1. Explain and translate the following words into Chinese (20%):

(1) totipotency
(2) transpiration
(3) C3, C4, CAM
(4) photorespiration
(5) Calvin cycle
(6) Carpel, Petal, Sepal, petiole
(7) Mycorrhizae
(8) self-incompatability
(9) dioecious
(10) autotroph

2. Read one of the research articles provided to you and answer the following questions:

(1) Translate the abstract into Chinese (20%).
(2) State very briefly, the overall problems that the investigators are studying and the importance of the topic. Within the context of the overall problems, what specific objectives or questions are the paper addressing (10%)?
(3) What kinds of approaches and methods have the authors used to address their problems and questions. Highlight the important methods used in the paper (20%).
(4) Describe the results and conclusions of the paper and of the experiments from which these conclusions were drawn (in other words, what did the authors learn and how did they learn it?). For each conclusion of the paper, there will be supporting data in the form of a figure or table. It is important to understand what is being shown in each of these (30%).

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research article

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LETTERS

Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice

Kenong Xu[1], Xia Xu[1], Takeshi Fukao[2], Patrick Canlas[1], Reycel Maghirang-Rodriguez[3], Sigrid Heuer[3], Abdelbagi M. Ismail[3], Julia Bailey-Serres[2], Pamela C. Ronald[1] & David J. Mackill[3]

Most *Oryza sativa* cultivars die within a week of complete submergence—a major constraint to rice production in south and southeast Asia that causes annual losses of over US$1 billion and affects disproportionately the poorest farmers in the world[1,2]. A few cultivars, such as the *O. sativa* ssp. *indica* cultivar FR13A, are highly tolerant and survive up to two weeks of complete submergence owing to a major quantitative trait locus designated *Submergence 1* (*Sub1*) near the centromere of chromosome 9 (refs 3–6). Here we describe the identification of a cluster of three genes at the *Sub1* locus, encoding putative ethylene response factors. Two of these genes, *Sub1B* and *Sub1C*, are invariably present in the *Sub1* region of all rice accessions analysed. In contrast, the presence of *Sub1A* is variable. A survey identified two alleles within those *indica* varieties that possess this gene: a tolerance-specific allele named *Sub1A-1* and an intolerance-specific allele named *Sub1A-2*. Overexpression of *Sub1A-1* in a submergence-intolerant *O. sativa* ssp. *japonica* conferred enhanced tolerance to the plants, downregulation of *Sub1C* and upregulation of *Alcohol dehydrogenase 1* (*Adh1*), indicating that *Sub1A-1* is a primary determinant of submergence tolerance. The FR13A *Sub1* locus was introgressed into a widely grown Asian rice cultivar using marker-assisted selection. The new variety maintains the high yield and other agronomic properties of the recurrent parent and is tolerant to submergence. Cultivation of this variety is expected to provide protection against damaging floods and increase crop security for farmers.

Submergence of plants inhibits aerobic respiration and photosynthesis, and stimulates a variety of responses that can enhance survival, such as a switch from aerobic to anaerobic respiration[7]. In contrast to deep-water rice cultivars that avoid submergence stress by growing above the water surface and thereby restoring gas exchange[8], submergence-tolerant rice can survive 10–14 days of complete submergence and renew growth when the water subsides[9]—although the duration of survival is also influenced by environmental factors such as water turbidity, temperature and light levels[10]. The *Sub1* locus was mapped to an interval of 0.06 centimorgans on chromosome 9 using a mapping population (DX202) of 4,022 plants developed from the hybridization of a tolerant *indica* derivative of the FR13A cultivar (IR40931-26) and the intolerant *japonica* cultivar M-202 (Supplementary Fig. 1a, b; Supplementary Tables 1–3). Physical coverage of this region was obtained with five overlapping bacterial artificial chromosome (BAC) clones derived from submergence-intolerant *indica* rice varieties and a nearly complete contig of 13 binary clones from IR40931-26 (Supplementary Fig. 1b). The *Sub1* region, bordered by the markers GR25K and SSR1A, physically spans over 182 kilobases (kb). This interval encodes three genes containing ethylene-response-factor (ERF) domains and designated *Sub1A*, *Sub1B* and *Sub1C*, ten non-ERF genes including four transcribed and six hypothetical protein-coding genes, and >50% retrotransposon-related sequences (Fig. 1a; Supplementary Fig. 1; Supplementary Table 4). The corresponding region of the *japonica* genome represented by the sequenced variety Nipponbare spans 142 kb and is considerably rearranged. Notably, *Sub1A* is absent from the Nipponbare genome[11]. Recombination was suppressed in this region in the mapping population, as revealed by the 10.7-fold higher-than-average recombination ratio (3,030 kb cM^{-1} in the *Sub1* region versus 282 kb cM^{-1} for the entire genome)[5,12]. This could reflect the proximity of the *Sub1* locus to the centromere and/or the presence of genomic rearrangements that have altered continuity in this region in the two rice subspecies[13].

Plant proteins that contain ERF domains are known regulators of abiotic and biotic stress responses[14,15]. The accumulation of *Sub1A* and *Sub1C* messenger RNAs was strongly but transiently promoted by submergence and further reduced on de-submergence in seedling leaves of tolerant FR13A (Fig. 1b). *Sub1C* mRNA induction was earlier and more pronounced in intolerant Nipponbare compared with FR13A (Fig. 1b), suggesting that the rapid induction of *Sub1A* limits expression of *Sub1C*. *Adh1* gene transcript levels were more strongly induced in the tolerant line, indicating that *Sub1A* may

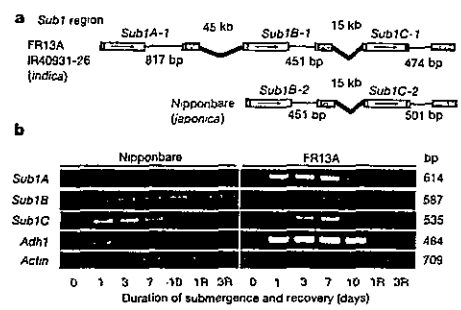

Figure 1 | *Sub1* region gene composition and submergence-induced mRNA accumulation in rice. **a**, ERF gene structure and organization in tolerant *indica* (IR40931-26) and intolerant *japonica* (Nipponbare). Arrows: direction of transcription; shaded boxes: untranslated regions; open boxes: coding sequence; thin lines: introns; thick lines: intergenic regions. Gene structure was determined by comparison of genomic and cDNA sequences of *Sub1B* (AK106057, AK068688) and *Sub1C* (AK060090, AK072749). **b**, Semi-quantitative RT–PCR assessment of gene transcript levels in shoot tissue from tolerant (FR13A) and intolerant (Nipponbare) genotypes following 1–10-d submergence and a subsequent 1–3-d recovery (1R and 3R, respectively).

[1]Department of Plant Pathology, University of California, Davis, California 95616, USA. [2]Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California, Riverside, California 92521-0124, USA. [3]International Rice Research Institute, DAPO Box 7777, Metro Manila, The Philippines

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positively regulate certain acclimation responses (Fig. 1b). In contrast, *Sub1B* transcripts increased only slightly during submergence (Fig. 1b; Supplementary Fig. 2a). The ten non-ERF genes in the *indica Sub1* region showed no evidence of expression in seedling leaves before or during submergence in IR40931-26 or the intolerant variety M-202 (data not shown). These results indicated that the three ERF-domain-containing genes, particularly *Sub1A*, were strong candidates for the genetic determinant controlling submergence tolerance.

The three SUB1 proteins each possess a single copy of the 56-amino-acid DNA-binding domain characteristic of the ERF subfamily of the plant *Apetela2*-like transcription factors (Supplementary Fig. 3). SUB1B and SUB1C have the two signature amino acids—alanine and aspartic acid—at positions 13 and 18, respectively, of the ERF domain that are characteristic of the B2 subgroup of ERF proteins[16]. In contrast, SUB1A has a serine at position 13, as found in two members of the B1b subgroup[15], but falls in the same clade as the other two SUB1 proteins[14]. Within the ERF domain, SUB1A shares 87.7% and 77.2% sequence identity with SUB1B and SUB1C, respectively (Supplementary Fig. 3). A full-length *Sub1A* transcript of 1312 nucleotides was obtained from mRNA of submerged IR40931-26 plants by reverse transcription–polymerase chain reaction (RT–PCR). The transcript consists of a 5′ untranslated region (UTR) of 149 nt, an open reading frame (ORF) of 846 nucleotides encoding a deduced protein of 281 amino acids, and a 3′ UTR of 317 nucleotides (Fig. 1a; Supplementary Fig. 4). The 5′ end of the *Sub1A* complementary DNA includes 30 nucleotides of ORF not predicted by gene-prediction algorithms. The *Sub1A* gene has one intron of 817 base pairs (bp) and two exons, the second exon comprising only 11 bp including the stop codon. A similar genomic structure was found for *Sub1B* (second exon 15 bp), whereas *Sub1C* has only one intron, located in the 3′ UTR (Fig. 1a).

A survey of *Sub1* locus haplotypes in 17 *indica* and four *japonica* varieties identified two *Sub1A*, nine *Sub1B* and seven *Sub1C* alleles on the basis of variation in amino-acid sequence (Table 1; Supplementary Figs 3, 5 and 6). The *Sub1A-1* and *Sub1C-1* alleles are limited to all six submergence-tolerant accessions, three of which were independently isolated submergence-tolerant varieties, including FR13A. There was no *Sub1B* allele identified as being specific to submergence tolerance. Variations in putative mitogen-activated protein kinase (MAPK) sites distinguish the tolerant and intolerant alleles of *Sub1A* and *Sub1C*. In the tolerant *Sub1A-1* allele a single nucleotide polymorphism at position 556 is responsible for a Pro 186 (intolerant) to Ser 186 (tolerant) substitution in a MAPK site (PXS/TP, where "X" is any amino acid[17]). Conversely, the *Sub*1C-1 allele of tolerant lines lacks a MAPK phosphorylation site (underlined) present in the alleles of the intolerant accessions (tolerant: SPPP$_{175}$PEQPAAPV; intolerant: SL/PPT$_{175}$PPPP/E(P)$_{0-3}$; (where P can be 0 or 3 in intolerant rice varieties) Table 1; Supplementary Figs 3 and 6). These potential phosphorylation sites are located in variable regions immediately carboxy-terminal to the ERF domain and may be of significance as phosphorylation can modulate DNA binding by ERF proteins[14,18].

The allelic survey further revealed that *Sub1A* is absent from five out of seventeen *indica* varieties and all four *japonica* varieties analysed, including the fully sequenced genome of Nipponbare[11] (Table 1). Assay of gene expression in a selection of *indica* varieties with *Sub1A* revealed that submergence tolerance is correlated with possession of the strongly submergence-induced *Sub1A-1* and intolerance is associated with the poorly submergence-induced *Sub1A-2* or complete absence of this gene (Fig. 1b; Supplementary Fig. 2a). Nucleotide polymorphisms in the *Sub1A* alleles, including the 5′-flanking regions (Supplementary Fig. 4), could be responsible for their differential expression. In contrast, submergence tolerance is correlated with limited induction of *Sub1C* under submergence, whereas intolerance is associated with high levels of *Sub1C* mRNA (Fig. 1b; Supplementary Fig. 2a). Together, these data suggested that stable transformation of *japonica* rice with an ectopically expressed *Sub1A-1* would downregulate *Sub1C* and confer submergence tolerance.

To test this hypothesis, we transformed the intolerant *japonica* variety Liaogeng with a *Sub1A-1* full-length cDNA under the control of the maize *Ubiquitin1* promoter[19,20] (*Ubi:Sub1A*). A screen of seedlings after 11 days of submergence identified four T_1 families, derived from independent T_0 *Ubi:Sub1A*$^+$ lines, with submergence-tolerant transgenic individuals, and progeny from two families were examined in detail. T_1 families 1 and 3 showed a correlation between high expression of the *Sub1A-1* transgene and submergence tolerance (Fig. 2c; Supplementary Fig. 2b). As observed in the FR13A descendant IR40931-26, tolerant *Sub1A-1*$^+$ plants showed a significant impairment of shoot elongation under submergence compared with the intolerant parent Liaogeng and non-transgenic siblings (Fig. 2a, b; Supplementary Fig. 2c). *Ubi:Sub1A-1* conferred a pleiotropic phenotype including reduced plant height under normal and submergence conditions (Fig. 2a, b) and enhanced expression of *Adh1* under normal growth conditions (Fig. 2c). Under submergence, the transgenic progeny showed reduced *Sub1C* (Fig. 2d) and enhanced *Adh1* (Supplementary Fig. 2d) mRNA accumulation concomitant with increased survival, as characteristic of tolerant *indica* varieties (Fig. 1b; Supplementary Fig. 2a). Although ectopic expression of *Ubi:Sub1A-1* reduced plant height, submergence tolerance was independent of plant height at the time of inundation (Fig. 2a; Supplementary Fig. 2c). It is known that entrapment of ethylene during submergence leads to decreased abscisic acid levels and increased gibberellin sensitivity, and has ramifications on cellular metabolism as well as stem and leaf elongation[10,21,22]. *Sub1* genotype controls ethylene and gibberillin mediated changes in gene expression including regulation of genes that control carbohydrate consumption and

Table 1 | Haplotypes of the *Sub1* locus based on alleles of the ERF-like genes in rice varieties

Line or cultivar	Submergence phenotype	Subspecies	*Sub1A* allele	*Sub1B* allele	*Sub1C* allele
FR13A, IR40931-26, DX18-121, IR48930	Tolerant	*indica*	A-1	B-1	C-1
Goda Heenati	Tolerant	*indica*	A-1	B-6	C-1
Kurkaruppan	Tolerant	*indica*	A-1	B-3	C-1
LMNIII	ND	*indica*	A-2	B-1	C-4
Teqing, CO39, IR64, IR64-M6D6-933-1-2, 93-11	Intolerant	*indica*	A-2	B-1, B-7	C-3, C-5
IR24, IRBB21, Swarna*	Intolerant	*indica*	Absent	B-8, B-5	C-6
IR50	Intolerant	*indica*	Absent	B-9	C-7
Habiganj aman	Intolerant	*indica*	Absent	B-4	C-6
Nipponbare, Liaogeng, M-202, Taipei309	Intolerant	*japonica*	Absent	B-2	C-2

Allele designations were based on the amino-acid sequence of the putative proteins (Supplementary Figs 3, 5 and 6). The submergence-tolerant *indica*-like variety FR13A is from Orissa, in eastern India. DX18-121 is an *indica/japonica* hybrid derivative. The submergence-tolerant varieties Kurkaruppan and Goda Heenati are from Sri Lanka. IR48930, IR40931-26 and DX18-121 are derivatives of FR13A. The primary locus conferring tolerance in FR13A and Kurkaruppan was reported to be similar but different from Goda Heenati[20]. However, submergence tolerance in Goda Heenati is also largely controlled by the *Sub1* locus (K.X. and D.J.M. unpublished data). Molecular marker studies indicate considerable divergence between Goda Heenati and FR13A (D.J.M. unpublished data). GenBank accessions of 93-11 containing *Sub1A*, *Sub1B* and *Sub1C* are AAAA01009971, AAAA01020021 and AAAA01005744, respectively. ND, not determined. The varieties are grouped based primarily on common alleles of Sub1A and Sub1C.
* Swarna lacks *Sub1A* and its alleles of *Sub1B* and *Sub1C* were not determined.

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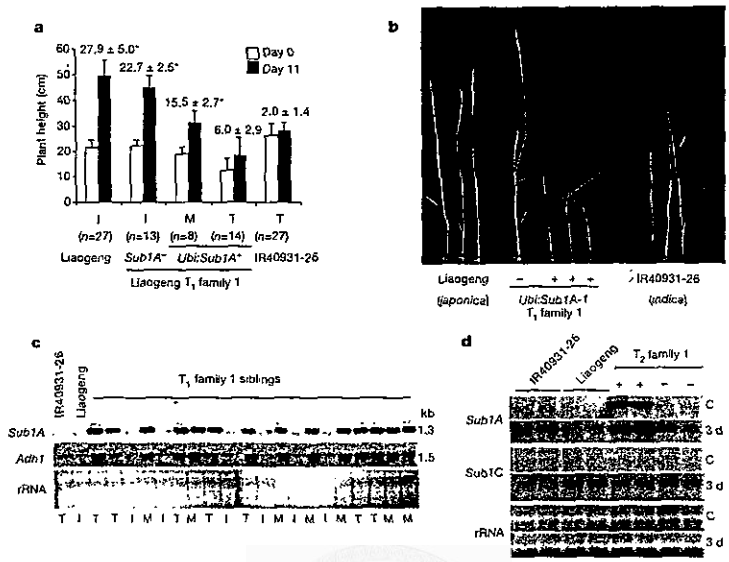

Figure 2 | Characterization of submergence response in transgenic rice ectopically expressing *Sub1A-1*. **a**, Comparison of plant height before submergence and after 11 days of submergence of the controls (non-transformed Liaogeng and IR40931-26) and siblings from T_1 family 1, including non-transgenic (*Sub1A*$^-$) intolerant (I) plants, and transgenic (*Sub1A*$^+$) intermediate tolerant (M) and tolerant (T) plants. At day 0 *Sub1A*$^+$ plants were significantly shorter than *Sub1A*$^-$ plants ($P < 0.0001$, unpaired *t*-test). Values above the bars are the mean and s.d. of the change in plant height following submergence. Error bars are also s.d. An asterisk indicates a significant increase in plant height in response to submergence at day 11 ($P < 0.0001$, unpaired *t*-test). **b**, Comparison of plant height of T_1 family 1 siblings and control plants following 11 days of submergence. **c**, Northern blot analysis of *Sub1A-1* and *Adh1* mRNA levels in shoot tissue harvested 10 days following de-submergence in T_1 family 1 siblings and control plants. Hybridization was performed with rice *Sub1A-1* and *Adh1* cDNA probes. **d**, Northern blot analysis of *Sub1A* and *Sub1C* mRNA levels in shoot tissue harvested immediately following submergence for 3 days or in control non-submerged tissue (C) from four T_2 siblings generated by self-pollination of a T_1 family 1 hemizygote. Siblings with the *Ubi:Sub1A* transgene (+); siblings lacking the transgene (−). Hybridizations were to *Sub1A-1* cDNA and *Sub1C-1* 3′-UTR probes, respectively.

cell elongation[23]. A genetic interaction between *Sub1A* and *Sub1C* may be of relevance because antagonistic relationships between ERFs have been recognized[15,24,25]. Detailed analyses of the function of *Sub1A* and *Sub1C* in the submergence response by targeted RNA interference (RNAi) and overexpression constructs are ongoing.

We used polymorphic molecular markers for *Sub1* with markers that flanked the locus to introgress the *Sub1* genes into the widely grown Indian variety Swarna, which lacks *Sub1A*. *Sub1* markers were used for selection of tolerant progenies, in combination with 5–12 background markers for each of the 12 rice chromosomes[26–28]. Marker assisted selection (MAS) application in the first backcross generation (BC_1F_1) was used to identify individual plants with the fewest IR49830-7-1-2-3 (FR13A descendant) chromosomal segments. Selected BC_1F_1 plants were used to generate BC_2F_3 and BC_3F_2 Swarna-*Sub1* lines that were genotypically identical to Swarna, except for the *Sub1* haplotype and adjacent markers. Both Swarna-*Sub1* lines showed strong submergence tolerance (Fig. 3). Field trials with the BC_2F_3 plants grown under control (non-submerged) conditions in the Philippines indicated no differences between the two varieties in terms of yield (Swarna: 6.3 ± 0.1 t ha^{-1}; Swarna-*Sub1*: 6.4 ± 0.1 t ha^{-1}), plant height (Swarna: 105 ± 1.4 cm; Swarna-*Sub1*: 106 ± 1.2 cm), harvest index (both 0.35) and grain quality as indicated by amylose content (Swarna: 26.4%; Swarna-*Sub1*: 25.9%). Development of submergence-tolerant varieties using these procedures is at an advanced stage for Laos, Bangladesh and India, and has already been reported in Thailand[29].

The results presented here confirm that submergence tolerance is conferred by a haplotype of the complex *Sub1* locus, with ectopic

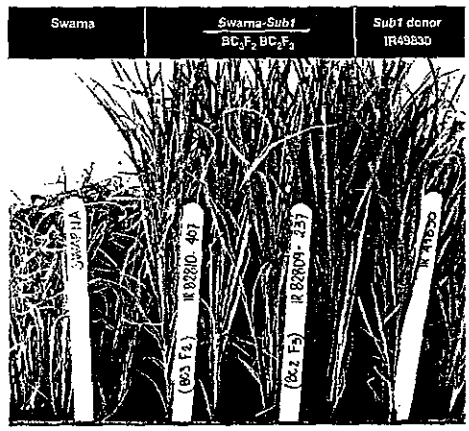

Figure 3 | Introgression of the FR13A *Sub1* haplotype into an intolerant variety by MAS confers submergence tolerance. The *Sub1* region donor line IR49830 (an FR13A derivative) was introduced into the submergence-intolerant *indica* variety Swarna by backcrossing (BC) with MAS using markers for the *Sub1* region (SSR1, RM316, RM464, RM464A, RM219 and RM524) and the 12 chromosomes[26–27]. Individual F_1 plants were selected from BC_1, BC_2 and BC_3 that carried the FR13A *Sub1* haplotype with the least IR49830 background. Fourteen-day-old seedlings were submerged for 14 d and photographed 14 d after de-submergence.

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Sub1A-1 expression being sufficient to enhance tolerance. The finding of the identical *Sub1* haplotype in accessions from submergence-prone areas in Sri Lanka and eastern India (Table 1) suggests that rice grains from submergence-tolerant plants may have been transported over 1,000 km and subsequently introgressed into local varieties, further indicating the agronomic importance of the *Sub1* locus.

METHODS

***Sub1* characterization.** Details of rice genotypes, growth and treatment conditions, and mapping are provided in Supplementary Methods. An F_2 mapping population of 4,022 plants expanded from DX202 (ref. 4), derived from a cross between DX18-121 (a tolerant line derived from *indica* IR40931-26, a descendant of FR13A) and the intolerant *japonica* cultivar M-202, was used. Submergence treatment was conducted as described previously[4]. The fine-mapping of the *Sub1* locus was accomplished with 24 markers specific to the *Sub1* region. For gene expression analyses, total RNA was isolated from seedling leaves and analysed as detailed in Supplementary Methods.

Generation of submergence-tolerant rice. To overexpress *Sub1A-1*, a binary construct Ubi:Sub1A-1-C1300 carrying the full-length Sub1A-1 cDNA was transformed into an intolerant *japonica* cultivar, Liaogeng, using *Agrobacterium tumefaciens* (EHA105) as described in Chern *et al.*[20]. Integration of the *Ubi:Sub1A-1* transgene was verified by PCR using a maize *Ubiquitin1* promoter-specific primer and a *Sub1A* specific primer. Submergence tolerance and gene expression in the transgenic rice was evaluated as described in Supplementary Methods. Swarna-*Sub1* was produced by crossing the Indian variety Swarna to the FR13A descendant IR49830-7-1-2-3, followed by subsequent backcrossing to Swarna. MAS of progeny with polymorphic markers was performed by PCR analysis of genomic DNA.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Sequences were submitted to GenBank/EMBL/DDJB under accession numbers DQ011597–DQ011607 and DQ453964–DQ453966. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to P.C.R. (pcronald@ucdavis.edu) or D.J.M. (d.mackill@cgiar.org).

27. The change in Coulomb failure stress is defined as $\Delta CFS = \Delta\sigma_s + \mu\Delta\sigma_n$, where $\Delta\sigma_s$ is the change in shear stress, $\Delta\sigma_n$ is the change in effective normal stress, and μ is the coefficient of effective internal friction (*28*). We use $\mu = 0.4$.
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FT Protein Movement Contributes to Long-Distance Signaling in Floral Induction of *Arabidopsis*

Laurent Corbesier,[1] Coral Vincent,[1]* Seonghoe Jang,[1]* Fabio Fornara,[1] Qingzhi Fan,[2] Iain Searle,[1] Antonis Giakountis,[1] Sara Farrona,[1] Lionel Gissot,[2] Colin Turnbull,[2] George Coupland[1]†

In plants, seasonal changes in day length are perceived in leaves, which initiate long-distance signaling that induces flowering at the shoot apex. The identity of the long-distance signal has yet to be determined. In *Arabidopsis*, activation of *FLOWERING LOCUS T* (*FT*) transcription in leaf vascular tissue (phloem) induces flowering. We found that *FT* messenger RNA is required only transiently in the leaf. In addition, FT fusion proteins expressed specifically in phloem cells move to the apex and move long distances between grafted plants. Finally, we provide evidence that FT does not activate an intermediate messenger in leaves. We conclude that FT protein acts as a long-distance signal that induces *Arabidopsis* flowering.

Perception of day length takes place in the leaf, whereas flowers are formed by the shoot apical meristem at the apex of the shoot (*1*, *2*). A long-distance signal, called florigen or the floral stimulus, has been demonstrated to be transmitted through the phloem vascular system from the leaves to the meristem, although the identity of this signal has remained unclear since the 1930s. Molecular-genetic approaches in *Arabidopsis* have defined a regulatory pathway that promotes flowering in response to long days (LDs) and have suggested how this pathway responds to day length (*3*–*5*). Under LDs, the CONSTANS (CO) transcriptional regulator activates transcription of *FLOWERING LOCUS T* (*FT*) in the vascular tissue of leaves (*6*–*8*). *FT* encodes a small protein with similarity to RAF-kinase inhibitors that acts at the meristem together with the transcription factor FD to activate transcription of the floral meristem identity gene *APETALA1* (*7*, *9*–*11*). *FT* is expressed in the leaves in response to photoperiod, but FT protein

[1]Max Planck Institute for Plant Breeding Research, Carl von Linne Weg 10, D-50829 Cologne, Germany. [2]Division of Biology, Imperial College London, Wye Campus, Wye, Kent TN25 5AH, UK.
*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: coupland@mpiz-koeln.mpg.de

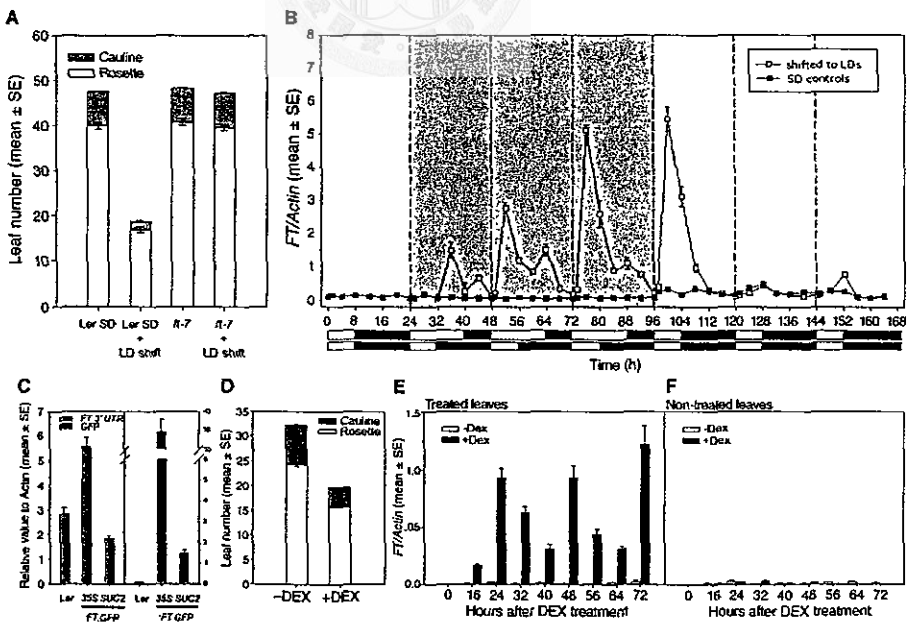

Fig. 1. Regulation of *FT* mRNA in leaves during flowering. **(A)** Flowering time of wild-type *Ler* and *ft-7* plants grown for 2 weeks under SD and exposed to three inductive LDs before return to SDs. **(B)** Expression of *FT* mRNA during 7 days comprising one SD followed by three LDs and then three subsequent SDs. *FT* mRNA expression in the SD-grown controls is also shown. RNA was tested every 4 hours. The inserted three LDs are shaded. Below the graph, bars show the duration of day (white) and night (black) for the shift experiment (top) and the control experiment (bottom). **(C)** Endogenous *FT* mRNA [FT 3′ untranslated region (UTR)] and *FT:GFP* mRNA (GFP) expression in 14-day-old *Ler*, *35S:FT:GFP*, and *SUC2:FT:GFP* plants. **(D)** Leaf number at flowering of *CO:CO:GR*, *co-2* plants treated (+DEX) or not treated (−DEX) with dexamethasone. Plants were grown for 2 weeks in SD conditions and then shifted to LDs for 4 days. Dexamethasone was applied during the LD treatment. **(E** and **F)** *FT* mRNA expression in treated **(E)** and nontreated **(F)** leaves of *CO:CO:GR* plants.

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acts in the meristem to promote gene expression, suggesting that a product of *FT* may be transported to the meristem as the floral stimulus (*6*, *7*, *9*). Experiments indicating that *FT* mRNA comprises the transmissible signal have recently been retracted (*12*). Furthermore, the floral stimulus, but no detectable mRNA of genes similar to *FT*, crossed the junction between grafted tomato plants (*13*). We examined the requirement for *FT* expression in the leaves during floral induction and explored the possibility that FT protein comprises the floral stimulus.

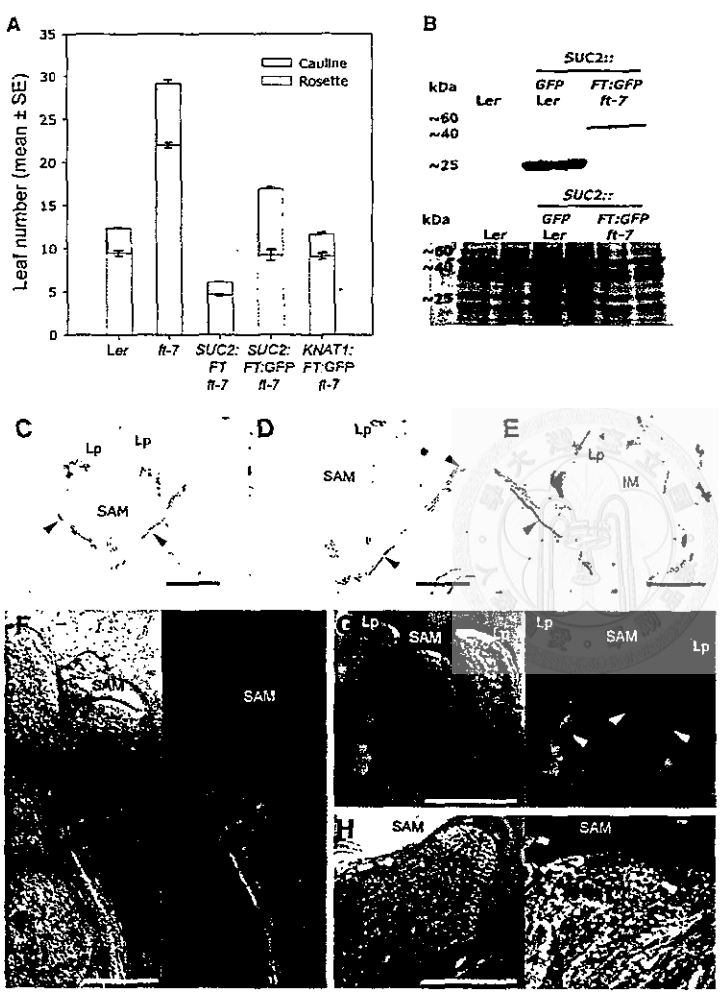

Fig. 2. Analysis of FT:GFP protein distribution in *SUC2:FT:GFP ft-7*. (**A**) Flowering time expressed as total leaf number (rosette and cauline) of representative transformants grown in LDs and compared with *Ler* and *ft-7*. (**B**) Western blot analysis showing expression of the intact FT:GFP fusion protein in *SUC2:FT:GFP ft-7* plants. *SUC2:GFP Ler* and *Ler* were used as positive and negative controls, respectively. The Comassie-stained gel acts as loading control. (**C** and **D**) In situ hybridization of apices of *SUC2:FT:GFP ft-7* plants grown for 8 extended short days (ESDs) (C) and 10 ESDs (D) and probed with a chimeric RNA fragment spanning the junction between *FT* and *GFP* in *FT:GFP*. The hybridization signal is restricted to the mature phloem (arrowheads). (**E**) In situ hybridization of a 12-ESD-old *SUC2:CO co-2* apex probed with *FT*. (**F** to **H**) Confocal analysis of the distribution of the GFP fluorescence produced by the FT:GFP fusion protein in the apical region of *SUC2:FT:GFP ft-7* transgenic plants. Images on the right show GFP signals separated from background emissions. (F) Six-day-old vegetative plant and [(G) and (H)] 10-day-old plant that is induced to flower [fluorescence is detected in the provascular tissue and at the base of the shoot apical meristem (SAM); arrowhead]. In (H), a leaf primordium flanking the SAM was removed to facilitate visualization. Lp, leaf primordium; IM, inflorescence meristem. Scale bars, 50 μm in (C) to (E), (G), and (H); 25 μm in (F).

First, we tested whether stable induction of *FT* expression in the leaves of *Arabidopsis* is required for flowering. *Perilla* leaves exposed to appropriate photoperiods produce the floral stimulus permanently (*14*, *15*). Short day (SD)–grown *Arabidopsis* plants exposed to three LDs and then returned to SDs flowered much earlier than plants exposed only to SDs (*16*) [Fig. 1A and supporting online material (SOM) text]. *FT* expression rises during the first LD after a shift from SDs (*17*). We tested whether this increase is stable by analyzing expression of *CO* and *FT* mRNA every 4 hours for 7 days, covering the shift from SDs to LDs and back to SDs (Fig. 1B and fig. S1A). In control plants grown only in SDs, *FT* mRNA abundance remained low (Fig. 1B). In contrast, in plants exposed to three LDs, *FT* mRNA abundance was increased in each of the three LDs. However, after return to SDs, *FT* mRNA levels fell after 1 day to the low level characteristic of SD-grown plants (Fig. 1B). Therefore, in these conditions, *FT* mRNA expression is not stably maintained after exposure to LDs. However, expression of endogenous *FT* mRNA was increased in the leaves of plants in which *FT* was substantially overexpressed from a transgene (Fig. 1C). We concluded that *FT* mRNA expression at wild-type levels in the leaves for 3 days is sufficient to stably induce flowering at the shoot apical meristem and that under these conditions *FT* expression in the leaves is not maintained.

In some plants, leaves that have not been exposed to inductive day lengths can be indirectly induced to form the floral stimulus. For example, grafting a plant exposed to inductive day lengths to a second noninduced plant can cause the second plant to produce the floral stimulus (*2*, *14*). To test whether *FT* expression is induced indirectly in leaves of *Arabidopsis*, we constructed a fusion of the *CO* promoter to a gene encoding a translational fusion between CO and the rat glucocorticoid receptor binding domain (*CO:CO:GR*), and we introduced this into the *co-2* mutant. In these plants, CO activity is induced by addition of the steroid dexamethasone (dex) only under LDs, during which the *CO* mRNA accumulates in the light (*18*–*20*). Application of dex to a single leaf induced flowering and increased the amount of *FT* mRNA in the leaves to which dex was added (Fig. 1, D to F, and fig. S1C). However, no difference in *FT* mRNA abundance was detected between the untreated leaves of plants treated with dex and similar leaves from untreated plants (Fig. 1F). Therefore, no detectable indirect activation of *FT* mRNA expression occurs in *Arabidopsis* leaves under the inductive conditions used in this experiment, and activation of *FT* in a single leaf is sufficient to induce flowering.

Next, we compared the spatial distribution of *FT* mRNA and protein, exploiting transgenic plants expressing FT and FT fusion proteins from heterologous promoters exclusively in the phloem companion cells, where *CO* and *FT* are expressed in wild-type plants (*6*, *21*). The use of well-characterized

heterologous promoters prevented difficulties associated with the low abundance of *FT* mRNA in the vascular tissue of wild-type plants (*6*, *10*, *11*). The promoter of the *SUCROSE TRANSPORTER 2* (*SUC2*) gene of *Arabidopsis* is active specifically in the phloem companion cells (*22*), whereas the promoter of the *KNAT1* gene is active in the shoot apical meristem, and expression of *FT* from these promoters causes early flowering of *co-2* mutants (*6*). A gene fusion comprising *FT* and *GREEN FLUORESCENT PROTEIN* (*GFP*) was constructed and expressed from the *SUC2*, *FT*, and *KNAT1* promoters. Introduction of *SUC2:FT:GFP*, *KNAT1:FT:GFP*, and *FT:FT:GFP* into *ft-7* mutants caused these plants to flower much earlier than *ft-7*, although slightly later than *SUC2:FT ft-7* or *FT:FT ft-7* (Fig. 2A and fig. S2). Protein was extracted from seedlings of *SUC2:FT:GFP* and *SUC2:GFP* plants and probed with a GFP antibody. The fusion protein was present in *SUC2:FT:GFP* plants, and importantly no free GFP protein was detected (Fig. 2B). Taken together, these results indicate that FT:GFP promotes flowering, although it is slightly less active than the wild-type FT protein.

The spatial distribution of FT:GFP protein and mRNA were then compared in *SUC2:FT:GFP* plants. *FT:GFP* and *FT* mRNAs were strongly detected in the mature phloem tissue where the *SUC2* promoter is active, but no mRNA was detected in the shoot apical meristem or protophloem (Fig. 2, C to E). The distribution of FT:GFP protein was then tested by confocal microscopy. In 6-day-old plants, which had not undergone the transition to flowering, FT:GFP was detected in the vascular tissue of the shoot (Fig. 2F). In 10-day-old plants, which were about to undergo the floral transition and had not yet formed floral primordia, FT:GFP was also detected in the provasculature at the shoot apex and at the base of the shoot apical meristem (Fig. 2, G and H). FT:GFP was detected in provasculature and apical tissues in which FT:GFP mRNA was not detected (compare Fig. 2, D and G). These results suggest that FT:GFP protein moves from the phloem companion cells to the meristem (SOM text). Such movement could occur through symplastic unloading from the phloem into the apical meristem region (*23*).

To test for movement of FT:GFP protein over longer distances, transgenic *SUC2:FT:GFP ft-7* plants were grafted to *ft-7* mutants. Sugars and other contents of the phloem sieve elements are transported from mature leaves down to the root and upward to the shoot apex. First, the aerial parts of *SUC2:FT:GFP* seedlings were grafted to *ft-7* roots. After grafting, FT:GFP protein was detected across the graft junction and in the vasculature of the *ft-7* root stock, which represents a strong sink for contents of the phloem (Fig. 3, A and B). No *FT:GFP* mRNA could be detected in these root stocks by reverse transcription polymerase chain reaction after 40 cycles of amplification (Fig. 3C). A *SUC2:FT:GFP* shoot was then grafted as a donor to

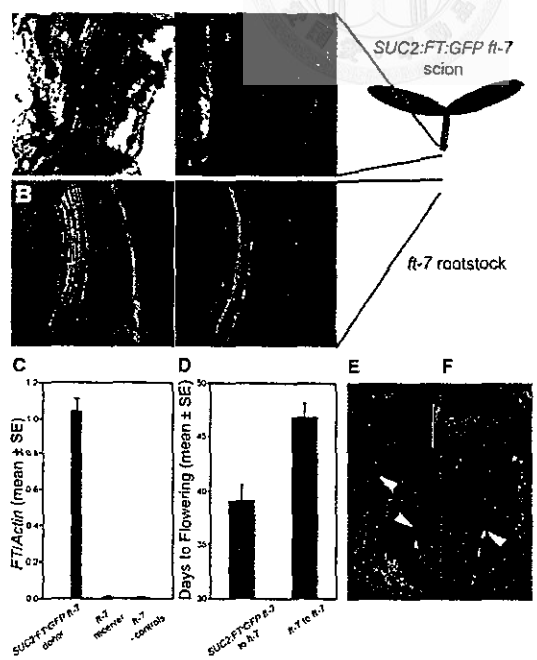

Fig. 3. Grafting of *SUC2:FT:GFP ft-7* plants to *ft-7* mutants. (**A** to **C**) Root grafting: Distribution of the FT:GFP fusion protein and *FT:GFP* mRNA. Confocal analysis of the distribution of FT:GFP fusion protein demonstrates that the protein is able to cross a graft junction (**A**) and can be detected in the vascular bundles of the *ft-7* root stock (**B**). The images on the right in (**A**) and (**B**) show GFP signals separated from background emissions. (**C**) *FT* cDNA amplification from the roots of *SUC2:FT:GFP ft-7* donor plants, *ft-7* root stock (labeled receiver) and *ft-7* controls. No difference was detected between the *ft-7* root stocks and *ft-7* controls. (**D**) Flowering time of *ft-7* mutants grafted to *SUC2:FT:GFP* or to *ft-7* donors. (**E** and **F**) Shoot grafting: Distribution of the FT:GFP fusion protein in the apical region of the *SUC2:FT:GFP ft-7* donor (**E**) and grafted *ft-7* receiver (**F**). The fusion protein can be detected in the vasculature of the donor and receiver (arrowheads).

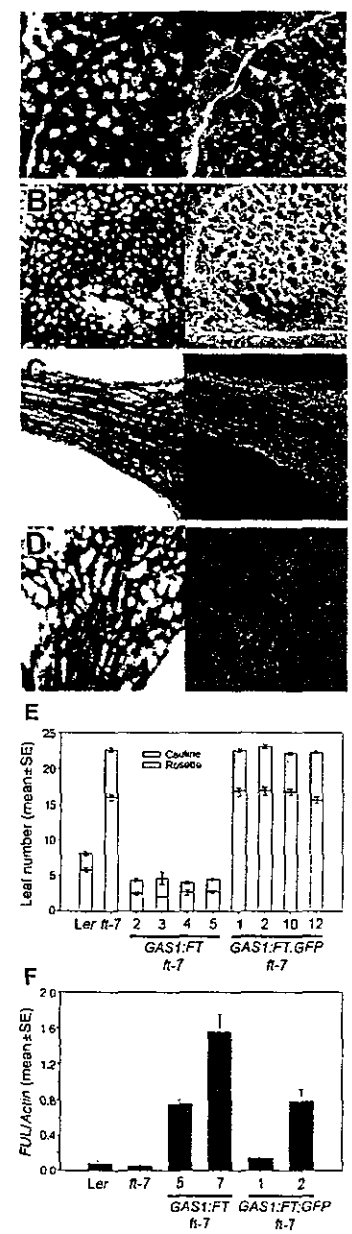

Fig. 4. Expression of FT:GFP in the minor veins alters gene expression patterns but does not induce flowering. (**A** to **D**) Confocal images of leaves expressing *GAS1:FT:GFP:GFP ft-7*. The GFP signal is detected in the minor veins [arrows in (**A**) and (**B**)] but not in the petiole (**C**) or the midrib (**D**). (**E**) Flowering time of *GAS1:FT ft-7* and *GAS1:FT:GFP ft-7* as compared with *Ler* and *ft-7* grown in LDs. (**F**) *FUL* expression in leaves of the same plants.

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an *ft-7* shoot receiver. These receiver shoots flowered slightly earlier than receiver shoots on control grafts (Fig. 3D and fig. S3), as observed previously for grafts of wild-type plants to *ft-7* mutants (*24*), and FT:GFP protein was clearly detected in the vascular tissue of the shoot receiver (Fig. 3, E and F). The grafting experiments support long-distance movement of FT:GFP protein in the phloem.

Two general models could explain the role of FT in floral induction. The first proposes that a product of *FT* expressed in the leaves moves to the meristem and initiates flowering through the activation of flowering-time genes such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) (*7*, *25*, *26*). Our data support movement of the protein. The second model suggests that *FT* expression in the leaves activates a second messenger, which is transmitted to the apex and induces flowering, perhaps through activation of *FT* genes or genes similar to *FT* in the meristem. We refer to this second model as a relay model: FT protein could move along with a second messenger but not comprise a signal.

We used transgenic plants expressing FT and FT:GFP from additional phloem promoters to test the relay model. The *GALACTINOL SYNTHASE* (*GAS1*) promoter is active specifically in the phloem companion cells of the minor veins of leaves (*27*) and not in the companion cells of the shoot or major veins of the leaf. *GAS1:CO* promotes early flowering of *co-1* mutants (*28*). We constructed *GAS1:FT*, *GAS1:FT:GFP*, and *GAS1:FT:GFP:GFP* transgenes and introduced these into *ft-7* mutants. In plants expressing the fusion proteins, GFP was detected only in the minor veins of the leaves (Fig. 4, A to D). *GAS1:FT* complemented the *ft-7* mutation, and the transgenic plants flowered earlier than did wild-type plants (Fig. 4E). However, *GAS1:FT:GFP ft-7* plants were as late flowering as *ft-7* mutants (Fig. 4E). Nevertheless, FT:GFP is biochemically active in the leaves of *GAS1:FT:GFP* plants. Expression of *FRUITFULL* (*FUL*) mRNA is increased in the leaves of transgenic *Arabidopsis* plants that express high levels of *FT* mRNA (*29*). *FUL* mRNA levels were higher in *GAS1:FT ft-7* and *GAS1:FT:GFP ft-7* than in wild-type plants and *ft-7* mutants (Fig. 4F). Thus FT:GFP is active in the leaves of *GAS1:FT:GFP* plants, but in contrast to *GAS1:FT* or *SUC2:FT:GFP*, this construct does not promote flowering. The larger FT:GFP protein may move less effectively to the meristem from the minor veins than from the larger veins in which *SUC2* is also active, or downloading from the companion cells to the minor veins may be differentially regulated compared with downloading to major veins. Thus, FT:GFP activity in the leaves of *GAS1:FT:GFP* plants was not sufficient to promote flowering, arguing for direct movement of an *FT* product to the meristem.

We conclude (i) that during floral induction of *Arabidopsis*, transient expression of *FT* in a single leaf is sufficient to induce flowering and (ii) that in response to *FT* expression, a signal moves from the leaves to the meristem. This signal is unlikely to be a second messenger activated by FT in the leaves given that *GAS1:FT:GFP* is active in leaves but does not promote flowering (Fig. 4). In contrast, we propose that FT protein is transported through the phloem to the meristem. Our data provide evidence for movement of FT:GFP from the phloem companion cells of *SUC2:FT:GFP* plants to the meristem that correlates with flowering, and of FT:GFP protein across graft junctions, consistent with the detection of proteins similar to FT in the phloem of *Brassica napus* plants (*30*). The data in the Report by Tamaki *et al.* (*31*) demonstrate that this function of FT is highly conserved in rice. The presence of a wide range of different proteins in phloem sap suggests that long-distance movement of proteins is the basis of other signaling processes in plants (*23*), in addition to the shorter-distance movement of proteins between neighboring cells (*32*) and previous indications of the importance of long-distance mRNA movement (*33*, *34*).

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Hd3a Protein Is a Mobile Flowering Signal in Rice

Shojiro Tamaki, Shoichi Matsuo, Hann Ling Wong, Shuji Yokoi,* Ko Shimamoto†

Florigen, the mobile signal that moves from an induced leaf to the shoot apex and causes flowering, has eluded identification since it was first proposed 70 years ago. Understanding the nature of the mobile flowering signal would provide a key insight into the molecular mechanism of floral induction. Recent studies suggest that the *Arabidopsis FLOWERING LOCUS T* (*FT*) gene is a candidate for encoding florigen. We show that the protein encoded by *Hd3a*, a rice ortholog of *FT*, moves from the leaf to the shoot apical meristem and induces flowering in rice. These results suggest that the Hd3a protein may be the rice florigen.

The flowering time of plants is determined by a number of environmental factors (*1*–*3*), among which day length (photoperiod) is a major factor (*4*). On the basis of the day length, which promotes flowering, plants are grouped into two major classes: long-day (LD) and short-day (SD) plants. *Arabidopsis* is a LD plant and rice is a SD plant. *FT* is a major floral activator (*5*, *6*), which is expressed in the vascular tissue of leaves (*7*, *8*). FT protein interacts with a transcription factor FD, which is expressed only in the shoot apical meristem (SAM) (*9*, *10*). The difference in expression site implies that FT protein must move to the SAM to interact with FD for flower induction. Therefore, *FT* is a primary candidate for encoding florigen (*11*), a mobile flowering signal.

Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma 630-0101, Japan.

*Present address: Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan.
†To whom correspondence should be addressed. E-mail: simamoto@bs.naist.jp

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