by *CO*. Loss of *FT* causes delay in flowering, whereas overexpression of *FT* results in precocious flowering independent of *CO* or photoperiod. *FT* acts in part downstream of *CO* and mediates signals for flowering in an antagonistic manner with its homologous gene, *TERMINAL FLOWER1* (*TFL1*).

In higher plants, flowering—the transition from vegetative to reproductive growth phase—is controlled via several interacting pathways influenced by both endogenous factors and environmental conditions. In *Arabidopsis*, a photoperiod-dependent pathway promotes flowering in response to an inductive long-day (LD) photoperiod, whereas an autonomous pathway functions independently of the photoperiod and other environmental conditions (*1*). Recent studies suggest that the *FT* gene may be regulated via both photoperiod-dependent and autonomous pathways and may act redundantly with *LFY* in promoting flowering (*2*).

We identified the *FT* gene by transferred DNA (T-DNA) tagging (*3*). The predicted *FT* open reading frame encodes a protein with similarity to the *TFL1* gene product (Fig. 1, A and B) (*4*). *FT* and *TFL1* are members of a gene family in *Arabidopsis* (Fig. 1, B and C) (*5*). Putative *FT* orthologs in other species were found in databases (Fig. 1, B and C) (*6*). *FT* and *TFL1* represent two clades that may have branched before the diversification of angiosperms (Fig. 1C). *FT* was expressed in all tissues in seedlings and mature plants (Fig. 2A). The *FT* mRNA level gradually increased with time under both LD and short-day (SD) photoperiods (Fig. 2, B and C). Under LD conditions, expression was first detected on day 4 and plateaued around day 6, preceding floral commitment around days 9 and 10 (*7*). Up-regulation of *FT* expression was delayed and reduced under SD conditions (Fig. 2C).

CO is up-regulated under LD conditions, and it activates meristem identity genes (*8*, *9*). We investigated whether *CO* regulates *FT*. In the *co-1* mutant, up-regulation of *FT* expression was delayed (Fig. 2C), suggesting that it may require *CO* during the early vegetative phase. In contrast, the *FT* mRNA level was not affected in *fha-1*, which lacks cryptochrome 2 (*10*); *fca-1*, which is defective in the autonomous pathway (*11*); or *fwa-2*, which is similar to *ft* in terms of genetic interactions with *LFY* and *AP1* (*2*) (Fig. 2D). When CO activity was induced from the CO–glucocorticoid receptor (CO:GR) fusion protein by treating *35S::CO:GR; co-2* plants (*9*) with dexamethasone (dex), up-regulation of *FT* expression was observed within 12 hours (Fig. 2E). The *FT* mRNA level was elevated in dex-treated *35S::CO:GR; co-2* plants, but not in plants with similar precocious flowering phenotype due to overexpression of *LFY* (*12*), *AP1* (*13*), or *TSF* (see below) (Fig. 2F). Therefore, up-regulation of *FT* expression upon induction of *CO* activity was not a secondary effect of induced acceleration of flowering. Because up-regulation of *FT* expression eventually occurs in the *co* mutant and in the SD photoperiod, there is probably a photoperiod-independent pathway for *FT* up-regulation. Although the physiological relevance is not clear, early transient expression of *FT* was observed in the *co* mutant and in the SD photoperiod (Fig. 2C).

Constitutive overexpression of *CO* causes photoperiod-independent precocious flowering (*9*). If *FT* promotes flowering under the control of *CO*, then constitutive overexpression of *FT* should result in flowering, independent of the photoperiod and *CO* function. All transgenic lines that overexpress *FT* (*35S::FT*) flowered early with determinate inflorescence similar to *tfl1*, as did *35S::CO* plants (*9*) (Fig. 3, A and B). Overexpression of *TSF* or *CiFT* results in the same phenotype (*14*). Early flowering was correlated with *FT* mRNA accumulation: Plants with the highest level flowered with only two rosette leaves (*14*). Neither the SD photoperiod nor *co-1* affected the

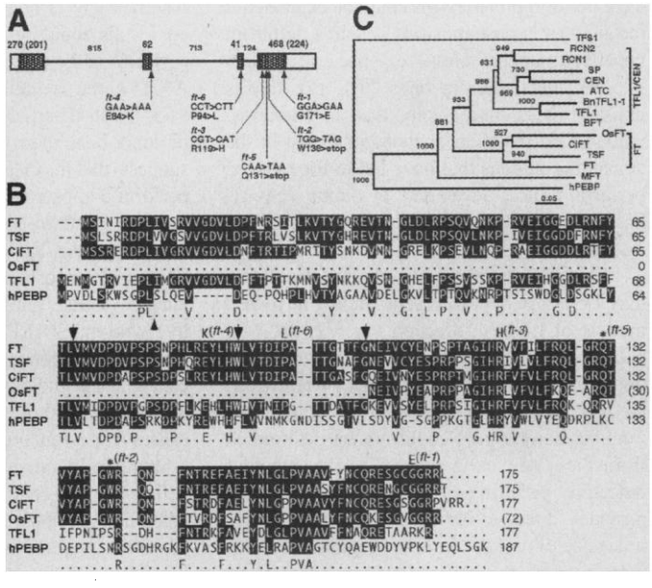

Fig. 1. Structure of the *FT* gene and sequence comparison. (**A**) Schematic diagram of the *FT* locus. Boxes and lines are exons and introns of indicated length (open reading frames are hatched box segments). Mutations in six alleles are shown below. (**B**) Comparison of amino acid sequences (*24*) for FT (*3*), TSF (*5*), CiFT (*6*), OsFT (*6*), TFL1 (*4*), and human PEBP (hPEBP) (*23*). Below the sequences is a consensus. Substitutions or terminations (asterisks) in the six alleles are shown. Vertical arrows, introns conserved between *FT* and *TFL1*; vertical arrowhead, cleavage site in hPEBP to generate the HCNP (underlined) (*23*). (**C**) A phylogenetic tree, constructed by the neighbor-joining method, for 13 representative proteins from plants, hPEBP, and *Saccharomyces* TFS1 (GenBank accession number X62105), RCN1 and RCN2 from rice (*25*), SP (GenBank accession number U84140), CEN (GenBank accession number S81193), and BnTFL1-1 (GenBank accession number AB017525). Bootstrap values are shown on each branch.

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early-flowering phenotype of transgenic plants (Table 1), which indicates that *FT* regulates flowering by acting in part downstream of *CO*. Consistent with this observation, *ft-1* partially suppressed the precocious flowering phenotype of *35S::CO* (*15*). In contrast, the semidominant *fwa-2* mutation, which did not affect *FT* expression (Fig. 1D), partially suppressed the precocious flowering phenotype of *35S::FT* (Table 1). Thus, *FWA* may interfere with pathways downstream of *FT* (*14*).

The SD photoperiod and *co-1* actually enhanced the precocious flowering phenotype in *35S::FT* plants (Table 1). The LD photoperiod or *CO* may also enhance the expression of genes antagonistic to *FT*. One candidate for such a gene is *TFL1*, which is up-regulated by the LD photoperiod and *CO* (*4*, *9*). *FT* and *TFL1* play opposite roles in flowering, as loss of function and ectopic overexpression of these genes results in nearly opposite phenotypes [this study and (*16*–*19*)]. Haploinsufficiency of *FT* in the *35S::TFL1* background (Table 1) and of *TFL1* in the wild-type background (*17*) suggests the importance of the balance between the two. This is further supported by the enhancement of the phenotype of *35S::FT* by *tfl1* and of the phenotype of *35S::TFL1* by *ft* (Table 1 and Fig. 3C).

Because a loss-of-function mutation in one gene had phenotypic effect even in an excess of the other's activity, their mutual antagonism may not simply be due to each blocking the other's activity. *35S::FT; 35S::TFL1* plants and *ft; tfl1* plants showed a phenotype similar to that of *35S::FT* and *ft* plants, respectively (*2*) (Table 1), which suggests that the level of *FT* activity is likely more important in the timing of flowering.

Hyperactivity of the *FT*-mediated pathway alone was not sufficient to induce flowering immediately upon germination, even in the absence of the antagonistic activity of *TFL1* (Fig. 3C). Because *FT* may function in parallel with *LFY* (*2*), we investigated whether the combined activity of these genes is a limiting factor for induction of flowering. *35S::FT/–; 35S::LFY/–* plants germinated normally and had cotyledons and hypocotyls indistinguishable from those of the wild type (Fig. 3, D and E). However, a terminal flower with one or two bracts developed within 3 days after germination, replacing the complex shoot system observed in the wild type (Fig. 3, D to G, and Table 1). Thus, a simultaneous excess of *FT* and *LFY* activity from the very beginning of post-embryonic development induces flowering with almost no intervening vegetative phase. In contrast, neither *35S::FT; 35S::API* nor *35S::LFY; 35S::API* plants showed such an extreme phenotype (*20*, *21*).

Our results and those of others (*2*, *9*, *19*) suggest that *FT* and *TFL1* mediate signals for floral transition in part downstream of *CO* in an antagonistic manner (*14*). What, then, are the biochemical functions of FT and TFL1? They belong to a family of possibly membrane-associated proteins that includes phosphatidylethanolamine-binding protein (PEBP) (*22*). Interestingly, PEBP in humans and rats is a precursor of hippocampal cholinergic neurostimulating peptide (HCNP) (*23*) (Fig. 1B). Whether the function of FT and TFL1 involves the generation of peptide molecules as the transmissible signal is an interesting question.

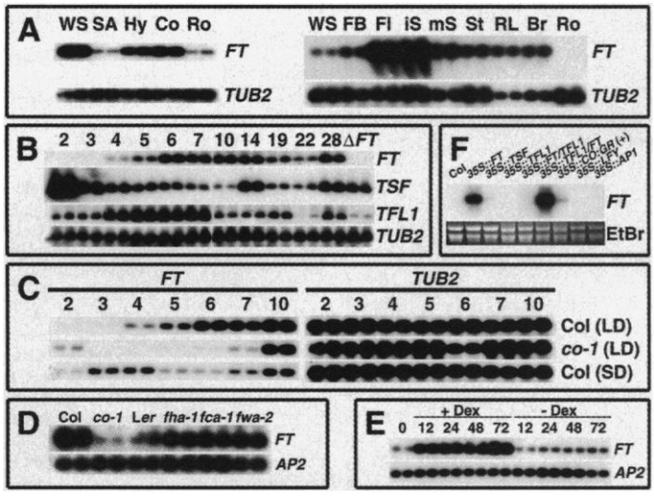

Fig. 2. Analysis of *FT* expression. (**A** to **E**) Reverse transcription polymerase chain reaction analysis (*14*). Duplicate lanes for each sample represent duplicate reactions. A fragment of a β-tubulin gene (*TUB2*) or *APETALA2* (*AP2*) was amplified as a control. Numbers in (B) and (C) indicate days of incubation in a growth chamber (germination on day 1). (A) Expression in LD-grown seedlings on day 6 (left) and mature plants (right). WS, whole seedling; SA, shoot apex; Hy, hypocotyls; Co, cotyledons; Ro, roots; FB, floral buds; Fl, flowers; iS, immature siliques; mS, mature siliques; St, stems; RL, rosette leaves, Br, bracts. (B) Temporal profile of expression of *FT*, *TSF*, and *TFL1* genes in aerial parts of LD-grown plants. Δ*FT* indicates *FT* deletion line (*3*) on day 14. (C) Effect of photoperiods and *co-1* on *FT* expression (LD, 16 hours light/8 hours dark; SD, 8 hours light/16 hours dark). (D) Effect of late-flowering mutations on the level of *FT* expression on day 7, when the level in the wild type plateaued [see (B)]; *co-1* is in the Col background, and *fha-1*, *fca-1*, and *fwa-2* are in the *Ler* background. (E) Up-regulation of *FT* expression by activation of the CO-GR fusion protein (*26*). CO-GR fusion protein was activated by application of dex. Numbers indicate hours after application of dex (+Dex) or the solvent (–Dex); rRNA stained with ethidium bromide (EtBr) is shown as a loading control. (**F**) *FT* expression in transgenic plants overexpressing various genes. RNA blot analysis of 10 μg of total RNA. *FT/TFL1* and *TFL1/FT* are chimeric genes coding for FT(1–63)/TFL1(67–177) and TFL1(1–66)/FT(64–175) fusion proteins, respectively. *35S::CO:GR* (+) indicates *35S::CO:GR; co-2* plants 4 days after application of dex (*26*).

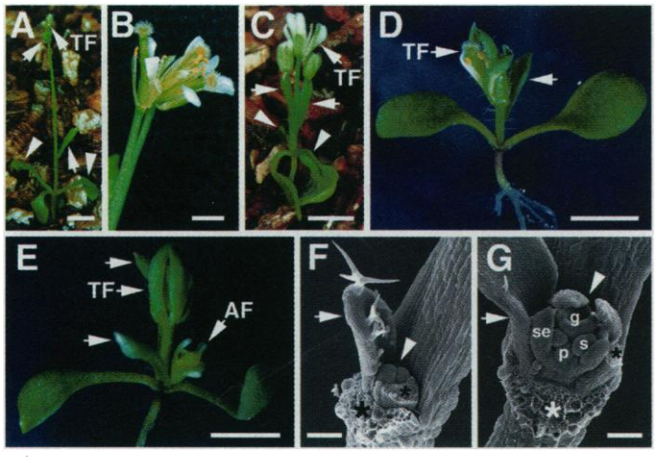

Fig. 3. Phenotype of transgenic plants. (**A**) A *35S::FT* plant. (**B**) A "terminal flower" on the primary inflorescence of a *35S::FT* plant. (**C**) A *35S::FT; tfl1-17* plant with single terminal flowers replacing the primary and secondary inflorescences; *tfl1-17* is an RNA-null allele as the result of a 1-kb deletion in *TFL1* (*20*). (**D** and **E**) *35S::FT/–; 35S::LFY/–* plants. In (A) through (E), the arrowhead indicates a rosette leaf; arrow, bract; TF, terminal flower; AF, axillary flower. (**F** and **G**) Shoot apex of a 3-day-old seedling of *35S::FT/–* (F) and *35S::FT/–; 35S::LFY/–* (G). The arrow indicates one of the first two leaves or bracts left intact; the arrowhead indicates the shoot apex in between. One cotyledon (large asterisk) and one leaf or bract (small asterisk) were removed. In *35S::FT/–; 35S::LFY/–*, the shoot apical meristem itself was transformed into a single terminal flower (se, sepal; p, petal; s, stamen; g, gynoecium), whereas it had two bract primordia and the shoot apical meristem between them in *35S::FT/–*. Scale bars, 5 mm (A, C, D, and E), 2.5 mm (B), 100 μm (F and G).

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Table 1. Flowering times of transgenic and mutant plants.

Genotype*	Rosette leaves or leaves‖	SD	Range	*n*
	Experiment 1			
Wild type, LD	10.9	1.9	9–15	11
Wild type, SD	21.0	2.1	18–25	21
35S::FT, LD†‡	2.2	0.4	2–3	20
35S::FT, SD†	2.0	0.2	2–3	30
tfl1-17, LD	8.0	1.1	6–9	5
35S::FT; tfl1-17, LD‡	2.0	0	2	44
35S::TFL1, LD	18.5	2.7	12–25	63
35S::FT/−; 35S::TFL1/−, LD	4.0	0	4	3
	Experiment 2			
Wild type	11.3	1.9	8–15	61
co-1	19.9	2.4	15–25	43
35S::FT§	3.8	0.5	3–5	57
35S::FT; co-1§	3.4	0.6	2–4	70
	Experiment 3			
Wild type (L)	7.6	0.7	7–9	10
fwa-2/fwa-2 (L)	17.6	0.9	16–19	12
FWA+/FWA+; 35S::FT/− (L/C)	4.6	0.5	4–5	8
FWA+/fwa-2; 35S::FT/− (L/C)	10.1	1.0	9–12	7
	Experiment 4			
Wild type (L)	9.8	1.6	8–12	6
ft-3/ft-3 (L)	19.0	1.2	18–21	7
FT+/FT+; 35S::TFL1/− (L/C)	11.0	0.8	10–12	3
FT+/ft-3; 35S::TFL1/− (L/C)	17.7	0.5	17–18	3
ft-3/ft-3; 35S::TFL1/− (L/C)	38.0	3.7	32–42	4
	Experiment 5			
Wild type	10.4	1.0	9–12	20
35S::FT	5.3	0.5	5–6	43
Wild type (L)	10.9	1.3	8–13	29
35S::LFY (L)	7.1	0.8	6–8	7
35S::FT × wild type (L) F_1	5.8	0.7	5–7	6
35S::FT × *35S::LFY* (L) F_1	1.9	0.2	1–2	15

*Genetic background: L, Landsberg *er* (L*er*); L/C, F_1 between L*er* and Columbia (Col); otherwise, Col. Transgenic lines used were YK#11-1 (a strong line, experiments 1 and 5) and YK#1-5C (a weak line, experiments 2 and 3) of *35S::FT*, *35S::TFL1* (KG#9-5) and *35S::LFY* (DW151.2.5L). SD, 8 hours light/16 hours dark cycle; LD, 16 hours light/8 hours dark cycle; otherwise, continuous light conditions. In each experiment, there was a statistically significant difference (Student's *t* test, $P < 0.001$) among genotypes or conditions compared including the three pairs marked †, ‡, and §. ‖The number of rosette leaves (experiments 1 to 4) or leaves (experiment 5) as an indicator of flowering time (*16*).

deletion in the *FT-FAS1* region was identified. The bacterial artificial chromosome (BAC) clone F5I14 (GenBank accession number AC001229) was found to cover the deleted region. One candidate gene (*F5I14.3*) with similarity to *TFL1* (*4*) was examined in six *ft* alleles, including three new ones (*ft-4*, *ft-5*, and *ft-6* from ecotype Nossen) [this study and (*16*)], and a nucleotide substitution was found in all cases. The entire *F5I14.3* was deleted in vTAAT26C51. On the basis of these results, we concluded that *F5I14.3* is the *FT* gene. Sequences of cDNA were deposited in GenBank (accession numbers AB027504 and AB027505).

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27. We particularly thank D. Weigel for exchange of unpublished results and materials. We thank G. Coupland and Plant Bioscience Limited, M. Koornneef, M. Omura, M. Yanofsky, and the NSF-supported Arabidopsis Biological Resource Center for materials, and G. Coupland, M. Koornneef, and J. Kyozuka for communicating unpublished results. Supported by grants from the Ministry of Education, Science, Culture and Sports of Japan, and an RFTF grant from the Japan Society for the Promotion of Science (JSPS). Y.K. is a JSPS Research Fellow.

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Activation Tagging of the Floral Inducer *FT*

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FLOWERING LOCUS T (*FT*), which acts in parallel with the meristem-identity gene *LEAFY* (*LFY*) to induce flowering of *Arabidopsis*, was isolated by activation tagging. Like *LFY*, *FT* acts partially downstream of *CONSTANS* (*CO*), which promotes flowering in response to long days. Unlike many other floral regulators, the deduced sequence of the FT protein does not suggest that it directly controls transcription or transcript processing. Instead, it is similar to the sequence of TERMINAL FLOWER 1 (TFL1), an inhibitor of flowering that also shares sequence similarity with membrane-associated mammalian proteins.

The transition from the vegetative to the flowering phase of *Arabidopsis* is controlled by several genetic pathways that monitor the developmental state of the plant as well as environmental conditions (*1*). Despite the cloning of several *Arabidopsis* genes participating in these

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