A *cis* Element within *Flowering Locus T* mRNA Determines Its Mobility and Facilitates Trafficking of Heterologous Viral RNA[▽]

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The *Arabidopsis flowering locus T* (*FT*) gene encodes the mobile florigen essential for floral induction. While movement of the FT protein has been shown to occur within plants, systemic spread of *FT* mRNA remains to be unequivocally demonstrated. Utilizing novel RNA mobility assay vectors based on two distinct movement-defective viruses, *Potato virus X* and *Turnip crinkle virus*, and an agroinfiltration assay, we demonstrate that nontranslatable *FT* mRNA, independent of the FT protein, moves throughout *Nicotiana benthamiana* and mutant *Arabidopsis* plants and promotes systemic trafficking of viral and green fluorescence protein RNAs. Viral ectopic expression of FT induced flowering in the short-day *N. tabacum* Maryland Mammoth tobacco under long-day conditions. Recombinant *Potato virus X* bearing *FT* RNA spread and established systemic infection more quickly than the parental virus. The *cis*-acting element essential for RNA movement was mapped to the nucleotides 1 to 102 of the *FT* mRNA coding sequence. These data demonstrate that a plant self-mobile RNA molecule can mediate long-distance trafficking of heterologous RNAs and raise the possibility that *FT* RNA, along with the FT protein, may be involved in the spread of the floral stimulus throughout the plant.

RNA trafficking plays an important role in systemic signaling that controls plant development and defense against pathogen infection (25). Hundreds of RNA transcripts have been recently identified in phloem, suggesting phloem-mobile RNAs may act as long-distance signaling molecules in plants (4, 8). Indeed, systemic movement of a homeobox fusion transcript and *gibberellic acid-insensitive* RNA regulates leaf architecture (13, 19), a non-cell-autonomous mobile RNA represents a long-distance signal that modulates potato tuber formation (3), and small interfering RNAs are components of intercellular and systemic mobile signals for innate RNA silencing defense (9, 12, 14). RNA trafficking is also critical for plant viruses and viroids to establish systemic infection. It has been demonstrated that an RNA motif directs long-distance trafficking of a small naked RNA viroid (33, 44, 46). Moreover, a short RNA sequence is found to be involved in cell-to-cell movement of a plant viral RNA (24), and replication-independent viral RNA can move over long distances in plants (11).

In floral induction, the mobile florigen is encoded by the *Arabidopsis flowering locus T* (*FT*) gene. *FT* transcribes mRNA in the leaf, but its encoded FT protein functions in the shoot apices where flowers develop (1, 2, 40). The *Arabidopsis* FT protein and its orthologues have been shown to be involved in long-distance signaling in floral induction (7, 18, 22, 23, 29, 30, 37). However, whether *FT* mRNA is also capable of systemic spread remains to be demonstrated. We describe novel approaches which show that not only does *FT* RNA move over

long distances but, remarkably, also facilitates the systemic spread of heterologous green fluorescent protein (GFP) mRNA and different viral RNAs in plants. The *FT* RNA movement does not rely on the expression of the FT protein. The *FT* RNA mobility is determined by a *cis*-acting element localized within nucleotides 1 to 102 of the *FT* mRNA coding sequence.

MATERIALS AND METHODS

Construction of RMA vectors. The wild-type and mutant *Arabidopsis FT* genes were reverse transcription-PCR (RT-PCR) amplified using *Pfu* DNA polymerase and the primers PP354/PP356 or PP356/PP355, digested with BspEI and SalI, and cloned in-frame fused to the GFP coding sequence in the BspEI/SalI sites of PVX and PVX/GFP (38) to produce PVX/FT, PVX/mFT, PVX/GFP-FT, and PVX/GFP-mFT, respectively. Plasmid DNA of PVX/GFP, PVX/GFP-FT, and PVX/GFP-mFT were then digested with SalI and XhoI to remove the coat protein (CP) gene subgenomic RNA promoter and the CP gene and self-ligated to produce PVX/GFPΔCP, PVX/GFP-FTΔCP, and PVX/GFP-mFTΔCP. Expression of the *GFP* gene from PVX/GFPΔCP and the wild-type and mutated *GFP-FT* fusion gene from PVX/GFP-FTΔCP and PVX/GFP-mFTΔCP were under the control of an engineered CP subgenomic RNA promoter. For construction of TCV-based RNA mobility assay (RMA) vectors TCV/mFTΔCP, TCV/GFP-FTΔCP, and TCV/GFP-mFTΔCP, the *Arabidopsis FT* gene was RT-PCR amplified using *Pfu* DNA polymerase and the primers PP406 or PP407 and PP408, digested with BclI and PmeI, and cloned into the BglII/PmeI sites of TCV/ΔCP (34) or TCV/GFPΔCP (47). Using a similar PCR and cloning strategy, a series of TCV/trFTΔCP-based RMA vectors carrying truncated (tr) *FT* for mapping the *cis*-acting element required for *FT* RNA trafficking were constructed. All RMA constructs were confirmed by nucleotide sequencing. The primers used for the construction of RMA vectors are listed in Table 1.

RMAs. RNA transcripts from each movement-deficient recombinant PVX and TCV vector were produced by in vitro transcription as previously described (34), pretreated with RNase-free DNase (Promega), and then mechanically inoculated onto *N. benthamiana* or *A. thaliana ft-10* mutant plants at 5 to 6 or 15 to 16 leaves, respectively, in repeated experiments. *N. benthamiana* plants aged at only 24 days after sowing seeds and were too young to initiate flowering. For PVX-based RMA, total RNAs (50 ng) extracted from inoculated and newly growing young leaves separately collected at 7 days postinoculation (dpi) were pretreated with RNase-free DNase (Promega) and used for RT-PCR detection (30 cycles) (34) with the primers PP354 and PP356 for *FT*, PP371 and PP372 for *GFP*, PP269

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TABLE 1. Primers used in this study

Primer	Sequence (5′–3′)[a]	Origin and modification
PP82	CAGTGTTGGCTTGCAAACTAG	PVX
PP228	GGGTAAGTTTTCCGTATGTTG	GFP
PP267	AGAAAAGTCATGAAGGTTCTGCTAGCC ACGG	TCV
PP269	CAAGGTGCGCGAGGTTTACCAATC	PVX
PP271	CGGCTACCACATCCAAGGAAGG	18S rRNA
PP272	GAGCTGGAATTACCGCGGCTG	18S rRNA
PP354	TCAAGA**TCCGGA**A̲ATGTCTATAAATATAAG AGACCC	*FT*, BspEI
PP355	TCAAGA**TCCGGA**TAG̲TCTATAAATATAAG AGACCC	*FT*, BspEI
PP356	AGGAAGAA**GTCGAC**TAAAGTCTTCTTCCTC CGCAG	*FT*, SalI
PP371	ATGAGTAAAGGAGAAGAACTTTTCACT GGAG	GFP
PP372	TTTGTGTCCAAGAATGTTTCCATCTTC	GFP
PP373	GTATGCTGTTTCCGTTGTGATCTCTGTGAG	PVX
PP407	TTCAAG**TGATCA**G̲TAG̲TCTATAAATATAAG AGAC	*FT*, BclI
PP408	AAGGAA**GTTTAAAC**CTAAAGTCTTCTTCCT CCGCA	*FT*, PmeI
PP433	ATGCCTCTCCTACATACACTCAACACAG	TCV
PP434	GTCTACGACATGTCTCTCTTTGCAG	TCV
PP473a	TTGGCCATAAGTAACCTTTAG	*FT*
PP512	TCAAGA**TGATCA**TAG̲TCTATAAATATAAGA GACCC	*FT*, BclI
PP513	TTAGTCAC**GTTTAAAC**GGCCATAAGTAACC TTTAG	*FT*, PmeI
PP514	GTTACT**TGATCA**TAG̲AGAGAGGTGACTAA TGGCTT	*FT*, BclI
PP515	AGGAA**GTTTAAAC**CTAAAGTCTTCTTCCTC CGCAG	*FT*, PmeI
PP516	AGATCC**GTTTAAAC**CAAAGTATAGAAGTTC CTGAG	*FT*, PmeI
PP517	AACTTC**TGATCA**TG̲AGTTATGGTGGATCCA GATG	*FT*, BclI
PP518	ATCTCAG**TTTAAAC**GGTTGTTCCAGTTGTA GCAGG	*FT*, PmeI
PP519	ACAACT**TGATCA**TAG̲TTTGGCAATGAGATT GTGTG	*FT*, BclI
PP520	CACCCTG**GTTTAAAC**ACTGTTTGCCTGCCA AGCTG	*FT*, PmeI
PP521	GGCAGG**TGATCA**TG̲ATATGCACCAGGGTG GCGCCA	*FT*, BclI

[a] Introduced restriction endonuclease sites are in boldface, and the start and mutated nonsense stop codons are underlined.

TABLE 2. Mapping *cis*-acting element for *FT* RNA movement

RMA vector[a]	*FT* RNA 5′–3′ coordinates	Primer pair used for:		Predicted size (bp) of RT-PCR product[b]
		PCR and cloning	RT-PCR detection	
TCV/FTn102ΔCP*	1–102	PP512/PP513	PP267/PP513	375
TCV/FTn201ΔCP*	1–201	PP512/PP516	PP267/PP516	474
TCV/FTn300ΔCP*	1–300	PP512/PP518	PP267/PP518	573
TCV/FTn399ΔCP*	1–399	PP512/PP520	PP267/PP520	672
TCV/FT103cΔCP†	103–528	PP514/PP515	PP267/PP515	699
TCV/FT202cΔCP†	202–528	PP517/PP515	PP267/PP515	600
TCV/FT301cΔCP†	301–528	PP519/PP515	PP267/PP515	500
TCV/FT400cΔCP†	400–528	PP521/PP515	PP267/PP515	402

[a] *, A stop codon was introduced to replace the native *FT* start codon; †, a stop codon was placed immediately upstream of the truncated *FT* RNA. Thus, all truncated *FT* RNAs produced from the eight RMA vectors are nontranslatable.

[b] The predicted size of each RT-PCR product includes TCV and cloning sequences (273 bp) plus the corresponding truncated *FT* sequence.

leaves of 24-day-old *N. benthamiana* plants were syringe infiltrated with *Agrobacterium* cultures carrying 35S-GFP-poly(A) (34), 35S-GFP-FT-poly(A) and 35S-GFP-mFT-poly(A). At 4 days postinfiltration, agroinfiltrated leaf tissues and noninfiltrated newly developed young leaves were collected for protein and RNA analysis.

Induction of flowering by viral transient expression of the *Arabidopsis* FT protein. In three experiments, young short-day (SD) *N. tabacum* Maryland Mammoth plants were mock inoculated or infected with PVX/FT, PVX/mFT, PVX/GFP, or PVX/GFP-FT and maintained in an insect-free containment glasshouse at 25°C with a long-day (LD; 16-h) photoperiod. Local and systemic symptoms, including chlorotic lesions on inoculated leaves and mild chlorosis on young leaves, developed after 7 to 14 dpi. All PVX/FT-infected plants started to shoot at ~20 dpi and flowered at ~35 dpi; this was photographically recorded with a Nikon digital CoolPix 995 camera. Total RNAs (50 ng) extracted from systemically infected young leaves collected at 42 dpi were treated with RNase-free DNase (Promega) and used for RT-PCR detection (30 cycles) (34) with the primers PP82 and PP356 for virus-carried *FT* RNA. The resultant specific RT-PCR products were purified and verified by direct sequencing. The viral transient FT protein was analyzed by Western blotting detection.

Western blotting. To investigate GFP, FT, GFP-FT fusion protein, and PVX CP expression, total proteins were extracted from leaf tissues as described previously (15). Western blot analyses of protein aliquots (10 μg) were performed with polyclonal antibodies specifically raised against GFP, the *Arabidopsis* FT and PVX CP, and detected using a goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (Sigma) and BCIP/NBT substrates (Roche) as described previously (39).

RESULTS AND DISCUSSION

***Arabidopsis* FT RNA facilitates the movement of heterologous GFP and viral RNAs in *N. benthamiana*.** To investigate plant RNA trafficking, we developed a GFP-tagged movement-defective *Potato virus X* (PVX/GFPΔCP)-based RMA (Fig. 1A). Deletion of viral CP prevented PVX/GFPΔCP movement and restricted viral RNAs to single *N. benthamiana* epidermal cells. Thus, unlike the wild-type virus, PVX/GFPΔCP was incapable of accessing the phloem to move systemically (10). It should be noted that free GFP, once loaded from the mesophyll into the sieve tube, can move over long distances in the phloem (16, 17, 31, 32). PVX/GFP-FTΔCP and PVX/GFP-mFTΔCP had, respectively, the translatable or nontranslatable *Arabidopsis* FT that was fused in-frame to the GFP coding sequence (Fig. 1A). Individual epidermal cells expressing transient GFP or GFP-FT fusion protein from PVX/GFPΔCP, PVX/GFP-mFTΔCP, or PVX/GFP-FTΔCP showed green fluorescence (Fig. 1A). However, cell-to-cell movement of PVX/GFP-FTΔCP or PVX/GFP-mFTΔCP or intercellular spread of
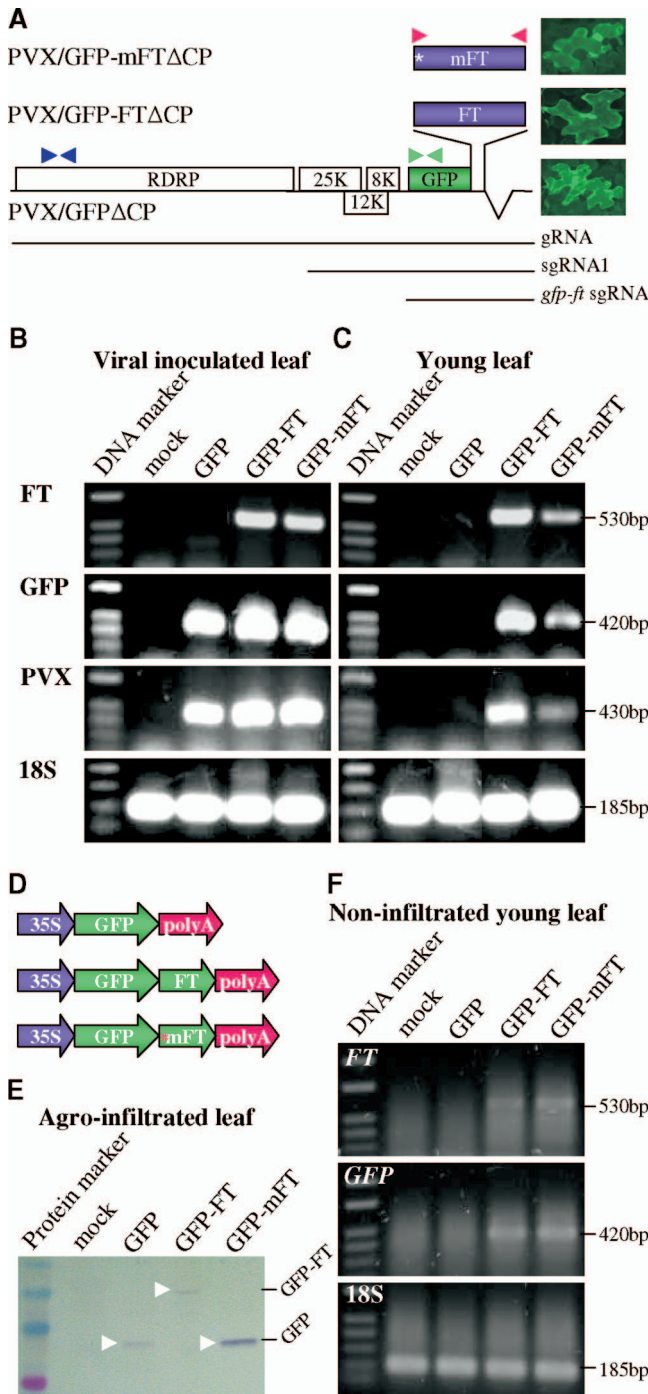
and PP373 for PVX, and PP271 and PP272 for 18S rRNA. Epidermal cells with GFP expression and the movement of GFP-tagged viruses were examined with a Zeiss Axiophot microscope with filters for GFP (excitation, 450 to 490 nm; long-pass emission, 520 nm) or under long-wavelength UV light (Upland UVP model B 100AP) through a yellow filter (Kodak no. 58) and photographed with a Nikon CoolPix 995 digital camera (38).

For TCV-based RMAs, inoculated, newly growing young leaves and shoot apices were carefully collected at 7 dpi from mock- and virus-inoculated *N. benthamiana* and mutant *ft-10 A. thaliana* plants for total RNA extraction. Total RNAs were treated with RNase-free DNase (Promega), and RT-PCR (30 cycles) detection was performed with the primers PP354 and PP356 for *FT*, PP371 and PP372 for *GFP*, PP433 and PP434 for TCV RNA, PP267 and PP473a for TCV-*mFT* RNA, PP267 and PP228 for TCV-GFP RNA, and PP271 and PP272 for 18S rRNA. The resultant specific TCV-mFT RT-PCR products were purified and verified by direct sequencing.

In two separate mapping experiments, plants were inoculated with recombinant viral RNAs that were produced by in vitro transcription from each TCV/trFTΔCP-based RMA vectors and pretreated with RNase-free DNase (Promega). Total RNAs were extracted at 7 dpi from inoculated and uninoculated young leaves, pretreated with RNase-free DNase (Promega), and used for RT-PCR (30 cycles) assays with primer sets listed in Table 2.

Transient agroinfiltration assay of RNA mobility. The *GFP-FT* and *GFP-mFT* fusion genes were isolated from PVX/GFP-FT and PVX/GFP-mFT and inserted into between the *Cauliflower mosaic virus* 35S promoter and terminator-poly(A) signal sequence of a binary vector in *Agrobacterium tumefaciens* LBA4404 (34) to produce 35S-GFP-FT-poly(A) and 35S-GFP-mFT-poly(A). In two experiments,

GFP or GFP-FT among epidermal cells was not detected by epifluorescence microscopy (Fig. 1A). We also examined the upper leaves for systemic movement of GFP or GFP-FT protein and these recombinant viruses but failed to observe any GFP in any type of cells.

We then used a more sensitive RT-PCR assay to test for RNA movement. Accumulation of recombinant viral RNAs from each vector was readily detected in inoculated leaves by RT-PCR using *FT*-, *GFP*-, and PVX-specific primers (Fig. 1A and B). However, no spread of genomic RNA (gRNA) and subgenomic RNA (sgRNA) of PVX/GFPΔCP to newly formed young leaves occurred (Fig. 1C). In striking contrast, inclusion of *FT* RNA in PVX/GFP-FTΔCP enabled *GFP-FT* sgRNA and PVX/GFP-FTΔCP gRNA to move systemically from inoculated leaves to young leaves (Fig. 1C). Furthermore, PVX/GFP-mFTΔCP gRNA and sgRNAs that contained nonsense mutations precluding FT protein synthesis also spread systemically (Fig. 1C), demonstrating that nontranslatable *mFT* RNA was able to move and promote long-distance trafficking of heterologous *GFP* and PVX RNAs. *FT* and *mFT* possessed similar abilities to facilitate the spread of heterologous RNAs, although the levels of mobile RNAs detected in the systemic leaves varied between plants for the *FT* and *mFT* constructs. The absence of GFP signal in neighboring epidermal cells and upper leaves after *FT* RNA-mediated movement from lower parts into younger tissues suggests that CP may be required for vascular exit, which could be a novel role for CP in viral movement, although we cannot exclude the possibility that it is due to the limited sensitivity of fluorescence detection of free GFP and GFP-FT fusion proteins.

FT and *mFT* RNA movement and *FT*-mediated heterologous RNA trafficking were also demonstrated in a distinct *Turnip crinkle virus* (TCV)-based RMA (Fig. 2). In a similar experimental design, we used a GFP-tagged movement-deficient TCVΔCP-based RMA vector (Fig. 2A) and also showed that the *Arabidopsis FT* RNA promoted systemic movement of TCV RNA. RT-PCR detection of *FT*, *GFP*, and TCV RNA and 18S rRNA (18S) in inoculated leaves (Fig. 2B) and particularly in the newly formed young leaves and shoot apices (Fig. 2C) of *N. benthamiana* plants infected with TCV/GFPΔCP, TCV/GFP-FTΔCP, or TCV/GFP-mFTΔCP mirrored the results obtained with the PVX-based RMA.

Mobility of *Arabidopsis FT* RNA is independent of viral RNA sequences. The self-mobility of *FT* RNA and the associated heterologous RNAs was further confirmed by an agroinfil-

FIG. 1. (A) PVX-based RMA vectors. Viral 7.0-kb genomic gRNA, 2.6-kb subgenomic sgRNA1, and 1.4-kb *GFP-FT* sgRNA are indicated. The CP gene was deleted. The positions of a stop codon (*) replacing *FT* start codon in PVX/GFP-mFTΔCP, and three sets of RT-PCR primers for the detection of *FT* (▶◀ [red], PP354/PP356), *GFP* (▶◀ [green], PP371/PP372), or PVX (▶◀ [blue], PP269/PP373) RNAs are indicated. Individual epidermal cells expressing free GFP or GFP-FT fusion protein showed green fluorescence. (B and C) RT-PCR analysis of *FT*, *GFP*, and PVX RNA and 18S rRNA in inoculated and newly growing young leaves, including shoot apices of *N. benthamiana*. RNAs were extracted from leaves of plants mock inoculated (mock) or inoculated with PVX/GFPΔCP (GFP), PVX/GFP-FTΔCP (GFP-FT), or PVX/GFP-mFTΔCP (GFP-mFT). The 1-kb DNA ladder and sizes of RT-PCR products are indicated. (D) Construction of 35S promoter-controlled *FT* gene expression cassettes for a transient agroinfiltration assay of RNA mobility. The position of an introduced stop codon (*) replacing the *FT* gene start codon in 35S-GFP-mFT-poly(A) is indicated. (E) Expression of free GFP or GFP-FT fusion protein. Total proteins were extracted from *N. benthamiana* leaves infiltrated with agrobacteria carrying the 35S-controlled gene expression cassette and were analyzed by Western blotting with a GFP-specific antibody. The positions of free GFP and GFP-FT fusion protein are indicated. (F) *FT* and *GFP* mRNAs in nonagroinfiltrated newly developed systemic young leaves were analyzed by RT-PCR. Samples are from plants with mock infiltration (mock) or infiltrated with agrobacteria carrying 35S-GFP-poly(A) (GFP), 35S-GFP-FT-poly(A) (GFP-FT), or 35S-GFP-mFT-poly(A) (GFP-mFT). The sizes of RT-PCR products, free and GFP-FT fusion proteins, and the prestained protein markers or 1-kb DNA ladder are indicated.
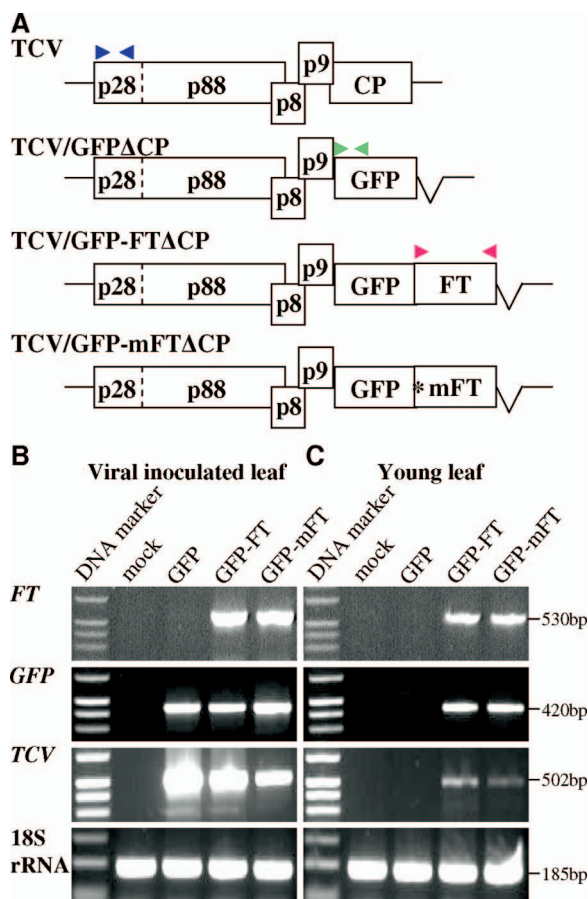
FIG. 2. The *Arabidopsis FT* RNA supports TCV RNA movement. (A) GFP-tagged movement-deficient TCV-based RMA vectors. The position of an introduced stop codon (*) replacing the *FT* gene start codon in TCV/GFP-mFTΔCP and three sets of RT-PCR primers for detections of *FT* (▶◀ [red], PP354/PP356), *GFP* (▶◀ [green], PP371/PP372) or TCV (▶◀ [blue], PP433/PP434) are indicated. (B and C) RT-PCR analysis of *FT*, *GFP* and TCV RNA and 18S rRNA (18S) in inoculated (B) and newly formed young leaves and shoot apices (C) of *N. benthamiana* plants. Total RNAs used as RT-PCR templates were extracted from inoculated or young leaves of plants with mock inoculation (mock) or inoculated with RNA transcripts from TCV/GFPΔCP (GFP), TCV/GFP-FTΔCP (GFP-FT), or TCV/GFP-mFTΔCP (GFP-mFT). The 1-kb DNA ladder and the sizes of RT-PCR products are indicated.

tration assay. *N. benthamiana* leaves were infiltrated with *Agrobacterium tumefaciens* carrying the 35S promoter-controlled *GFP* or *GFP*-tagged *FT* gene expression cassettes, respectively (Fig. 1D). Western blot analysis showed that expression of free GFP occurred in leaves infiltrated with agrobacterium carrying 35S-GFP-poly(A) and 35S-GFP-mFT-poly(A), while the larger GFP-FT fusion protein was expressed from 35S-GFP-FT-poly(A) (Fig. 1E). These data clearly demonstrated that the replacement of the initiation codon with a stop codon in the *FT* open reading frame blocked the translation of FT protein from the 35S-GFP-mFT construct. We also examined the *Arabidopsis FT* sequence and found no internal in-frame start codons; thus, it is unlikely that *mFT* RNA would be translated from internal initiation. Consistent with the results obtained from the viral RMAs, following agroinfiltration,

both *FT* (*mFT*) and *FT* (*mFT*)-*GFP* RNAs spread systemically to young leaves that had developed on the plant after the agroinfiltration (Fig. 1F). It should be pointed out that systemic movement of *FT* (*mFT*) and *FT* (*mFT*)-*GFP* RNA seems to be stronger in virus-based RMAs than in the agroinflitration assays. This may be due to differences in the site (nucleus versus cytoplasm) and the means of production of the mobile RNA molecules (35S promoter-controlled transcription versus recombinant viral RNA replication and transcription) in agroinfiltrated cells compared to viral inoculated cells. Nevertheless, our data indicate that no viral sequences and proteins are necessary for systemic spread of *FT* RNA.

Viral transient expression of *Arabidopsis* FT protein promotes floral induction. *Arabidopsis FT* is required to induce flowering. However, it did not stimulate early flowering in day neutral *N. benthamiana* expressing GFP-FT, probably due to interference by the GFP fusion. To test this, we infected SD *N. tabacum* Maryland Mammoth tobacco (21) with PVX/FT (Fig. 3A) and PVX/GFP-FT (see Fig. 6A). Viral ectopic expression of the GFP-FT fusion protein was unable to induce flowering under noninducing LD conditions (Fig. 3B and C); however, all Maryland Mammoth plants infected with PVX/FT that had the capacity to produce free FT protein flowered in LD, while control plants infected with either PVX/mFT carrying a mutated nontranslatable *FT* mRNA or PVX/GFP remained vegetative (Fig. 3B and C). Viral delivery of wild-type and mutated *FT* RNA was readily detected in systemically infected leaves by RT-PCR and further confirmed by direct sequencing of the specific RT-PCR products (Fig. 3D). Free FT protein expressed from PVX/FT but not from PVX/mFT was also detected by Western blotting with an antiserum specifically raised against FT (Fig. 4D). The fact that PVX/FT could induce flowering but PVX/mFT could not shows that the *Arabidopsis FT* RNA alone is not sufficient to initiate flowering but that its protein product expressed from PVX/FT is necessary for floral induction.

Systemic movement of *FT* RNA does not require FT protein. The virus-based RMAs, together with the agroinfiltration assay, demonstrate that *Arabidopsis FT* RNA not only is self-mobile but also can mediate systemic trafficking of heterologous RNAs in *N. benthamiana*. To eliminate the possibility that endogenous plant-derived FT protein might be involved in facilitation of the *FT* RNA movement, we used the TCV-based RMA vector TCV/mFTΔCP containing a nontranslatable *FT* gene (Fig. 4A). Deletion of the CP gene in TCVΔCP prevents its spreading from infected cells to distal parts of plants (6). However, in contrast to the PVX-based RMA, TCV is able to infect *A. thaliana* (36) and TCVΔCP can establish local infection in this host (unpublished data). We inoculated the *Arabidopsis ft-10* T-DNA insertion mutant (43) with RNA transcripts of TCV/mFTΔCP or TCV/GFPΔCP. In inoculated leaves, accumulation of recombinant viral RNAs from both RMA vectors was readily detected by RT-PCR, while no specific recombinant viral RNA was detectable in mock-inoculated control plants (Fig. 4B). Not surprisingly, no systemic spread of TCV/GFPΔCP RNA to noninoculated leaves occurred. However, the nontranslatable mutant *mFT* RNA in TCV/mFTΔCP moved and facilitated long-distance movement of viral RNAs to young leaves and the shoot apices (Fig. 4B). The nonsense mutation prohibiting FT protein synthesis from
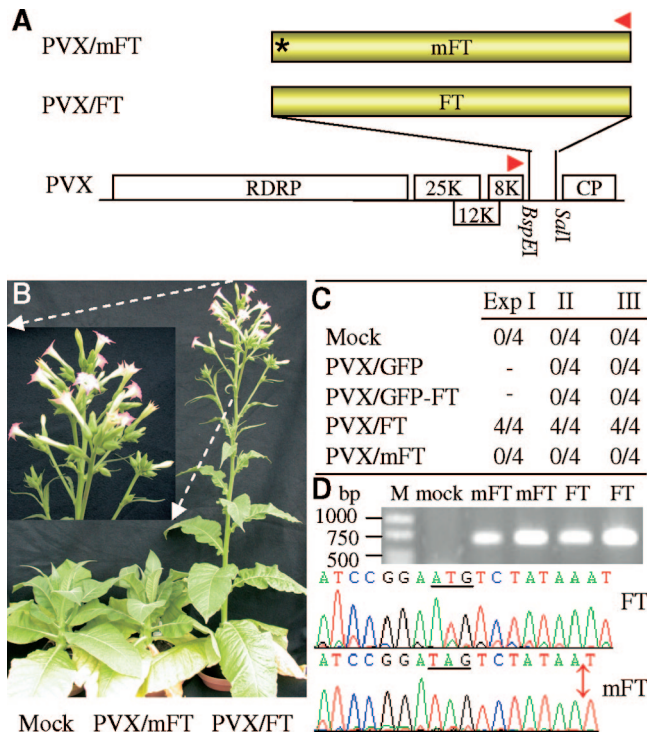
FIG. 3. Ectopic expression of *FT* induces flowering. (A) The translatable and mutated (*) nontranslatable *Arabidopsis* FT coding sequences were cloned into wild-type PVX vector to produce PVX/FT and PVX/mFT, respectively. (B and C) Floral induction caused by viral expression of FT protein. Young SD *N. tabacum* Maryland Mammoth plants were mock inoculated or infected with PVX/FT or PVX/mFT and grown under a noninducing LD photoperiod. Twelve plants infected by PVX/FT in three separate experiments started bolting at ~20 dpi, flowered at ~35 dpi, and were photographed at 42 dpi (B and inset image). Tobacco mock inoculated or infected with PVX/mFT, PVX/GFP, or PVX/GFP-FT did not flower (B and C). (D) Detection of viral transient *FT* RNA. Viral transient *FT* RNA was detected by RT-PCR using primers PP82 (▶) and PP356 (◀) in systemic young leaves from two separate plants infected with PVX/mFT (mFT) or PVX/FT (FT) but not in a mock-infected plant (mock). The position and the sizes of 1-kb DNA ladder (lane M) are indicated. Direct sequencing of RT-PCR products (648 bp) verified the presence of virally expressed wild-type and mutant *FT* RNA in flowering and nonflowering plants, respectively. The native *FT* ATG (underlined) in PVX/FT and its TAG replacement (underlined) together with a nucleotide deletion (double-arrow) in PVX/mFT are indicated.
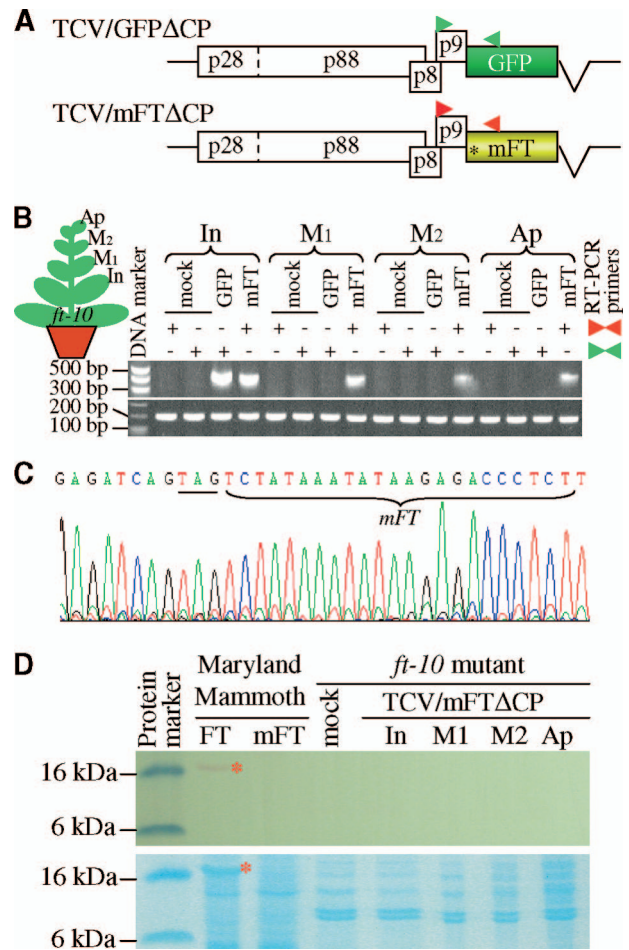

FIG. 4. (A) Movement-deficient TCV-based RMA vectors. The parental TCV vector and long-distance movement-deficient TCV/GFPΔCP were previously constructed (34). The position of the introduced stop codon (*) replacing ATG in the *FT* gene in TCV/mFTΔCP and two sets of RT-PCR primers are indicated. (B and C) RT-PCR analysis of *FT-TCV* and *GFP-TCV* RNA (top panel) using primers PP267/PP473a (▶◀ [green]) or PP267/PP228 (▶◀ [red]) and 18S rRNA (bottom panel) in inoculated (In) and newly formed young leaves (M1 and M2) and shoot apices (Ap) of *ft-10 A. thaliana* mutant plants. RNAs were extracted from tissues of plants mock-inoculated (mock) or inoculated with TCV/GFPΔCP (GFP) or TCV/mFTΔCP (mFT). The positions and sizes of the 1-kb DNA ladder are indicated. (C) Direct sequencing of *mFT* RT-PCR products (372 bp) generated from total RNA extracted from young leaves of *Arabidopsis* mutant plants inoculated with TCV/mFTΔCP. (D) Western blot analysis indicated no endogenous or viral transient FT protein was expressed in the mock-inoculated leaves (mock) or in different leaves (In, M1, and M2) and shoot apices (AP) of *ft-10* plants inoculated with TCV/mFTΔCP. Viral ectopic expression of FT protein (red asterisk) was detected in *N. tabacum* Maryland Mammoth plants infected with PVX/FT (FT) but not with PVX/mFT (mFT), a finding consistent with that the FT protein is required for floral induction (Fig. 3B). Coomassie blue-stained gel indicates the equal loading of protein samples. The positions and sizes of the protein markers are indicated.

TCV/mFTΔCP was maintained in the *Arabidopsis* mutant (Fig. 4C). Moreover, no viral transiently expressed or endogenous FT protein was detected in different leaf tissues of mock-inoculated and virus-treated *ft-10* mutant plants, a finding consistent with the mutation in *ft-10* (Fig. 4D). Long-distance trafficking of virus-derived m*FT* RNA from inoculated leaves to noninoculated leaves and the shoot apices was also detected in *ft-1* mutant plants (X. Liu, C. Li, S. Jackson, and Y. Hong, unpublished data). On the other hand, when fusing GFP downstream of FT, the nonsense mutation also eliminates GFP expression (K. Zhang and Y. Hong, unpublished data). Thus, we conclude that the systemic *FT* RNA trafficking does not require the FT protein.

Mapping of the *cis*-acting element within *Arabidopsis* FT RNA. To elucidate what controls long-distance spread of *FT*

RNA, we constructed a series of TCV/ΔCP-based RMA vectors carrying truncated nontranslatable *FT* fragments (Fig. 5A). We found that viral-derived *FT* RNAs from TCV/FTn102ΔCP, TCV/FTn201ΔCP TCV/FTn300ΔCP and TCV/FTn399ΔCP were capable of movement and facilitating sys-
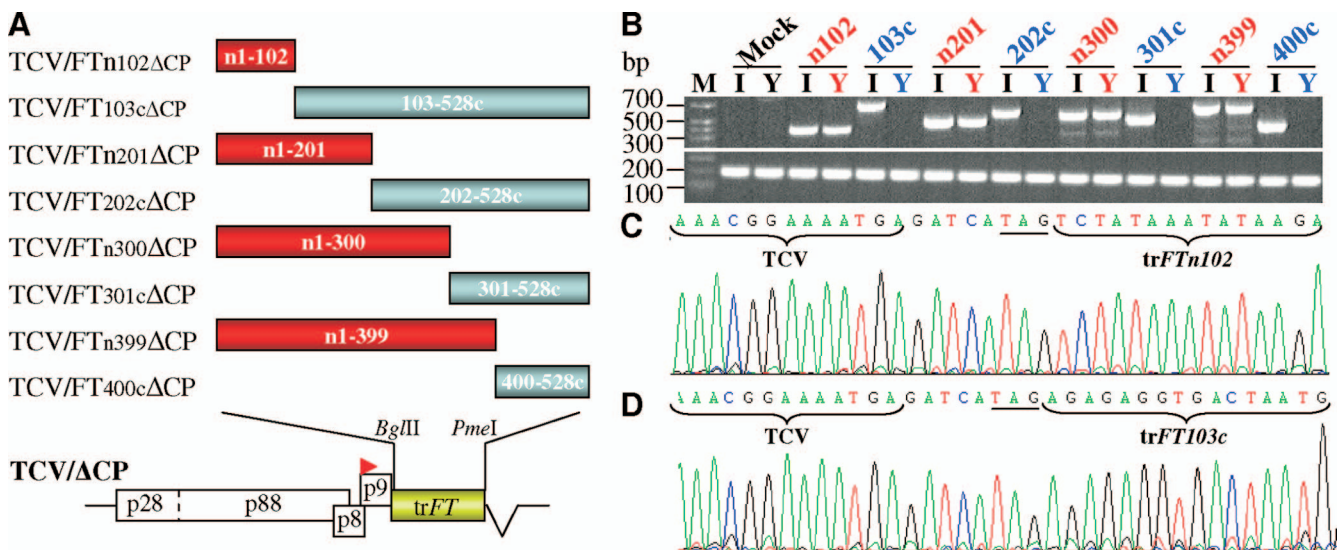
FIG. 5. Functional mapping of the *cis*-acting element that controls the *FT* RNA movement. (A) TCV/trFTΔCP-based RMA vectors carrying truncated (tr) nontranslatable *Arabidopsis FT*s. (B) Detection of virus-derived *FT* RNA (top panel) by RT-PCR in systemic young leaves (Y) from plants inoculated with TCV/FTn102ΔCP (n102), TCV/FTn201ΔCP (n201), TCV/FTn300ΔCP (n300), or TCV/FTn399ΔCP (n399); but not from plants inoculated with TCV/FT103cΔCP (103c), TCV/FT202cΔCP (202c), TCV/FT301cΔCP (301c) or TCV/FT400cΔCP (400c). Recombinant viral RNA of each TCV/trFTΔCP was readily detectible in inoculated leaves (I). No specific virus-derived FT RNA was detected in mock-inoculated plants. RT-PCR analysis of 18S rRNA (bottom panel) is included as an RNA control. (C and D) Direct sequencing of RT-PCR products verified the presence of virus-derived truncated *FT* RNA. Example sequence panels are shown for truncated *FT* RNA expressed in the young leaves of plants inoculated by TCV/FTn102ΔCP (C) or in the TCV/FT103cΔCP-inoculated leaves (D). The TAG stop codon is underlined and TCV and virus-derived *FT* sequences are indicated.

temic movement of TCV RNA. This was evident by positive RT-PCR detections of TCV and virus-derived *FT* RNAs in the noninoculated, newly developing and growing young leaves (Fig. 5B). All of the truncated *FT* RNAs possess nucleotides 1 to 102 of *FT* RNA (Fig. 5A). In striking contrast, *FT* RNAs generated from TCV/FT103cΔCP, TCV/FT202cΔCP, TCV/FT301cΔCP, and TCV/FT400cΔCP that lack the 5′-terminal 102 nucleotides were completely immobile. Consequently, RT-PCRs failed to detect any virus-derived *FT*-related RNA in the young leaves of virus-treated plants although recombinant viral RNAs accumulated in the inoculated leaves (Fig. 5B). Direct sequencing of RT-PCR products confirmed the identities of the mobile and immobile TCV and virus-derived *FT* RNA molecules (Fig. 5C and D). Thus, the element required for systemic *FT* RNA movement was unequivocally mapped to the 102-nucleotide sequence at the 5′ terminus of *FT* mRNA.

It should be noted that TCV without CP moves readily in *dcl2/dcl4* mutated *Arabidopsis* that is defective in RNA silencing. Thus, the TCV CP is mainly providing a silencing-suppression function which allows virus movement, although it may also be offering some movement support (28). This is consistent with the idea that the CP silencing-suppressor possesses a differential role in viral intercellular spread (35). However, the *Arabidopsis FT* RNA and indeed FT protein have no effect on RNA silencing (K. Zhang, C. Li, and Y. Hong, unpublished data). Therefore, *FT* RNA is only providing movement function and the *FT* RNA-mediated systemic spread of TCV seems to operate with a distinct mechanism.

The *Arabidopsis FT* RNA enhances systemic spread of PVX. The translational and nontranslational *Arabidopsis* FT coding sequences were fused in-frame with the GFP coding sequence

of PVX/GFP to produce PVX/GFP-FT and PVX/GFP-mFT (Fig. 6A), respectively. In repeated experiments, 24-day-old *Nicotiana benthamiana* plants were mock inoculated (Fig. 6B) or infected with RNA transcripts produced by in vitro transcription from PVX/GFP (Fig. 6C), PVX/GFP-FT (Fig. 6D), or PVX/GFP-mFT (Fig. 6E). Local infection of the inoculated leaves induced GFP-expressing green lesions (ca. 10 lesions per inoculated leaf) 3 to 5 dpi. However, only plants challenged with PVX/GFP-FT or PVX/GFP-mFT quickly established systemic infection at 7 dpi, showing GFP green fluorescence in newly developing young leaves. Development of systemic symptoms in plants infected by PVX/GFP took 2 to 3 extra days. Furthermore, the accumulations of free GFP and GFP-FT fusion proteins (Fig. 6F) and viral CP (Fig. 6G) in the inoculated and systemic young leaves after mock inoculation (mock) or infection of PVX/GFP (GFP), PVX/GFP-FT (GFP-FT), or PVX/GFP-mFT (GFP-mFT) were consistent with the development of viral symptoms. Western blot assays of total proteins extracted from inoculated and systemic young leaves using GFP- and PVX CP-antiserum indicated that only a trace amount of free GFP and PVX CP was detected in the inoculated leaves but not in the systemic young leaves of PVX/GFP-infected plants. However, viral CP as well as the GFP-FT fusion protein or free GFP expressed from PVX/GFP-FT or PVX/GFP-mFT, respectively, could be readily detected in both inoculated and systemic leaves.

We provide here compelling evidence that *Arabidopsis FT* RNA is capable of systemic movement and that *FT* RNA can also act as a *cis* transportation carrier for heterologous RNAs. The *FT* RNA mobile function is independent of the FT protein. Consistent with this idea, an engineered plant RNA virus
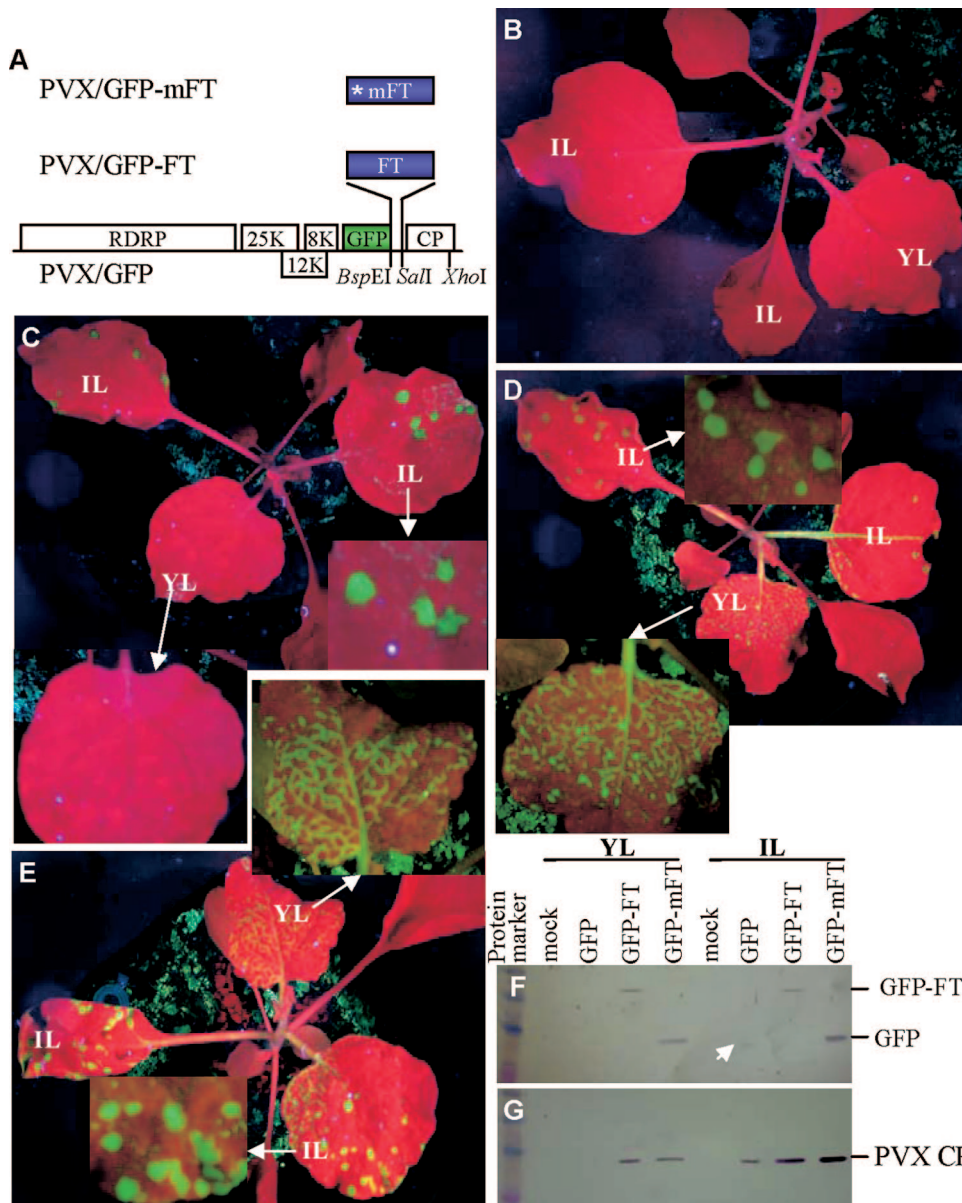
FIG. 6. *Arabidopsis FT* RNA enhances the systemic spread of PVX. PVX-based *FT* expression cassettes are schematically represented (A). The translatable and nontranslatable *Arabidopsis* FT coding sequences were fused in-frame with the GFP coding sequence of PVX/GFP by using the BspEI and SalI sites. The unique XhoI site used to produce CP-defective RMA vectors (Fig. 1A) is indicated. Twenty-four-day-old *N. benthamiana* plants were mock inoculated (B) or infected with PVX/GFP (C), PVX/GFP-FT (D), or PVX/GFP-mFT (E). (C to E) Local infection of inoculated leaves (IL) induced GFP-expressing lesions. However, only plants challenged with PVX/GFP-FT or PVX/GFP-mFT quickly established systemic infection at 7 dpi, showing GFP green fluorescence in newly developed young leaves (YL) (D and E). Moreover, protein were extracted from inoculated and systemic young leaves of plants with mock (mock) or viral infection, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and detected by Western blotting with antiserum raised against GFP (F) and PVX CP (G). The positions of free GFP, GFP-FT fusion protein, and the PVX CP are indicated. The production of viral CP, free GFP, and GFP-FT fusion proteins associated with PVX/GFP (GFP), PVX/GFP-FT (GFP-FT) or PVX/GFP-mFT (GFP-mFT) was consistent with symptom development. These data indicate that the *Arabidopsis FT* RNA with or without its translatable capacity enhances systemic spread of PVX.

carrying *Arabidopsis FT* RNA spread more quickly than its parental virus to establish infection of young tissues. It should be noted that the CP genes of PVX and TCV participate in virus-plant interactions and contribute to symptom development (5, 10, 20, 45); thus, it was not surprising that the CP-deleted recombinant viruses that were transported through the plant via *FT* RNA in *cis* could still not start a proper infection.

Moreover, we demonstrate that the systemic mobility of *FT* RNA is determined by a *cis*-acting element of 102 nucleotides at the 5′ terminus of *FT* RNA. The positive identification of *cis*-acting element for *FT* RNA trafficking provides a unique opportunity to dissect the molecular mechanism that governs cellular RNA signaling in plants. It is possible that the *cis*-RNA sequence may bind host proteins to form an RNA-protein

complex for *FT* RNA spread. Indeed, some plant and viral proteins can bind RNAs and facilitate their intercellular and long-distance trafficking (26, 27, 41, 42). The discovery that a host RNA molecule can mediate systemic trafficking of heterologous RNAs is also significant. In particular, facilitation of viral RNA movement by the *Arabidopsis FT* RNA raises the possibility that viruses and plants might have evolved similar mechanisms for systemic RNA trafficking.

There is now collective evidence that the *Arabidopsis* FT protein and its tomato SFT and rice Hd3a orthologs may act as a non-cell-autonomous flower-inducing signal that can move from the end of the vasculature into the meristematic tissue in the shoot apical meristem (7, 18, 22, 23, 29, 30, 37). Elegant experiments wherein the intercellular movement of FT protein is prevented by either a large C-terminal fusion and/or a nuclear localization signal have demonstrated that FT protein needs to move, and is sufficient on its own, to induce flowering (18, 29). FT- and Hd3a-GFP fusion proteins were found to cross a graft union from a donor scion and reach the apex of the recipient plant (7, 37), and FT-derived peptides have been identified in phloem exudates from *Cucurbita* (23), suggesting that the protein is transported through the phloem. Once the FT protein enters the apical meristem it interacts with the bZIP transcription factor FD to activate floral identity genes such as *APETELA 1* (1, 40). However, these studies do not definitely rule out a role for *FT* mRNA as part of the mobile florigenic signal. In this context, it is worthwhile noting that mRNA of the rice *FT* ortholog *Hd3a* has been detected at extremely low levels in shoot apices of rice even though the *Hd3a* promoter is not active in the shoot apical meristem (37). The evidence presented here that the *Arabidopsis FT* RNA is able to move systemically is in contrast to these recent reports where *FT* RNA movement could not be detected (7, 18, 22, 23, 29, 30), possibly due to the different host plants and experimental systems used. For example, high levels of viral *FT* expression, even from immobilized viruses in single cells, could increase the levels of systemic *FT* RNA over the detection limit. Our data raise the possibility that systemic movement of the *FT* RNA may also contribute to the long-distance florigen signaling, although the FT protein is still required to trigger flowering, as shown by the nonflowering of PVX/mFT-inoculated plants.

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