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Rapid paper

Long-Distance, Graft-Transmissible Action of *Arabidopsis* FLOWERING LOCUS T Protein to Promote Flowering

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Day length perceived by a leaf is a major environmental factor that controls the timing of flowering. It has been believed that a mobile, long-distance signal called florigen is produced in the leaf under inductive day length conditions, and is transported to the shoot apex where it triggers floral morphogenesis. Grafting experiments have shown that florigen is transmissible from a donor plant that has been subjected to inductive day length to an uninduced recipient plant. However, the nature of florigen has long remained elusive. *Arabidopsis FLOWERING LOCUS T* (*FT*) is expressed in cotyledons and leaves in response to inductive long days (LDs). FT protein, with a basic region/leucine zipper (bZIP) transcription factor FD, acts in the shoot apex to induce target meristem identity genes such as *APETALA1* (*AP1*) and initiates floral morphogenesis. Recent studies have provided evidence that the FT protein in *Arabidopsis* and corresponding proteins in other species are an important part of florigen. Our work shows that the *FT* activity, either from overexpressing or inducible transgenes or from the endogenous gene, to promote flowering is transmissible through a graft junction, and that an FT protein with a T7 tag is transported from a donor scion to the apical region of recipient stock plants and becomes detectable within a day or two. The sequence and structure of mRNA are not of critical importance for the long-distance action of the *FT* gene. These observations led to the conclusion that the FT protein, but not mRNA, is the essential component of florigen.

Keywords: *Arabidopsis* — Flowering — Florigen — FT — Graft — Long-distance signal.

Abbreviations: bZIP, basic region/leucine zipper; CBB, Coomassie brilliant blue; CL, continuous light; EGFP, enhanced green fluorescent protein; FT, FLOWERING LOCUS T; GFP, green fluorescent protein; GUS, β-glucuronidase; Hd3a, Heading date 3a; HSP, heat-shock protein; LD, long day; ORF, open reading frame; 35S, cauliflower mosaic virus 35S RNA promoter; SAM, shoot apical meristem; SD, short day; TFL1, TERMINAL FLOWER 1; UTR, untranslated region; ZT, zeitgeber time.

Introduction

Seasonal flowering is an important adaptive trait in plants that has direct consequences on reproductive success. One of the most important environmental factors that influence the timing of flowering is the change in day length. Plants monitor day length in the leaf to anticipate upcoming seasonal changes and initiate floral morphogenesis at the shoot apex (Imaizumi and Kay 2006). It has been believed that upon perception of a favorable or inductive day length, a mobile, long-distance signal called florigen is produced in leaves, and is then transported to the shoot apex where it triggers floral morphogenesis (Chailakhyan 1937, Zeevaart 1976). Classical experiments have suggested that florigen is transmissible from a donor plant that has been subjected to inductive day length to an uninduced recipient plant through a graft junction (Zeevaart 1976). Grafting experiments involving a variety of plants in terms of day length response have provided strong support for the existence of florigen and, in some cases, evidence for a common florigen among various species (Zeevaart 1976). However, despite efforts over many years, the nature of florigen has remained elusive, until recently.

FLOWERING LOCUS T (*FT*) is a potent promoter of flowering with a conserved role in the long-day (LD) plants *Arabidopsis* (Kardailsky et al. 1999, Kobayashi et al. 1999), wheat and barley (Yan et al. 2006) and possibly aspen (Böhlenius et al. 2006), and the short-day (SD) plants rice

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(Kojima et al. 2002) and Japanese morning glory (Hayama et al. 2007), as well as day-neutral plants such as tomato (Lifschitz et al. 2006). In *Arabidopsis*, transcription of *FT* is induced by LDs (Kardailsky et al. 1999, Kobayashi et al. 1999, Samach et al. 2000, Suárez-López et al. 2001, Yanovsky and Kay 2002) in the phloem tissues of cotyledons and leaves, but not in the shoot apex (Takada and Goto 2003, Yamaguchi et al. 2005). *FT* encodes a 20 kDa protein with homology to the mammalian protein called phosphatidylethanolamine binding protein (PEBP) or Raf kinase inhibitor protein (RKIP) (Kardailsky et al. 1999, Kobayashi et al. 1999, Ahn et al. 2006). The FT protein interacts with a basic region/leucine zipper (bZIP) transcription factor FD that is preferentially expressed in the shoot apex and activates transcription of meristem identity genes such as *APETALA1* (*AP1*) and *FRUITFULL* (*FUL*) (Abe et al. 2005, Wigge et al. 2005). *FT* ectopically expressed in the whole region or the outermost cell layer of the shoot apical meristem (SAM) can fully rescue the phenotype of *ft*, *ft; lfy* and *co* mutants (An et al. 2004, Abe et al. 2005). Inhibition of FT protein activity, primarily in the nucleus by a specific inhibitor protein FWA in the shoot apex, but not in the vasculature, delays flowering (Ikeda et al. 2007). These observations indicate that the FT protein acts in the shoot apex (Abe et al. 2005, Wigge et al. 2005, Ikeda et al. 2007), and led to the proposal that FT protein and/or mRNA are transported from cotyledons and leaves to the shoot apex (Abe et al. 2005, Wigge et al. 2005). A report of the failure to detect the transfer of mRNA of the tomato *FT* ortholog, *SINGLE FLOWER TRUSS* (*SFT*), through a graft junction (Lifschitz et al. 2006) and recent retraction of a paper that had demonstrated the transport of *FT* mRNA from the leaf to the shoot apex (Böhlenius et al. 2007) have made mRNA an unlikely candidate for the mobile signal, although positive disproof of the mRNA hypothesis still remains necessary.

Recent studies from several laboratories have provided strong evidence that the FT protein in *Arabidopsis* and corresponding proteins in other species are an important part of the mobile signal that promotes flowering. Transgenic plants expressing FT:green fluorescent protein (GFP) and Heading date 3a (Hd3a):GFP fusion proteins in the vascular tissues in *Arabidopsis* and rice, respectively, were used to demonstrate the presence of the respective proteins, observed as GFP fluorescence, in the shoot apex or its vicinity (Corbesier et al. 2007, Tamaki et al. 2007). Similar results have been obtained with transgenic *Arabidopsis* expressing Myc-tagged FT protein in the vasculature (Jaeger and Wigge 2007). The presence of FT proteins in the phloem sap has been unambiguously demonstrated in the cucurbits (Lin et al 2007). Also, transmission of FT:GFP protein through a graft junction from donor transgenic plants expressing the fusion protein in the vasculature to recipient *ft* plants was demonstrated, with concomitant promotion of flowering in the recipient plants (Corbesier et al. 2007). These results, considered together, led to the current view of FT protein transport from the leaf to the shoot apex via the phloem. However, key observations in *Arabidopsis* were based on the effect of cumulative, supposedly constitutive expression in the phloem tissues by the *SUCROSE TRANSPORTER 2* (*SUC2*) promoter from the young seedling stage to the time of the observation, thereby making analysis and interpretation of the temporal aspects of the transport difficult. Furthermore, while *FT* expression is restricted to minor veins in the leaf blade (Takada and Goto 2003, Yamaguchi et al. 2005), the *SUC2* promoter has strong activity near the end of the vasculature within a short distance from the shoot apex (see An et al. 2004, Corbesier at al. 2007, Jaeger and Wigge 2007). This makes it difficult to exclude the possibility of short-distance, cell-to-cell transport of the protein without entering into the phloem. In the case of rice, these problems were largely avoided by the use of *Hd3a*'s own promoter as well as the *rolC* promoter which has strong activity in the phloem (Tamaki et al. 2007). In addition, with the reputation of GFP for its ability for long-distance transport via phloem (Imlau et al. 1999) in mind, it seems that independent demonstrations based on approaches other than GFP fusion proteins are desirable.

Using a micrografting technique and a transient induction system, we show that the activity of *FT*, either from overexpressed or inducible transgenes, or from endogenous genes, to promote flowering is transmissible through a graft junction, and that FT protein with a T7 tag is transported from the donor scion to the apical region of the recipient stock plants and becomes detectable within a short period of a day or two. We also show that *FT* mRNA is available for translation in the vascular tissue and the sequence and structure of mRNA is not critically important for the long-distance action of the *FT* gene. Evidence against the possibility of autoregulation of *FT* expression, which may contribute to signal amplification, and of a relay mechanism in which *FT* acts as an indirect signal, is also presented.

Results

FT mRNA can act cell-autonomously in phloem tissues without autoregulation

To confirm that the *FT* transcripts, through translation, act cell-autonomously in the phloem tissues, we made a reporter construct in which the stop codon in a 10.9 kb genomic fragment of the *FT* locus was replaced with an open reading frame (ORF) of β-glucuronidase (GUS) to express an FT:GUS fusion protein (*gFT::GUS* AY#1) at the site of transcription and translation (Supplementary

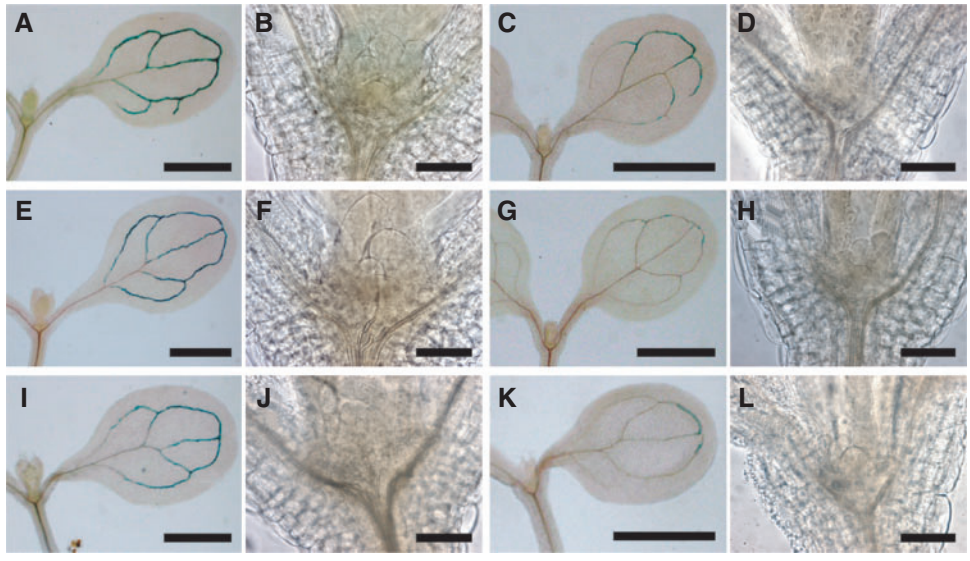

Fig. 1 Sites of transcription and translation of *FT* and absence of autoregulation. Expression of *pFT::GUS* (A–D, I–L) and *gFT::GUS* (E–H) in plants grown in LD (A, B, E, F, I and J) or SD (C, D, G, H, K and L) for 5 d. *pFT::GUS* is in either the wild-type (A–D) or *35S::FT* (YK#11-1) (I–L) background. Plants were fixed at ZT15 in LD or ZT7 in SD conditions, when *FT* mRNA levels are highest in the respective day length conditions. B, D, F, H, J and L are higher magnification images of the shoot apical region of A, C, E, G, I and K, respectively. Scale bars, 1 mm (A, C, E, G, I and K) and 0.1 mm (B, D, F, H, J and L).

Fig. S1 online). In *gFT::GUS* seedlings grown in LDs, GUS staining patterns were similar to the patterns of GUS expression under the control of the *FT* promoter [*pFT::GUS* (Takada and Goto 2003), see Supplementary Fig. S1] (Fig. 1A–H). This indicates that the *FT* transcript, with an inserted GUS ORF, can act cell-autonomously and is available for translation in the phloem tissues of cotyledons and leaves. No GUS staining was observed in the shoot apical region in *gFT::GUS* seedlings as in *pFT::GUS* seedlings (Fig. 1B, D, F, H; Takada and Goto 2003, Yamaguchi et al. 2005), suggesting the absence of expression (transcription and/or translation) in the shoot apex. Comparison of the *pFT::GUS* expression in wild-type and *FT*-overexpressing (by the cauliflower mosaic virus 35S (35S) promoter, *35S::FT*) backgrounds showed no ectopic induction of *pFT::GUS* expression, either in cotyledons and leaves or in the shoot apex, by *FT* ectopic overexpression (Fig. 1I–L compared with Fig. 1A–D). This suggests the absence of an autoregulatory loop of *FT* expression in either cotyledons and leaves, or in the shoot apex. As described below, experiments using a transient *FT* induction system also supported the absence of autoregulation (Fig. 8D).

Activity of the endogenous FT gene to promote flowering is graft-transmissible

It has recently been reported that the activity to promote flowering by overexpressed *SFT* in tomato and phloem-expressed *FT:GFP* in *Arabidopsis*, respectively, is transmissible through a graft junction (Lifschitz et al. 2006, Corbesier et al. 2007). We investigated further to determine if the activity of the endogenous *FT* gene to promote flowering is transmitted from a wild-type donor scion to a recipient *ft* mutant stock and partially rescues the late-flowering phenotype of the *ft* stock. Y-shaped grafts, in which scion and stock shoots are of equal size, were assembled on the hypocotyl of 4-day-old seedlings (Supplementary Fig. S2), and the flowering time of the stock plants was observed in successful grafts. We first confirmed that *ft-1* stock plants with a transgenic scion that overexpresses *FT* (*35S::FT* YK#11-1; Kobayashi et al. 1999) flowered earlier than *ft-1* stock plants with an *ft-1* scion (Fig. 2A, C, G, Supplementary Table S1). Grafting of a wild-type scion also resulted in small but significant promotion of flowering in *ft-1* stock plants (Fig. 2A, B, Supplementary Table S1), suggesting that the activity of the endogenous *FT* gene to promote flowering is transmitted through a graft junction. This is in contrast to tomato *SFT*, where grafting of a wild-type donor does not rescue *sft* mutant recipients (Lifschitz et al. 2006). No promotion of flowering was observed in *ft-1* stock plants with an *ft-1* scion, as compared with intact *ft-1* plants (Fig. 2A, D, Supplementary Table S1), indicating that grafting *per se* does not cause accelerated flowering.

In the next set of experiments, transgenic plants with an inducible *FT* transgene were used as donor scions. Transgenic *ft-1* plants which express FT protein with a T7 peptide added to the C-terminus (FT-T7) under control of the *Arabidopsis HEAT-SHOCK PROTEIN 18.2* (*HSP18.2*) promoter (Takahashi and Komeda 1989) (*HSP::FT-T7*;

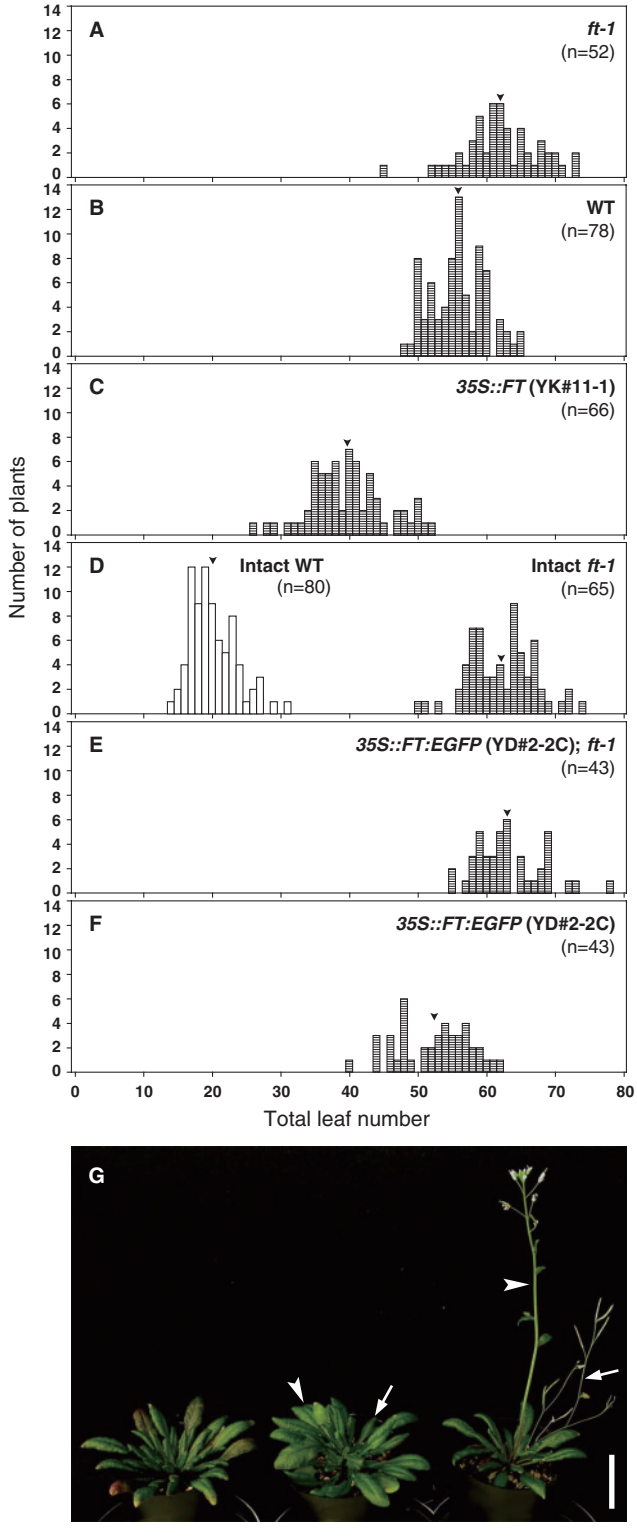

Fig. 2 Graft-transmissible action of the endogenous *FT* gene and *35S::FT* and *FT:EGFP* transgenes. Distribution of flowering time of *ft-1* recipient stock plants with a scion of (A) *ft-1*, (B) wild type (WT), (C) *35S::FT* (YK#11-1), (E) *35S::FT:EGFP* (YD#2-2C); *ft-1* and (F) *35S::FT:EGFP* (YD#2-2C), respectively, and (D) intact *ft-1* and WT plants. An arrowhead in each graph shows the average of the population. $P<0.0001$ (Student's *t*-test) for A vs. B, $P<0.0001$ for A vs. C, $P<0.0001$ for B vs. C, $P>0.1$ for A vs. D, $P>0.1$ for A vs. E, $P<0.0001$ for B vs. F. Statistics of the data are summarized in Supplementary Table S1. (G) A representative set of grafts. From left to right: an intact *ft-1* plant, an *ft-1* stock plant with an *ft-1* scion, and an *ft-1* stock plant with a *35S::FT* scion at the same age (52 d after surgery or equivalent). Arrows indicate an *ft-1* scion (middle) and a flowering *35S::FT* scion (right). Arrowheads indicate the stock *ft-1* plants, still in vegetative phase (middle) and flowering (right). Scale bar, 3 cm.

Supplementary Fig. S3) were grafted onto *ft-1* stock plants. Twenty-six days after the graft surgery, successful grafts were selected and subjected twice to a 2.5-h heat treatment at a 24-h interval to induce pulsed *FT* expression. Intact *ft-1* and *HSP::FT-T7* (MA#2); *ft-1* plants at a comparable age were subjected to the same heat treatment for comparison. Heat-treated intact *HSP::FT-T7; ft-1* plants flowered significantly earlier than untreated *HSP::FT-T7; ft-1* plants. In contrast, there were no differences in the flowering times between untreated and treated *ft-1* plants, or untreated *HSP::FT-T7; ft-1* and untreated *ft-1* plants (Supplementary Table S2), indicating that the heat treatment or presence of the transgene *per se* does not cause accelerated flowering. Heat treatment of grafts with an *HSP::FT-T7; ft-1* scion resulted in accelerated flowering in both *HSP::FT-T7; ft-1* scions and *ft-1* stocks, as compared with untreated intact *HSP::FT-T7; ft-1* plants and *ft-1* stocks of heat-treated *ft-1*/*ft-1* grafts, respectively (Fig. 3A–C, Supplementary Table S2). These results indicate that the effects of transient induction of the *FT* activity in the donor scion are transmissible through a graft junction. Furthermore, a weak correlation [$r=0.59$; $P<0.0001$ (*t*-test)] was observed between the total leaf number (an indicator of the flowering time) of the recipient *ft-1* stock and that of the donor *HSP::FT-T7; ft-1* scion on the same grafts (Fig. 3D). In contrast, no correlation [$r=0.13$; $P>0.4$ (*t*-test)] was observed in the number of leaves between the donor *ft-1* scion and the recipient *ft-1* stock on the same grafts in the control experiments (Fig. 3D). A similar relationship between the strength of the donor scion and the degree of rescue in the recipient *ft-1* stocks was observed by comparing the results with *35S::FT* (YK#11-1), *35S::FT* (YK#1-5C) and the wild type as a scion (Figs. 2, 7).

Transport of FT protein from the donor stock to the shoot apical region of the recipient scion through a graft junction within a short period

Evidence in support of transmission of FT:GFP fusion protein through a graft junction was recently reported (Corbesier et al. 2007). We further tested the long-distance transport of FT protein from the donor stock to the shoot apical region of the recipient scion through a graft junction.

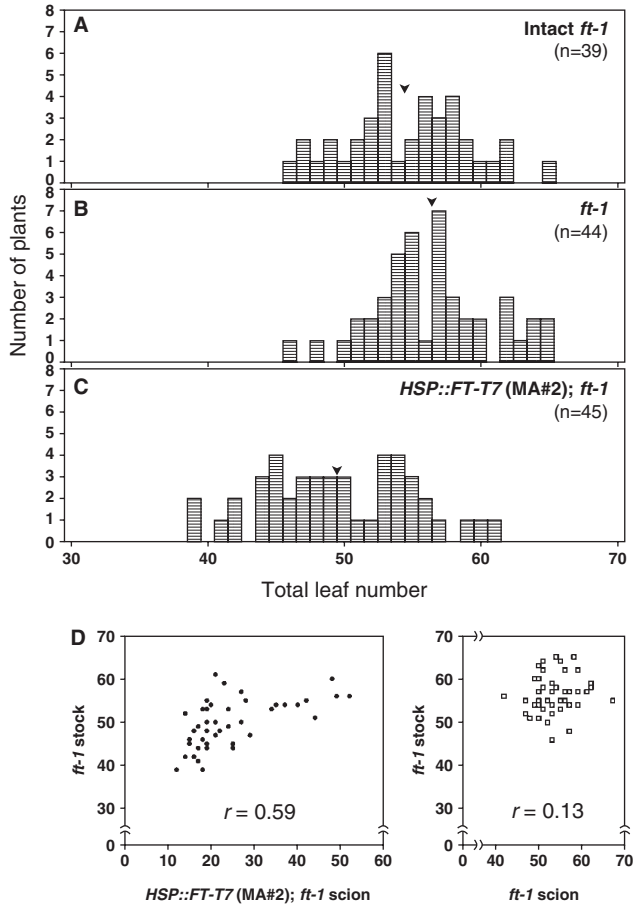

Fig. 3 Graft-transmissible action of the *HSP::FT-T7* transgene upon heat treatment. Distribution of the flowering time of (A) heat-treated, intact *ft-1* plants, (B) *ft-1* recipient stock plants of heat-treated *ft-1/ft-1* grafts and (C) *ft-1* recipient stock plants of heat-treated *HSP::FT-T7* (MA#2); *ft-1/ft-1* grafts. An arrowhead in each graph shows the average of the population. $P > 0.03$ (Student's *t*-test) for A vs. B, $P < 0.0001$ for A vs. C. Additional data and statistics of the data are summarized in Supplementary Table S2. (D) Relationship between the total leaf number of the donor scion plants and that of the recipient stock plants in heat-treated *HSP::FT-T7; ft-1/ft-1* grafts (left) and heat-treated *ft-1/ft-1* grafts (right). The linear correlation coefficient (*r*) is shown in each graph.

The T7 peptide was chosen as a tag rather than GFP, for two reasons. First, it is likely that the small T7 tag interferes with the FT protein function much more weakly than does a GFP tag. Secondly, GFP itself has a reputation for long-distance transport via phloem (Imlau et al. 1999). Transgenic plants overexpressing FT-T7 protein in the *ft-1* background (*35S::FT-T7* YD#2; *ft-1*) were grafted onto *ft-1* stocks in a Y-shaped graft. Grafting of *35S::FT-T7; ft-1* resulted in partial rescue of the late-flowering phenotype of the *ft-1* stock plants (Table 1), confirming that the activity of the *35S::FT-T7* transgene to promote flowering is transmitted through a graft junction. To detect the transported FT-T7 protein, shoot apical regions (see Materials and Methods) of the *ft-1* stock plants of successful grafts were collected 4 weeks after the graft surgery, by which time functional continuity of the phloem, as judged from the vascular *rolC::GUS* expression pattern and trafficking of fluorescent dyes and enhanced GFP (EGFP), was firmly established in our experimental conditions (see Materials and Methods; M. Notaguchi, Y. Daimon, M. Abe and T. Araki, submitted). Floral transition of the *ft-1* stock plants was observed much later (corresponding to the sixth or seventh week). To circumvent equivocal results caused by cross-reaction of the antibody with unrelated proteins observed in the preliminary experiments, two-dimensional PAGE was performed for protein separation. As expected, a spot corresponding to the expected size (21,161 Da) and pI (7.75) of the FT-T7 protein, which was not observed in intact *ft-1* plants, was detected in the extract of the donor *35S::FT-T7; ft-1* scions (Fig. 4A). A spot of the same relative position was detected in the extract of shoot apices from the recipient *ft-1* stock plants (Fig. 4A).

Next, to investigate temporal aspects of the transport, *HSP::FT-T7; ft-1* plants were grafted onto *ft-1* stock plants. Twenty-six days after the graft surgery, the whole grafts were subjected twice to 2.5-h heat treatments at a 24-h interval to induce pulsed *FT* expression, and the shoot apical regions of the *ft-1* stock plants were collected 24 h after the second heat treatment was completed. As described above, this treatment resulted in significant acceleration of flowering of the *ft-1* stocks with an *HSP::FT-T7; ft-1* scion (Fig. 3, Supplementary Table S2), indicating that the treatment was effective in causing the generation and transport of a sufficient amount of the floral stimulus. A spot corresponding to the FT-T7 protein was detected in the extract of shoot apices from the *ft-1* stock plants (Fig. 4B). These results suggest that the FT-T7 protein can be transported from the donor transgenic scion to the shoot apical region of the recipient stock plants through a graft junction within 24–48 h after the induced expression.

Table 1 Graft-transmissible promotion of flowering by *35S::FT-T7*

Graft combination, scion/stock	Total leaf number, average ± SD (range)[a]	*n*	Student's *t*-test, *P*-value[b]
ft-1/ft-1	63.2 ± 4.2 (55–73)	25	–
35S::FT-T7 (YD#2); *ft-1/ft-1*	48.6 ± 6.2 (36–56)	18	8.5×10^{-12}

Plants were grown under CL on soil.
[a]Total leaf number of *ft-1* recipient stock plants.
[b]Student's *t*-test with *ft-1/ft-1*.

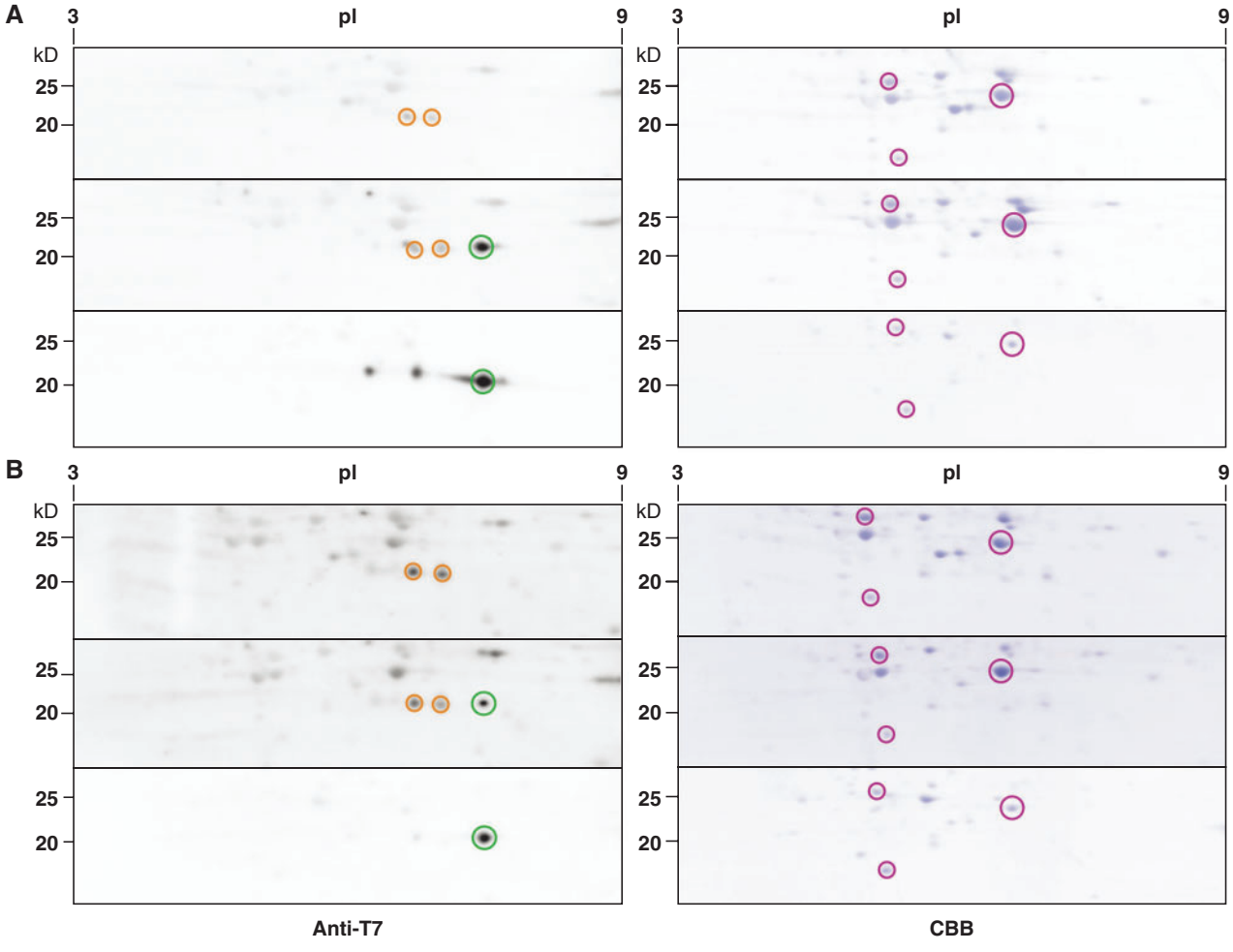

Fig. 4 Detection of transported FT-T7 protein in the shoot apex of recipient *ft-1* stock plants. (A) Detection of FT-T7 protein in shoot apices of the recipient *ft-1* stock plants of *35S::FT-T7* (YD#2); *ft-1*/*ft-1* grafts. Proteins from 30 shoot apices of intact *ft-1* plants (top panel), 30 shoot apices of the recipient *ft-1* stock plants of *35S::FT-T7* (YD#2); *ft-1*/*ft-1* grafts (middle panel) and aerial parts (equivalent of a tenth of a plant) of *35S::FT-T7* (YD#2) scions (bottom panel), respectively, were separated. (B) Detection of FT-T7 protein in shoot apices of the recipient *ft-1* stock plants of heat-treated *HSP::FT-T7* (MA#2); *ft-1*/*ft-1* grafts. Proteins from 30 shoot apices of intact *ft-1* plants (top panel), 30 shoot apices of the recipient *ft-1* stock plants of heat-treated *HSP::FT-T7* (MA#2); *ft-1*/*ft-1* grafts (middle panel) and aerial parts (equivalent of a tenth of a plant) of heat-treated *HSP::FT-T7* (MA#2); *ft-1* scions (bottom panel), respectively, were separated. Left column (anti-T7), immunoblot with anti-T7 antibody; right column (CBB), Coomassie brilliant blue staining. Only a lower molecular weight portion of two-dimensional polyacrylamide gels with the whole width for isoelectric focusing is shown. Green circles indicate the FT-T7 spot. Orange and purple circles depict reference spots for precise alignment of the gel images.

In accordance with previous reports (Lifschitz et al. 2006, Corbesier et al. 2007), *FT-T7* mRNA was not detected in the shoot apical region of the *ft-1* stock plants with a scion of *35S::FT-T7; ft-1* or *HSP::FT-T7; ft-1* (Supplementary Fig. S4A, B).

Long-distance action of FT to promote flowering does not depend on mRNA sequence

To test the importance of mRNA *per se* for long-distance action, we investigated whether an extensive sequence and structural alteration of mRNA affects the ability of the long-distance action of *FT* to promote flowering. The sequence and structure of *FT* mRNA were extensively altered without affecting the translated protein sequence by introducing synonymous substitutions in 171 of 175 codons in a single construct according to the 'resurrection method' (Mori and Dohi 2005) and replacing 5′- and 3′-untranslated regions (UTRs) with foreign sequences (*synonymous FT*, *synFT*) (Fig. 5A, Supplementary Fig. S3). This alteration resulted in only 63.8% sequence identity between *FT* and *synFT* ORFs (Fig. 5A). This is roughly comparable with the 60.5% sequence identity between *FT*

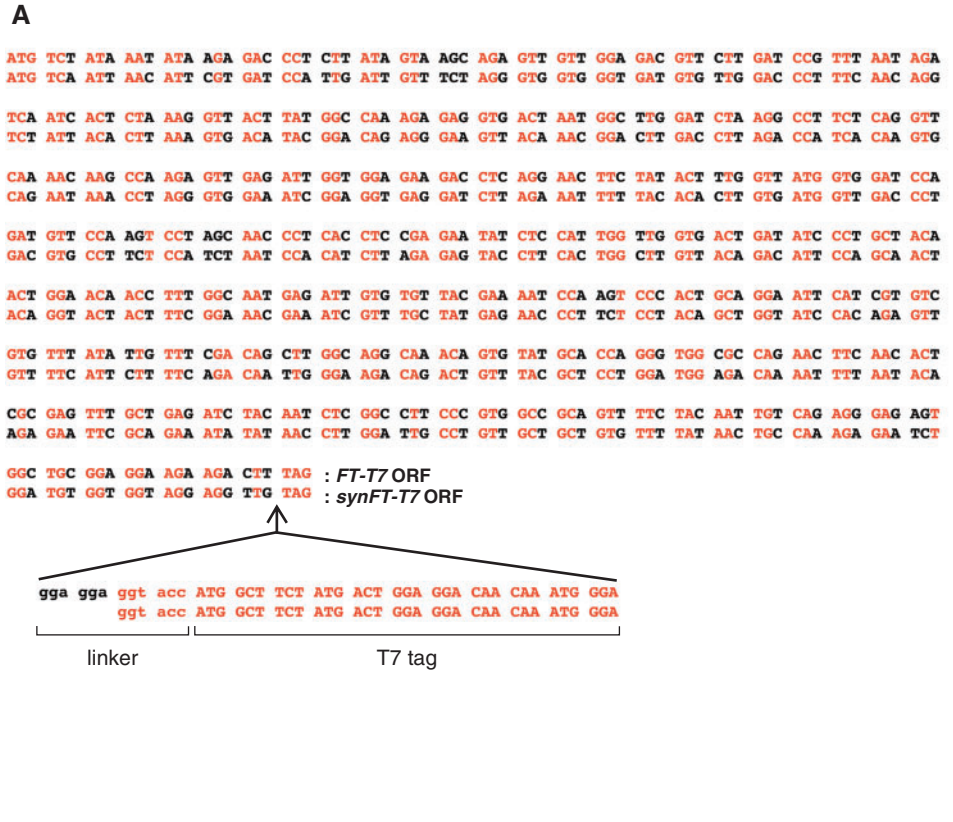

Fig. 5 Sequence and predicted secondary structure of *FT-T7* and *synFT-T7* ORFs. (A) ORF sequences of *FT* (upper) and *synFT* (lower) with sequences for the T7 tag and a linker are shown. Black letters indicate positions with different nucleotides, red letters indicate positions with an identical nucleotide. (B, C) Predicted secondary structures with the lowest free energy for (B) *FT* ORF ($\Delta G = -207.37$ kcal mol^{-1}) and (C) *synFT* ORF ($\Delta G = -196.53$ kcal mol^{-1}). Prediction was performed using an mfold (version 3.2) program available at http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi. which utilizes a free energy minimization algorithm (Zuker 2003).

and its antagonistic homolog, *TERMINAL FLOWER 1* (*TFL1*) (Bradley et al. 1997, Kardailsky et al. 1999, Kobayashi et al. 1999), which presumably acts in a short distance within the shoot apex (Conti and Bradley 2007). The *FT* and *synFT* ORFs share at most 5-bp matches (Fig. 5A), whereas *FT* and *TFL1* ORFs share much longer matches. As expected, predicted secondary structures with the lowest free energy were quite different between the *FT* ORF and the *synFT* ORF (Fig. 5B, C). To monitor protein accumulation, FT was expressed as a T7-tagged protein in both the *FT* and *synFT* constructs (*FT-T7* and *synFT-T7*) (Fig. 5A, Supplementary Fig. S3). Since synonymous substitutions can result in different protein conformations and activities by affecting translational efficiency and peptide chain folding (Kimchi-Sarfaty et al. 2007), it was confirmed that the *synFT-T7* is functional by complementation of *ft-1* (Table 2). The abilities for long-distance action of these modified versions of *FT* were tested in grafting experiments.

We examined whether a *35S::synFT-T7* scion promotes flowering of *ft-1* stock plants. To exclude the effect of the endogenous wild-type *FT* gene (see Fig. 2A, B, Supplementary Table S1), *35S::synFT-T7* in the *ft-1* mutant background was used as a scion so that the *FT* activity was provided only from the *synFT-T7* transgene. Transgenic lines of *35S::synFT-T7; ft-1* and *35S::FT-T7; ft-1*, with similar levels of FT-T7 protein accumulation, were chosen for comparison (Fig. 6D). Grafting of a *35S::synFT-T7* (MA#7); *ft-1* scion promoted flowering of the *ft-1* stock plants to a similar degree as the *35S::FT-T7* (YD#7); *ft-1* scion (Fig. 6A–C, Supplementary Table S3). The same results were obtained in another experiment with a larger number of grafts using *35S::synFT-T7* (MA#4); *ft-1* and *35S::FT* (YK#1-5C) as a scion (Fig. 7A–C, Supplementary Table S4). These results indicate that extensive alteration of the mRNA sequence and structure does not abolish the graft-transmissible action of the *FT* gene. As expected, *synFT-T7* mRNA was not detected in the shoot apical region of the *ft-1* stock plants with a *35S::synFT-T7; ft-1* scion (Supplementary Fig. S4C).

The ability for long-distance action of the *synFT-T7* was further confirmed by examining the effect of local transient induction of a *synFT-T7* expression in a single leaf blade. Using a heat shock-inducible *HSP18.2* promoter,

Table 2 Flowering times of *35S::synFT-T7* transgenic plants

Genotype	No. of rosette leaves, average ± SD (range)	No. of cauline leaves, average ± SD (range)	*n*
Wild type	12.0 ± 1.5 (10–15)	2.6 ± 0.9 (1–4)	15
35S::synFT-T7 (MA#2); *ft-1*	3.0 ± 0.3 (2–4)	1.0 ± 0 (1)	18
35S::synFT-T7 (MA#4); *ft-1*	2.4 ± 0.5 (2–3)	0.9 ± 0.6 (0–2)	22
35S::synFT-T7 (MA#7); *ft-1*	2.4 ± 0.5 (2–3)	0.7 ± 0.5 (0–1)	19
35S::synFT-T7 (MA#8); *ft-1*	4.2 ± 0.5 (3–5)	0.8 ± 0.4 (0–1)	16

Plants were grown under LD on soil.

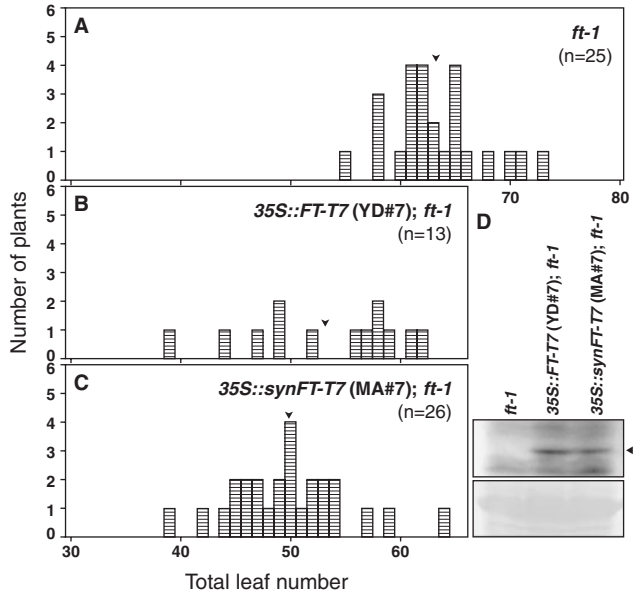

Fig. 6 Graft-transmissible action of *35S::FT-T7* and *35S::synFT-T7* transgenes with comparable FT-T7 protein levels. Distribution of the flowering time of *ft-1* recipient stock plants with a scion of (A) *ft-1*, (B) *35S::FT-T7* (YD#7); *ft-1* and (C) *35S::synFT-T7* (MA#7); *ft-1*. An arrowhead in each graph shows the average of the population. $P < 0.0001$ (Student's *t*-test) for A vs. B, $P < 0.0001$ for A vs. C. Statistics of the data are summarized in Supplementary Table S3. (D) Comparison of levels of FT-T7 protein accumulation in *35S::FT-T7* (YD#7); *ft-1* and *35S::synFT-T7* (MA#7); *ft-1*. Immunoblot with anti-T7 antibody (upper panel) and Ponceau S staining (lower panel). An arrowhead indicates FT-T7 protein.

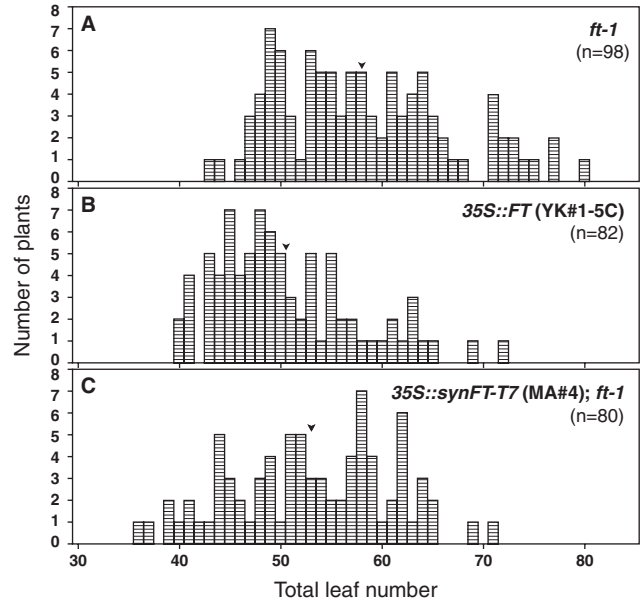

Fig. 7 Graft-transmissible action of *35S::FT* and *35S::synFT-T7* transgenes. Distribution of flowering time of *ft-1* recipient stock plants with a scion of (A) *ft-1*, (B) *35S::FT* (YK#1-5C) and (C) *35S::synFT-T7* (MA#4); *ft-1*. An arrowhead in each graph shows the average of the population. $P < 0.0001$ (Student's *t*-test) for A vs. B, $P < 0.0001$ for A vs. C. Statistics of the data are summarized in Supplementary Table S4.

a local transient induction system similar to the one originally reported in a recently retracted paper (see Böhlenius et al. 2007) was developed (Supplementary Fig. S5, Fig. 8A–C). Transient transcriptional induction of *synFT-T7* and accumulation of the FT-T7 protein after heat treatment were confirmed (Fig. 8D, Supplementary Fig. S6). Consistent with the observations of no effect of *35S::FT* on the *pFT::GUS* expression described above (Fig. 1A–D, I–L), induction of expression of the endogenous *FT* was not observed by transient expression of FT-T7 protein from the *HSP::synFT-T7* transgene (Fig. 8D).

Three-week-old SD-grown plants carrying the *HSP::FT-T7*, *HSP::synFT-T7* or *HSP::GUS* transgene were subjected to the single-leaf heat treatment. Flowering was not observed during the first week after the treatment, but was observed in *HSP::FT-T7* and *HSP::synFT-T7* plants 2 weeks after the treatment, while untreated plants and treated *HSP::FT:GUS* plants remained vegetative (Fig. 8F, G). Heat-treated *HSP::FT-T7* and *HSP::synFT-T7* lines with similar levels of FT-T7 protein induction [*HSP::FT-T7* (MA#2 and MA#20), *HSP::synFT-T7* (MA#2 and MA#9); Fig. 8E] flowered with a similar number of leaves (Fig. 8H). This, taken together with the results of the grafting experiments, suggests that *synFT-T7* is as effective as *FT-T7* in the long-distance action to promote flowering.

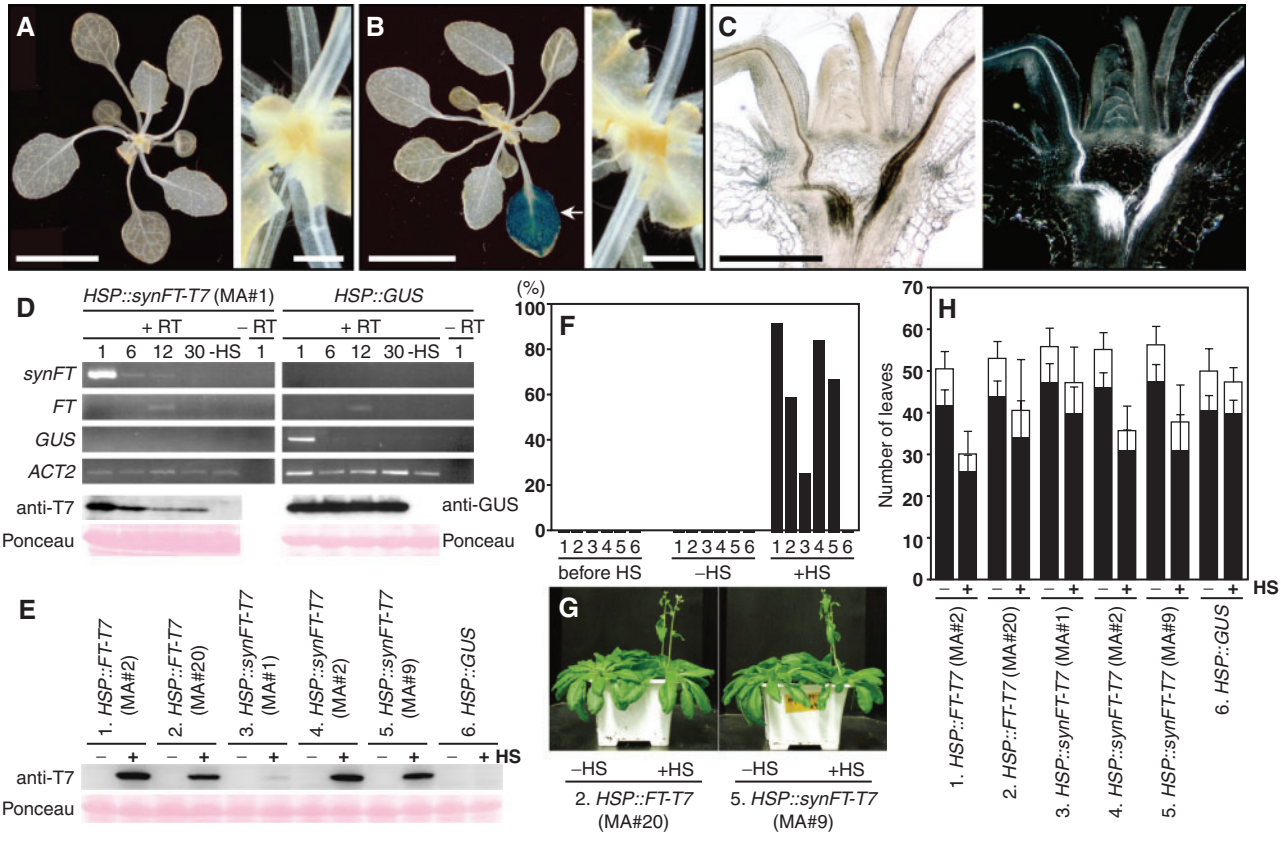

Fig. 8 Long-distance action of locally induced *FT-T7* and *synFT-T7*. (A–C) Local induction of the HSP promoter activity by single-leaf heat treatment. GUS staining of untreated (A) or treated (with subsequent incubation at 22°C for 1 d in SD conditions) (B) plants of 3-week-old *HSP::FT:GUS* (AY#12). The white arrow in (B) indicates the treated leaf. Whole plants (left panels) and higher magnification images of the shoot apical region (right panels). (C) Section of the shoot apex of the plant shown in (B) viewed in bright (left) and dark (right) field. Scale bars, 1 cm (A and B, left panel), 1 mm (A and B, right panel) and 0.5 mm (C). (D) Transient induction of *synFT-T7* and GUS expression in *HSP::synFT-T7* and *HSP::GUS* transgenic lines after whole-plant heat treatment ('HS'). RNA and proteins were extracted from treated plants at the indicated times after the treatment or untreated plants at the time corresponding to 30 h after the treatment. Accumulation of *synFT*, endogenous *FT* and *GUS* mRNA, and FT-T7 and GUS proteins was analyzed. *ACTIN 2* (*ACT2*) was amplified for reference. Note that 12 h after HS corresponds to ZT14 when expression of the endogenous *FT* is near its peak level. (E) FT-T7 protein accumulation in *HSP::synFT-T7* and *HSP::FT-T7* transgenic lines with (+) or without (–) whole-plant heat treatment. Proteins were extracted 1 h after the end of 2-h HS. Protein extract from one-seedling equivalent was loaded per lane. (F–H) Flowering phenotype of 1, 2, *HSP::FT-T7* (MA#2 and #20); 3–5, *HSP::synFT-T7* (MA#1, #2 and #9); 6, *HSP::GUS* after single-leaf heat treatment. (F) Percentage of flowering in plants at the time of single-leaf heat treatment (before HS) (3 weeks in SDs), untreated controls (–HS) (5 weeks in SDs) and 2 weeks after the treatment (+HS) (5 weeks in SDs) (1–5, $n = 11$–12; 6, $n = 6$). (G) Representative plants of treated (+HS) and untreated (–HS) *HSP::FT-T7* and *HSP::synFT-T7* lines with similar levels of induction of FT-T7 protein (see E). (H) Flowering time of plants with (+) or without (–) HS treatment shown in (F). The numbers of rosette (solid) and rosette + cauline (open) leaves are shown as the average ± SD (bar).

Addition of an EGFP tag to the FT protein limits the graft-transmissible action without affecting local function in leaves and in the shoot apex

In contrast to the addition of a small T7 tag to the protein (FT-T7) and extensive alteration of the sequence and structure of mRNA (*synFT*), both of which had no severe effect, modification of the FT protein by addition of a large tag of EGFP (27 kDa) with a short linker (FT:EGFP; see Supplementary Fig. S3 for the linker sequence) reduced the graft-transmissible effect of *FT* to promote flowering. Apparent transmission of strong promotion of flowering by *35S::FT:EGFP* in the *ft-1* background (*35S::FT:EGFP* (YD#2-2C); *ft-1*, Table 3) to the *ft-1* stock plants was not observed (Fig. 2A, E, Supplementary Table S1; see also Supplementary Fig. S7A, B and Supplementary Table S5 for another set of results). Since the local expression of *FT:EGFP* in the shoot apex by the *FD* promoter [*FD::FT:EGFP* (YD#11f); see Abe et al. (2005) for the expression pattern] strongly promoted flowering (Table 3), an EGFP tag does not interfere with the *FT* function in the shoot apex when the fusion protein is present in the shoot apex. In addition, since increased expression of *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and *FUL*,

Table 3 Flowering times of transgenic plants expressing FT:EGFP fusion protein

Genotype	No. of rosette leaves, average ± SD (range)	No. of cauline leaves, average ± SD (range)	*n*
Wild type	16.2 ± 2.5 (11–24)	4.0 ± 1.1 (2–7)	80
35S::FT:EGFP (YD#2-2C)	4.7 ± 0.6 (3–6)	1.3 ± 0.5 (0–3)	52
ft-1	52.2 ± 4.6 (42–64)	10.0 ± 1.0 (8–12)	65
35S::FT:EGFP (YD#2-2C); *ft-1*	4. 5 ± 0.7 (3–6)	1.7 ± 0.6 (1–3)	73
Wild type	15.1 ± 1.8 (13–19)	3.7 ± 0.8 (2–5)	19
35S::FT:EGFP (YD#2-2C) (strong)	3.7 ± 0.6 (3–5)	1.4 ± 0.5 (1–2)	14
35S::FT:EGFP (YD#1-2C) (weak)	8.5 ± 1.6 (6–11)	2.5 ± 0.6 (2–3)	15
FD::FT:EGFP (YD#11f)	6.4 ± 1.0 (5–8)	1.1 ± 0.6 (0–2)	25

Plants were grown under CL on soil.

previously reported potential downstream target genes in cotyledons and leaves (Michaels et al. 2005, Teper-Bamnolker and Samach 2005, Yoo et al. 2005), was observed in *35S::FT:EGFP; ft-1* plants (Supplementary Fig. S8), FT:EGFP protein also retained its biochemical activity in cotyledons and leaves. Interestingly, slight enhancement of the graft-transmissible effect of the endogenous wild-type *FT* gene to promote flowering (Fig. 2A, B, Supplementary Table S1) by the same *35S::FT:EGFP* transgene was observed (Fig. 2B, F, Supplementary Table S1; see also Supplementary Fig. S7C, D and Supplementary Table S5 for another set of results).

Discussion

Recent works from several laboratories have provided strong evidence that the FT protein in *Arabidopsis* and corresponding proteins in other species are an important part of the mobile signal that promotes flowering (Corbesier et al. 2007, Jaeger and Wigge 2007, Lin et al. 2007, Mathieu et al. 2007, Tamaki et al. 2007). However, before a firm conclusion can be reached, there remains some room for further investigation. The temporal or dynamic aspects of transport have not yet been analyzed. In some key experiments, the possibility of short-distance, cell-to-cell transport of tagged FT proteins without entering into the phloem has not been excluded. Finally, with the reputation of GFP for its ability for long-distance trafficking via the phloem (Imlau et al. 1999) in mind, it seems that independent demonstrations based on other approaches are still needed. In the present work, although there are minor differences, we showed that the essential conclusions of the previous reports are firmly supported.

The present work shows that the activity of *FT* to promote flowering, either from the overexpressing (by 35S promoter) or inducible (by *HSP18.2* promoter) transgenes or from the endogenous genes, is transmissible through a graft junction (Figs. 2, 3, Table 1), and that FT protein is transported from the donor scion to the apical region of the recipient stock plants within a short period of 24–28 h (Fig. 4). These findings are consistent with the recent reports, and extend their observations. Transport of the FT protein from the site of translation to the shoot apical region was unequivocally confirmed using the T7-tagged protein and immunoblotting after the 2D-PAGE, which are different approaches from those based on GFP-fusion proteins and fluorescent microscopy. It is important to note that detection of the tagged protein in the shoot apical region of the recipient plants, which do not carry the transgene for the protein, enabled the attainment of an unequivocal conclusion about the transport. Furthermore, by using an inducible transgene (*HSP::FT-T7*), temporal aspects of the transport were investigated and it was shown that FT-T7 protein can be detected in the shoot apex of the recipient stock plants within 24–48 h after the pulsed induction in the donor transgenic scion (Fig. 4). With further refinement of the experimental system for the single-leaf blade heat treatment of the *HSP::FT-T7* plants described in this work, it will be possible to perform detailed temporal analysis of the transport of the FT-T7 protein and the transmission of the activity to promote flowering. Whether transport of the FT protein with concomitant transmission of flowering activity is regulated by photoperiods, as recently suggested in the case of cucurbits (Lin et al. 2007), and/or restricted to some hours (e.g. early night) during a day/night cycle is an interesting question to be addressed.

Unlike a previous report (Corbesier et al. 2007), the FT:EGFP fusion protein with a short linker in the present work appears to have limited ability, if any, of graft-transmissible action to promote flowering (Fig. 2, Supplementary Fig. S7), although it retains a cell-autonomous function to activate downstream genes in the leaf (Supplementary Fig. S8) and promote flowering in the shoot apex (Table 3). Consistent with this, our efforts to detect EGFP fluorescence from the fusion protein in the shoot

apical region of the recipient *ft-1* stock plants with a *35S::FT:EGFP* scion failed (data not shown). One possible reason for this discrepancy may be the choice of the linker length and/or the sequence in our FT:EGFP construct, which was inappropriate in such a way that the efficiency of the transport of the fusion protein through a graft junction was reduced due to hindrance from the EGFP tag. Another reason may be differences between the promoters used in the two experiments. The phloem companion cell-specific *SUC2* promoter used in the previous report is likely to be more efficient in the production of the fusion protein at the site of uploading to the phloem element.

Although the view that *FT* mRNA constitutes an important part of the mobile signal has lost grounds for support (Lifschitz et al. 2006, Corbesier et al. 2007, Lin et al. 2007, Tamaki et al. 2007), positive disproof of the mRNA hypothesis does not exist, except for experiments using the artificial microRNA (amiRNA) against *FT* mRNA locally expressed in the shoot apex or in the phloem companion cells (Mathieu et al. 2007). The results using *synFT*, a synthetic *FT* gene with extensive nucleotide substitutions throughout the ORF and foreign UTRs (Fig. 5, Supplementary Fig. S3), clearly showed that the sequence (and structure) of mRNA is not critically important for the long-distance action of the *FT* gene (Figs. 6, 7). Since the transport of mRNA should depend on its sequence and/or the structure determined by the sequence (see Haywood et al. 2005 for an example), we conclude that mRNA is not an essential component of the mobile signal. Consistent with this and previous reports (Lifschitz et al. 2006, Corbesier et al. 2007, Lin et al. 2007, Tamaki et al. 2007), repeated efforts to detect transcripts from the donor transgenes (either authentic *FT* or *synFT*) in the recipient *ft* stock have not been successful (Supplementary Fig. S4). In addition, as expected for the cell-autonomous function of *FT* mRNA for translation, no differences were observed between patterns of the GUS expression from *pFT::GUS* (promoter reporter expressing GUS mRNA) and *gFT::GUS* (reporter expressing FT:GUS fusion mRNA in a genomic context) (Fig. 1, Supplementary Fig. S1).

The absence of promotion of its own expression by *FT* (Figs. 1, 8D) suggests that the secondary induction and amplification of the primary induction signal through an autoregulatory loop is not likely to be the case in *Arabidopsis*. Also unlikely is a relay mechanism in which FT acts as an activator of the downstream mobile signal(s), a conclusion supported by the absence of graft-transmissible action of the FT:EGFP protein which retains a cell-autonomous function to activate downstream genes in the leaf (Fig. S8). This closely agrees with a previous report (Corbesier et al. 2007). In view of autoregulation of the long-distance action of the FT protein, it is noted that the FT:EGFP protein seems to facilitate the long-distance action of the endogenous wild-type *FT* gene (Fig. 2B, F; Supplementary Fig. S7C, D). Since this is unlikely through the transcriptional activation of *FT*, the FT:EGFP protein may act on the FT protein. Whether or not the FT protein can facilitate its own transport is an interesting question to be addressed.

Taken together, we propose that the FT protein, but not mRNA, is the essential component of the graft-transmissible *FT* mobile floral signal and that the FT protein may support its own long-distance action possibly through the facilitation of its transport. TWIN SISTER OF FT (TSF), the protein with the highest degree of similarity and partially redundant role with FT (Michaels et al. 2005, Yamaguchi et al. 2005), is likely to serve a similar role (Yamaguchi et al. 2005, Mathieu et al. 2007). It has been suggested recently that TFL1, another homolog of FT with an antagonistic role in flowering (Bradley et al. 1997, Kardailsky et al. 1999, Kobayashi et al. 1999, Hanzawa et al. 2005, Ahn et al. 2006), acts as a mobile signal in the SAM (Conti and Bradley 2007). Therefore, an interesting scenario is that the FT protein, expressed in the leaf, acts as a potent long-distance signal which overcomes the action of a negative, short-distance TFL1 signal within the shoot apex, enabling floral transition (Fig. 9). That *FT* is able to overcome the negative action of *TFL1* on flowering is supported by observations that the late-flowering phenotype of *35S::TFL1* plants was strongly suppressed by *FT* overexpression (Kardailsky et al. 1999, Kobayashi et al. 1999). Since both FT and TFL1 proteins can interact with a bZIP protein FD (Abe et al. 2005, Wigge et al. 2005), they are likely to compete for FD in the shoot apex (Ahn et al. 2006). Interaction with FT results in nuclear localization of FD as an FT–FD complex (Abe et al. 2005) and transcriptional activation of target genes such as *AP1* (Abe et al. 2005, Wigge et al. 2005, Ahn et al. 2006), while interaction with TFL1 results in the absence of target gene activation, possibly through the conversion of FD (as a TFL1–FD complex) into a repressor (Ahn et al. 2006) or prevention of FD acting in the nucleus. Recent findings have shown that TFL1 is a cytoplasmic protein (Conti and Bradley 2007), with the latter possibility being more probable. An FT protein may have a higher affinity for FD than TFL1 does, since TFL1–FD interaction seems weaker than FT–FD interaction in some experiments (Abe et al. 2005). In addition to FD, FT and TFL1 proteins are in competition for other common interacting partners in the SAM (Pnueli et al. 2001). Differential interaction with these partners by FT and TFL1 should play an important role in the regulation of flowering. Successful floral transition and patterned initiation of the floral primordia at the shoot apex should require precise temporal regulation and an intricate balance between the FT and the TFL1 proteins, and

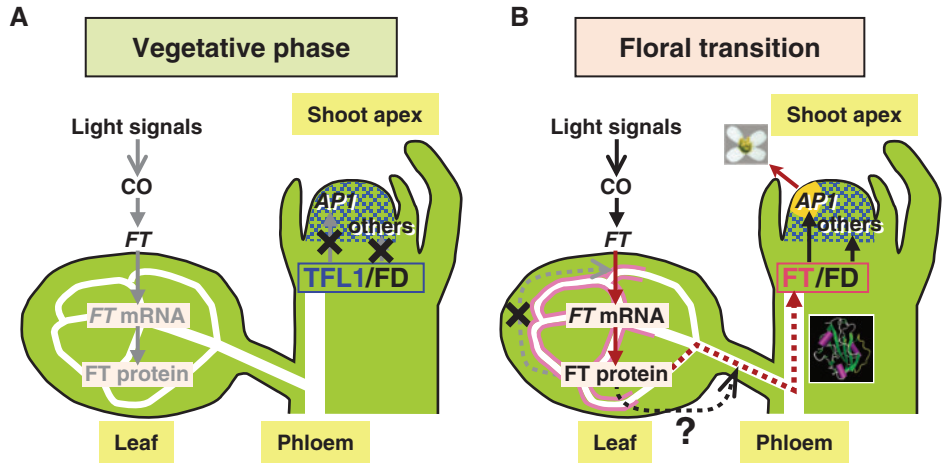

Fig. 9 A current model of the action of the FT florigen. (A) Vegetative phase. In the absence of proper light signals or at very young seedling stage, *FT* expression is absent. TFL1 protein is translated in the inner cells and becomes evenly distributed across the SAM (shown as blue shading). TFL1 protein interacts with a bZIP transcription factor FD and prevents FD from activating target genes for flowering. (B) Floral transition. Transcription of *FT* is induced in the phloem companion cells in the leaf (shown as pink lines around phloem represented by a white line) in response to light signals via action of CO protein. FT protein is translated and is transported to the shoot apex where it interacts with a bZIP transcription factor FD by competing against TFL1 protein (shown as blue shading). The FT–FD complex becomes localized in the nucleus and activates transcription of target genes such as a floral meristem identity gene, *AP1* (shown as yellow shading). *AP1*, in turn, specified the floral fate of nascent lateral meristems to initiate floral morphogenesis. FT protein does not promote its own transcription (a gray broken arrow with an 'X' on it) but may act *in trans* to facilitate its movement (a black broken arrow with a question mark). See text for details.

patterned spatial distribution of FT–FD vs. TFL1–FD as well as other protein interactions throughout the SAM. Understanding how all these are achieved is obviously an important problem to be tackled in the near future.

Materials and Methods

Plant materials and growth conditions

Columbia-0 (Col) was used as the wild type. *ft-1* (G171E) introgressed into a Col background [*ft-1*(Col)] was obtained from J. H. Ahn (Korea University, Korea). *35S::FT* (YK#11-1 and YK#1-5C) and *35S::FT:EGFP* (YD#2-2C) were previously described (Kobayashi et al. 1999, Abe et al. 2005). *pFT::GUS* (Takada and Goto 2003) and *HSP18.2::GUS* (referred to as *HSP::GUS*, line AHS9) (Takahashi and Komeda 1989) were obtained from K. Goto, and T. Takahashi and Y. Komeda, respectively. For expression analysis, plants were grown on 1/2× Murashige Skoog medium supplemented with 0.5% sucrose at 22°C under LD (16 h light/8 h dark) conditions with white fluorescent lights (~60 μmol m^{-2} s^{-1}) or SD (8 h light/16 h dark) conditions with white fluorescent lights (~100 μmol m^{-2} s^{-1}). For the single-leaf heat treatment and analysis of the flowering time phenotype, plants were grown on soil at 22°C under SD conditions.

Plasmid construction and plant transformation

All the plasmid constructs for plant transformation (shown in Supplementary Fig. S3) were generated using standard molecular biology procedures. *gFT::GUS* was constructed by replacing the stop codon of the *FT* coding sequence in a 10.9 kb genomic fragment, which contains a region sufficient for complementation of *ft* (Takada and Goto 2003), with the GUS (*uidA*) ORF (Supplementary Fig. S1). The 5′ end of the fragment is approximately 200 bp upstream of the 5′ end of the transcribed region of *FASCIATA1* (*FAS1*), which is transcribed in the opposite direction (Kaya et al. 2001). *35S::FT-T7* and *HSP::FT-T7* were constructed by fusing a 35S promoter and a promoter fragment (−853 to −1 relative to ATG) from an *Arabidopsis HSP18.2* gene (Takahashi and Komeda 1989), respectively, with a 5′-UTR plus FT-T7 ORF (Fig. 5A). The same *HSP18.2* promoter fragment was used for *HSP::FT:GUS* in which the *FT* ORF without the stop codon was fused to the GUS ORF to generate the FT:GUS fusion protein.

The sequence of the *synFT* ORF (Fig. 5A) was designed using a program developed by Mr. T. Tochihara (Ishikawa Prefectural University) and was synthesized essentially as described (Mori and Dohi 2005). The *35S::synFT-T7* and *HSP::synFT-T7* constructs were made with designs similar to the corresponding *FT* constructs (Supplementary Fig. S3). Constructs in binary vectors were introduced into the *Agrobacterium tumefaciens* strain pMP90 and were transformed into *Arabidopsis* plants by a floral-dip procedure (Clough and Bent 1998). Newly generated transgenic lines are listed in the Supplementary methods.

Grafting

ft-1 (Col) was used as a recipient stock. Transgenic plants with various *FT* constructs and wild-type plants were used as donor scions. As a control, *ft-1* (Col) was used as a donor scion in each set of the experiments. Grafting was performed essentially as described (Turnbull et al. 2002) with modifications for adaptation to the humid conditions found in our growth rooms. Four-day-old seedlings grown in continuous light (CL; ~60 μmol m^{-2} s^{-1}) conditions at 22°C were subjected to 'Y-graft' surgery. The graft was assembled on the hypocotyl of a recipient stock seedling (Supplementary Fig. S2). The resulting grafts were kept at 27°C for 5 d under CL conditions (~30 μmol m^{-2} s^{-1}) on wet filter paper in a

Petri dish to facilitate graft fusion and to suppress formation of adventitious roots. The surviving grafts were planted on soil and were grown under CL conditions (~40 μmol m^{-2} s^{-1}) at 22°C. In our experimental conditions, functional continuity of the phloem tissues between the scion and the stock, as judged by trafficking of phloem-specific tracer dyes and EGFP, was established by 2–3 weeks after the graft surgery (M. Notaguchi, Y. Daimon, M. Abe and T. Araki, submitted). Combinations of the grafts and the number of trials are shown in Supplementary Table S6.

Measurement of flowering time of stock plants

To avoid biased selection at the time of measurement of the flowering time, all the *ft-1* stock plants with a firm graft junction and a scion shoot of nearly equal size and vigor as the stock shoot after 2–3 weeks of post-grafting growth (Supplementary Fig. S2) were subject to measurement without further selection. The flowering time of the recipient *ft-1* stock plants was measured by counting the number of rosette and cauline leaves on the primary axis ('total leaf number') after inflorescence stem elongation (usually 50–100 d after grafting, depending on the graft combination). The flowering times were compared only among graft combinations from the same experimental set.

Single-leaf heat treatment

Plants grown in SD conditions for 3 weeks were subjected to a single-leaf heat treatment following a modified version of a protocol kindly provided by O. Nilsson (Umeå Plant Science Centre, Sweden). The leaf blade of a well-expanded leaf (usually from the fourth to the sixth leaf) was exposed to 37°C for 2 h [zeitgeber time (ZT) 0.25 to ZT2.25 or ZT2.25 to ZT4.25] by placing it in the water-filled space between a heated copper plate and a glass slide to ensure good contact is made (Supplementary Fig. S5). The copper plate was heated using a silicon rubber plate heater fixed under the plate, and was connected to a temperature controller (SHM-CONT2; Asahi Techno Glass, Japan), so that the final temperature of 37°C in the water-filled space was stably maintained. Local induction of the *HSP18.2* promoter activity by our experimental system was confirmed by treating the *HSP::GUS* and *HSP::FT:GUS* plants in the same way. For the 'whole-plant' heat treatment, 7-day-old seedlings grown in LDs at 22°C were incubated for 2 h (ZT0 to ZT2) at 37°C (Fig. 8D, E) or 40°C (Supplementary Fig. S6).

Heat treatment of grafts

HSP::FT-T7 (MA#2); *ft-1* plants were grafted onto *ft-1* stock plants as described above. On the 25th day of growth under CL conditions (~40 μmol m^{-2} s^{-1}) at 22°C after the graft surgery, leaves on the *ft-1* stock plants were removed to enhance the sink strength. From days 26 to 27, the entire grafts were subjected twice to a 2.5-h heat treatment (39°C) at a 24-h interval to induce pulsed *FT* expression. The shoot apical regions of the *ft-1* stock plants were collected for protein analysis 24 h after the second heat treatment on the 28th day.

Preparation of the shoot apical region for detection of FT-T7 protein

The shoot apical region for the detection of the FT-T7 protein was prepared by excising the aerial part of the plant and then removing cotyledons, leaves and hypocotyls as much as possible under a dissecting microscope. It includes the SAM, leaf primordia and the upper part of the compressed internodes.

GUS staining, reverse transcription–PCR, two-dimensional gel electrophoresis and immunoblotting

Procedures are described in the Supplementary methods.

Supplementary data

Supplementary data are available at PCP online.

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