Mobile *FT* mRNA contributes to the systemic florigen signalling in floral induction

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In inducing photoperiodic conditions, plants produce a signal dubbed "florigen" in leaves. Florigen moves through the phloem to the shoot apical meristem (SAM) where it induces flowering. In *Arabidopsis*, the FLOWERING LOCUS T (FT) protein acts as a component of this phloem-mobile signal. However whether the transportable *FT* mRNA also contributes to systemic florigen signaling remains to be elucidated. Using non-conventional approaches that exploit virus-induced RNA silencing and meristem exclusion of virus infection, we demonstrated that the *Arabidopsis FT* mRNA, independent of the FT protein, can move into the SAM. Viral ectopic expression of a non-translatable *FT* mRNA promoted earlier flowering in the short-day (SD) *Nicotiana tabacum* Maryland Mammoth tobacco in SD. These data suggest a possible role for *FT* mRNA in systemic floral signalling, and also demonstrate that *cis*-transportation of cellular mRNA into SAM and meristem exclusion of pathogenic RNAs are two mechanistically distinct processes.

Photoperiodic flowering plants perceive seasonal changes by producing a phloem-mobile signal, known as florigen, which is transported through the phloem translocation stream to the shoot apical meristem (SAM) where it induces floral development. Characterization of this systemic signal has attracted great interest since the concept of florigen was proposed in the 1930s [1, 2]. In *Arabidopsis* the *FLOWERING LOCUS T* (*FT*) gene plays a major role in the induction of flowering by photoperiod [3-6]. The FT protein [7-10] and its orthologs from rice [11], cucurbit [12] and tomato [13] have been shown to be a component of the systemic flowering signal. However, using novel RNA mobility assays based on two distinct movement-defective RNA viruses, *Potato virus X* (PVX) and *Turnip crinkle virus* (TCV), we have recently demonstrated that the *Arabidopsis FT* mRNA, independent of the FT protein, moves systemically through the plant and

in cis promotes trafficking of hetrologous RNAs from one leaf to another [14]. An RNA 'zip code' mapped to the 5' nucleotides 1 to 102 of the *FT* mRNA coding sequence with predicted stem-loop structures is responsible for its mobility [14]. These findings provide new insights about the nature of the mobile florigen [15, 16]. However, whether the *FT* mRNA is capable of moving into the shoot apex is still unknown. This is an important question that needs to be addressed in order to elucidate the biological significance of the systemic movement of *FT* mRNA in the context of long-distance florigenic signalling.

In plants, there are three types of mobile RNAs including pathogenic viral and viroid RNAs, small RNAs (siRNA and microRNA), and cellular RNA transcripts [17]. Indeed, thousands of cellular transcripts have been recently identified in the *Arabidopsis* phloem, suggesting that these RNAs are capable of long-distance trafficking and may function as systemic signalling molecules [18, 19]. However not all of the phloem-based transcripts moves into meristematic cells and tissues. Only a few cellular RNAs such as the tomato *KNOTTED1*-like homeobox fusion transcript and the RNA for *GIBBERELLIC ACID-INSENSITIVE* have so far been shown to move through the phloem and into the meristem in *Arabidopsis* and tomato [20-22]. These findings suggest that plants may have evolved a selective mechanism to enable phloem-mobile RNA to access the shoot apex. However, whether common or distinct mechanisms are involved in both the selective mersistem entry of cellular RNAs and the meristem exclusion of plant viral RNAs remains to be elucidated.

Using virus-induced gene (RNA) silencing (VIGS) and in situ immune-detection assay, we present evidence that the *Arabidopsis FT* mRNA can move into the shoot apex of *Nicotiana benthamiana* and enable recombinant PVXs carrying the *FT* mRNA to overcome meristem exclusion and enter the SAM. The *FT* mRNA-mediated meristem entry does not require the FT

protein. The underlying mechanism by which the *FT* mRNA moves and transports recombinant PVX into the shoot apex does not involve suppression of RNA silencing that is known to mediate meristem exclusion of plant viral RNAs [23-27]. Furthermore, viral transient expression of a non-translatable *FT* RNA is able to induce earlier flowering in the short-day (SD) *N. tabacum* Maryland Mammoth (MM) plants in inducing SD, which demonstrates that the mobile *FT* RNA plays a role in the induction of flowering.

Results

***Arabidopsis FT* RNA promotes VIGS in SAM.** Most plant cells and tissues are vulnerable to virus infection. However, the apical meristem is generally free of viral invasion [28]. Recent studies have demonstrated that RNA silencing plays an essential role in meristem exclusion of PVX and other viruses, and that VIGS can be effectively triggered by viruses in leaves, stems and roots, but not in the apices [23-27]. Exploiting these specific properties associated with virus infection and VIGS, we utilised a series of recombinant PVX constructs (Fig. 1a) and a non-conventional approach involving VIGS, to investigate whether *FT* mRNA could move into the shoot apex. In mock-inoculated GFP transgenic *N. benthamiana* line 16c plants, GFP green fluorescence was readily visible in leaves and stems of the whole plant under long-wavelength ultraviolet light (Fig. 1b). However inoculating transgenic plants with the PVX/GFP construct expressing GFP induced VIGS of GFP mRNA, and GFP-silenced tissues showed red chlorophyll auto-fluorescence in leaves and stems (Fig. 1c). Similarly, PVX/FT-GFP and PVX/mFT-GFP constructs, expressing a translatable and non-translatable *Arabidopsis FT* mRNA (the entire coding sequence of 525 nucleotides without any 5' or 3' UTR) fused in frame to the GFP coding sequence respectively (Fig. 1a), were also able to trigger local and systemic GFP RNA silencing

(Fig. 1d, e). PVX/GFP-mediated VIGS could not spread into the shoot apex and the surrounding emerging leaves, in which the endogenous transgenic GFP expression remained strong (Fig. 1f). In striking contrast, both PVX/FT-GFP and PVX/mFT-GFP were able to promote GFP mRNA silencing in these tissues demonstrated by the remarkably weaker GFP fluorescence (Fig. 1f). Further examination by confocal microscopy confirmed GFP fluorescence in the shoot apices of line 16c plants which were either mock-inoculated (Fig. 1g) or infected with PVX/GFP (Fig. 1h), indicating that no VIGS of GFP mRNA occurred. However the PVX/FT-GFP and PVX/mFT-GFP constructs were able to enter the shoot apex and effectively induce VIGS of GFP expression (Fig. 1i, j). Moreover, PVX/GFP-FTn102 carrying the 102-nt *FT* RNA movement domain fused to GFP was also found to be capable of moving into and promoting VIGS of GEP expression in the shoot apex (Fig. S1). These phenomena were observed in all 6-9 plants treated with different recombinant viruses, suggesting that the *Arabidopsis FT* mRNA, independent of the FT protein, can move into the shoot apex and is also able to promote entry of associated plant viral RNAs into the shoot apex and induce VIGS there.

Arabidopsis FT **RNA overcomes meristem exclusion of PVX.** PVX/GFP, like wild-type PVX, infects *N. benthamiana* effectively. Systemic infection of plants appeared at 7 – 9 days post-inoculation (dpi). Extensive spread of PVX/GFP throughout infected plants at 12 dpi was readily detectable by GFP green fluorescence. However, in the shoot apex no GFP fluorescence was observed (Fig. S2). Exclusion of PVX/GFP from the SAM was further demonstrated by *in situ* immunocytochemical assays using an antibody specifically raised against the PVX coat protein (CP). CP-antibody probing of sections of 4 *N. benthamiana* plants with severe systemic symptoms following PVX/GFP infection at 12 dpi showed that PVX/GFP was transported via

the phloem to the base of the shoot apex where pink staining of the antibody was evident. PVX/GFP was absent, and no virus was detected, in the SAM of the same infected plants (Fig 2a). However, insertion of the *Arabidopsis FT* mRNA coding sequence (528 nucleotides including the stop codon) into the viral positive sense RNA genome enabled PVX/FT to invade the shoot apex. This recombinant virus was constantly found to be present in different types of cells and tissues (pink staining), including the meristematic layers of the apical dome and leaf primordia (Fig. 2b). Moreover, a non-translatable *FT* mRNA also enabled the entry of PVX/mFT into the shoot apex (Fig. 2c) of all infected plants.

Western analysis using specific antibodies raised against PVX CP, Arabidopsis FT or GFP, was used to confirm the presence/absence of these proteins in virus-infected plants. PVX CP was readily detected in all infected plants, but not in the mock-inoculated control (Fig. 3a), consistent with systemic viral symptom development observed in these plants. Whilst viral expression of GFP was only observed in plants infected with PVX/GFP (Fig. 3b), expression of the *Arabidopsis* 19.8 kDa FT protein, however, was only detectable in PVX/FT but not PVX/mFT or PVX/GFP infected plants (Fig. 3c). Considering the equal loading of total soluble cellular proteins in each lane (Fig. 3d), these data support the conclusion that the acquired mobility of PVX/FT and PVX/mFT into the shoot apex is directly associated with *FT* mRNA and is independent of FT protein.

Taken together, our data demonstrate that whilst PVX is normally excluded from the shoot apex, *FT* RNA, no matter whether it possesses the capability to translate the FT protein or not, functions *in cis* to overcome the viral meristem exclusion mechanism.

Ectopic expression of a non-translatable *Arabidopsis FT* mRNA triggers early flowering. To address the potential significance of *FT* RNA trafficking in floral induction, we exploited a virus-induced flowering assay in SD *N. tabacum* MM tobacco [14]. In SD inducing conditions, mock-inoculated MM plants bolted, produced floral buds and eventually open flowers (Fig. 4a). Remarkably, PVX/mFT-treated MM plants started to bolt approx 1-2 weeks earlier than mock inoculated control MM plants. Viral expression of the non-translatable *Arabidopsis FT* mRNA also resulted in early budding and flowering (Fig. 4a, Fig. 5a). RT-PCR analyses confirmed that the non-translatable *FT* mRNA was present in PVX/mFT-treated, but not mock-inoculated MM plants (Fig. 4b). The double mutations that preclude translation of the FT protein were maintained in the recombinant viral RNA, as shown by direct sequencing of the RT-PCR product (Fig. 4c). Consistent with these data, western blotting detected no *Arabidopsis* FT protein in these MM plants although the FT protein was readily detectable in MM plants infected with PVX/FT (Fig. 4d-f). On the other hand, in long-day (LD) non-inducing conditions, all MM plants that had been mock-inoculated or infected with PVX/mFT neither bolted nor flowered, and remained vegetative (Fig. 4g, h), although expression of wild-type *FT* mRNA and the free FT protein from PVX/FT was able to stimulate early flowering in both LD (Fig. 4i) and SD (Fig. 4j-l, Fig. 5b). These data suggest that the non-translatable *Arabidopsis FT* mRNA alone is not a direct inducer or gene expression regulator that is essential for MM plants to flower as it does not induce flowering in non-inducing LD conditions. Instead, the non-translatable *FT* mRNA promotes earlier flowering, perhaps by acting as a transporter that facilitates the long-distance trafficking of the endogenous tobacco FT protein from the leaf to the SAM where it triggers flowering in SD.

Discussion

Arabidopsis FT mRNA, independent of the FT protein, is capable of long distance movement and can act as a *cis* transportation carrier for heterologous RNAs [14]. In this report, we further demonstrate that *FT* mRNA can move into the SAM, and is also able to promote entry into the SAM of recombinant viruses that carry translatable (wild-type) or non-translatable *FT* mRNA sequences (Figs. 1-3). However, the underlying mechanism by which the *Arabidopsis FT* mRNA transports PVX into the shoot apex remains to be elucidated. Phloem-mobile cellular RNA is known to be selectively taken up into the shoot apex whilst most viruses and viral RNAs are excluded from this region. There is evidence that RNA silencing, a cellular antiviral surveillance system, is involved in viral meristem exclusion. Prevention of meristem invasion by PVX requires RDR6 that is involved in the biogenesis of siRNA which is an immediate response that restricts the systemic spread of viruses into the shoot meristem [26, 27]. Moreover, RNA viruses are able to invade the shoot apex in plants expressing viral suppressors of silencing [23, 24]. This raises the possibility that the *Arabidopsis FT* RNA may suppress silencing to enable entry of PVX/FT or PVX/mFT into the shoot apex. However because both PVX/GFP-FT and PVX/GFP-mFT were able to trigger RNA silencing in leaves, stems and shoot apices (Fig. 1), an silencing suppression mechanism is unlikely to be involved in the movement of these recombinant viruses into the SAM. This idea was further confirmed by a local RNA silencing assay (Supplementary Text; Fig. S3). Therefore, Arabidopsis *FT* RNA meristem entry occurs independently of RNA silencing suppression.

On the other hand, plants have evolved selective mechanisms that govern entry of RNA into meristems where germ cells arise after vegetative development has ceased [29]. Protection of the shoot apex from pathogenic RNA invasion and from random endogenous RNAs is essential

for plants to ensure the integrity of the germline. Our findings show that *FT* RNA overcomes selective meristem exclusion and suggest that the mechanism controlling the selective entry of endogenous plant RNAs into the SAM may be different from the silencing-mediated meristem exclusion of viral RNA (Fig. 1; Fig. S3). Such selective entry may require specific host proteins that bind to the endogenous RNA [30-33]. Thus, the *FT* mRNA may bind to an escort/gateway protein that moves it across.

There are numerous examples in animal and plant systems for intra- and inter-cellular mobile RNAs [34]. In animals, mRNAs are transported within the cell in a tightly regulated process to facilitate their function. For instance, the Staufen protein of *Drosophila* and the *bicoid* and *oskar* mRNAs involves RNA-protein, RNA-RNA and protein-protein interactions to determine their transportation, localization and repression of translation of the mRNAs in the egg cell [35, 36]. In plants, intra- and intercellular RNA trafficking also plays an important role in growth, development and responses to stresses [34]. It has been well documented that specific RNA motifs are required for viroid RNA movement [37]. Another example includes that the *Arabidopsis GA-INSENSITIVE* (*GAI*) RNA constitutes motifs that are essential for its own long distance movement and transportation of heterlogous RNA [38]. Moreover, an RNA-protein complex with six mRNAs and approx. 16 proteins that transports the pumpkin *GAI* RNA has been identified [38] and the key RNA-binding protein has recently been found to be a polypyrimidine tract binding protein, designated CmRBP50 [39]. Nevertheless, the precise nature of the mobile *FT* mRNA zip code and potential *FT* RNA-interacting cellular factors that may be required for the systemic florigenic signalling remains to be elucidated.

The long-distance transport of wild-type and mutant non-translatable *FT* mRNA within plants [14] and their ability to enter the meristem raises the question of the biological significance

of *FT* mRNA movement in systemic floral signalling. Both wild-type *FT* mRNA and FT protein have been detected in the shoot apex of rice at low levels, even though *FT* is not expressed there [11]. It has already been established that *Arabidopsis* FT and its tomato SFT, rice Hd3a and Cucurbit Cm-FTL1/2 protein orthologs [7-12] act as a component of the systemic flowering signal. FT-derived peptides have been identified in phloem exudates [12, 41], suggesting that the protein is transported through the phloem into the shoot apical meristem. Once FT is present in the shoot apex, it interacts with a basic region/leucine zipper transcription factor FD encoded by *FLOWERING LOCUS D* to activate floral identity genes such as *APETALA 1* and *SUPPRESSOR OF OVEREXPRESSION OF CO1,* the latter activates *LEAFY* and induces flowering [4, 6]. These data do not rule out a possible role for *FT* mRNA as part of, or in promoting movement of, the florigenic signal that moves from the leaf to the shoot apex to induce flowering. Indeed, viral ectopic expression of the wild-type and the non-translatable *Arabidopsis FT* mRNA promoted earlier floral induction in SD MM tobacco plants under the inductive SD conditions (Fig. 4; Fig. 5), suggesting both the wild-type and the mutant *FT* RNA contribute to the long-distance signalling in floral induction. A plausible explanation for this phenomenon could be that the viral-derived wild-type or non-translatable mutant *Arabidopsis FT* RNA could function as a carrier to enable a more efficient transport of the endogenous tobacco FT protein produced in SD from the leaf to the shoot apical meristem, thus promoting earlier flowering in the PVX/mFT-inoculated plants. Therefore, this mobile wild-type or mutant *Arabidopsis FT* RNA does not necessarily need to be translated into protein in the SAM to have a promotive effect on floral induction.

In summary, our data together with previous findings [14] suggest a possible role for *FT* mRNA in promoting the florigen movement from leaf to SAM to induce flowering. Although the

underpinning mechanism for this process remains to be elucidated, it is tempting to propose that *FT* mRNA may function as a protein transporter to transfer an integrated florigenic complex. Physical separation of *FT* mRNA and the FT protein or RNA/protein structural modifications could lead to disruption of the systemic florigen signalling [9, 10].

Methods

Details of methods and materials are provided in Supplementary Information.

1. Zeevaart, J. A. D. Physiology of Flower Formation. *Annu. Rev. Plant Physiol.* **27,** 321-348 (1976).
2. Corbesier, L. & Coupland, G. The quest for florigen: a review of recent progress. *J. Exp. Bot.* **57,** 3395-3403 (2006).
3. Tatada, S. & Goto, K. TERMINAL FLOWER2, an *Arabidopsis* homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of *FLOWERING LOCUS T* by CONSTANS in the vascular tissues of leaves to regulate flowering time. *Plant Cell* **15,** 2856-2865 (2003).
4. Abe, M. *et al.* FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309,** 1052-1056 (2005).
5. An, H. *et al.* CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* **131,** 3615-3526 (2004).
6. Wigge, P. A. *et al.* Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* **309,** 1056-1059 (2005).

7. Corbesier, L. *et al.* FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* **316,** 1030-1033 (2007).
8. Jaeger, K. E. & Wigge, P. A. FT protein acts as a long-range signal in *Arabidopsis. Curr. Biol.* **17,** 1050-1054 (2007).
9. Mathieu, J., Warthmann, N., Küttner, F. & Schmid, M. Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis. Curr. Biol.* **17,** 1055-1060 (2007).
10. Notaguchi, M. *et al.* Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering. *Plant Cell Physiol.* **49,** 1645-1658 (2008).
11. Tamaki, S., Matsuo, S., Wong, H. L., Yokoi, S. & Shimamoto, K. Hd3a protein is a mobile flowering signal in rice. *Science* **316,** 1033-1036 (2007).
12. Lin, M. K. *et al.* FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the Cucurbits. *Plant Cell* **19,** 1488-1506 (2007).
13. Lifschitz, E. *et al.* The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli**.** *Proc. Natl. Acad. Sci. USA* **103,** 6398-6403 (2006).
14. Li, C. *et al.* A *cis* element within *Flowering Locus T* mRNA determines its mobility and facilitates trafficking of heterologous viral RNA. *J. Virol.* **83,** 3540-3548 (2009).
15. Kragler, F. RNA in the phloem: A crisis or a return on investment? *Plant Sci* **178,** 99-104 (2010).
16. Yant, L., Mathieu, J. & Schmid, M. Just say no: floral repressors help *Arabidopsis* bide the time. *Curr. Opin. Plant Biol.* **12,** 580-586 (2009).

17. Kehr, J. & Buhtz, A. Long distance transport and movement of RNA through the phloem. *J. Exp. Bot.* **59,** 85-92 (2008).
18. Deeken, R. *et al.* Identification of *Arabidopsis* thaliana phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. *Plant J.* **55,** 746-759 (2008).
19. Hannapel, D. J. A model system of development regulated by the long-distance transport of mRNA. *J. Integr. Plant Biol.* **52,** 40-52 (2010).
20. Haywood, V., Yu, T. S., Huang, N. C. & Lucas, W. J. Phloem long-distance trafficking of GIBBERELLIC ACID-INSENSITIVE RNA regulates leaf development. *Plant J.* **42,** 49-68 (2005).
21. Kim, M., Canio, W., Kessler, S. & Sinha, N. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. *Science* **293,** 287-289 (2001).
22. Xoconostle-Cázares, B. *et al.* Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* **283,** 94-98 (1999).
23. Foster, T. M. *et al.* A Surveillance System Regulates Selective Entry of RNA into the Shoot Apex. *Plant Cell* **14,** 1497–1508 (2002).
24. Martín-Hernández, A. M. & Baulcombe, D. C. Tobacco rattle virus 16-kilodalton protein encodes a suppressor of RNA silencing that allows transient viral entry in meristem. *J. Virol.* 82, 4064-4071 (2008).
25. Mochizuki, T. & Ohki, S. T. Shoot meristem tissue of tobacco inoculated with Cucumber mosaic virus is infected with the virus and subsequently recovers from infection by RNA silencing. *J. Gen. Plant Pathol.* **70,** 363-366 (2004).

26. Qu, F. *et al.* RDR6 has a broad-spectrum but temperature-dependent antiviral defense role in *Nicotiana benthamiana*. *J. Virol.* **79,** 15209-15217 (2005).

27. Schwach, F., Vaistij, F. E., Jones, L. & Baulcombe, D. C. An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal. *Plant Physiol.* 138:1842-1852 (2005).

28. Matthews, R. E. F. Plant Virology, *Ed3*. Academic Press, San Diego (1990).

29. Lucas, W. J. Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virology* **344,** 169-184 (2006).

30. Lucas, W. J. *et al.* Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* **270,** 1980-1983 (1995).

31. Ruiz-Medrano, R., Xoconostle-Cázares, B., Lucas, W. J. Phloem long-distance transport of *CmNACP* mRNA: implications for supracellular regulation in plants. *Development* **126,** 4405-4419 (1999).

32. Irish, V. F. & Sussex, I. M. A fate map of the *Arabidopsis* embryonic shoot apical meristem. *Development* **115,** 745-753 (1992).

33. Kim, J. K., Rim, Y., Wang, J. & Jackson, D. A novel cell-to-cell trafficking assay indicates that KNOX homeodomain is necessary and sufficient for intercellular protein and mRNA trafficking. *Genes Dev.* **19,** 788-793 (2005).

34. Hannapel, D. J. A model system of development regulated by the long-distance transport of mRNA. *J. Integr. Plant Biol.* **52,** 40-52 (2010).

35. Ferrandon, D., Koch, I., Westhof, E. & Nusslein-Volhard, C. RNA-RNA interaction is required for the formation of specific *bicoid* mRNA 3' UTR-STAUFEN ribonucleoprotein particles. *EMBO J.* **16,** 1751-1758 (1997).

36. Elvira, G., Massie, B. & DesGroseillers, L. The zinc-finger protein ZFR is critical for Staufen 2 isoform specific nucleocytoplasmic shuttling in neurons. *J. Neurochem.* **96,** 105-117 (2006).

37. Wang, Y. & Ding, B. Viroids: Small probes for exploring the vast universe of RNA trafficking in plants. *J. Integr. Plant Biol.* **52,** 28-39 (2010).

38. Huang, N. C. & Yu, T. S. The sequences of *Arabidopsis GA-INSENSITIVE* RNA constitute the motifs that are necessary and sufficient for RNA long-distance trafficking. *Plant J.* **59,** 921-929 (2009).

39. Ham, B. K., Brandom, J. L., Xoconostle-Cazares, B., Ringgold, V., Lough, T. L. & Lucas, W. J. A polypyrimidine tract binding protein, pumpkin RBP50, forms the basis of a phloem-mobile ribonucleoprotein complex. *Plant Cell* **21**, 197–215 (2009).

40. Li, P., Ham, B. & Lucas, W. J. CmRBP50 protein phosphorylation is essential for assembly of a stable phloem-mobile high-affinity ribonucleoprotein complex. *J. Biol. Chem.* **286,** 23142-23149 (2011).

41. Giavalisco, P., Kapitza, K., Kolasa, A., Buhtz, A. & Kehr, J. Towards the proteome of *Brassica napus* phleom sap. *Proteomics* **6,** 896-909 (2006).

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Author contributions

C.L. and M.G. designed and performed experiments; N.S., H.Z. and X.Y. performed researches; T.O., Y.L., H.W. M.V. and S.J. contributed through discussion and revised paper. Y.H. initiated the project, designed and performed experiments and wrote the paper.

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Figure legends

Figure 1 | *Arabidopsis FT* RNA enables virus-induced RNA silencing (VIGS) in shoot apex. (**a**) A diagrammatic representation of recombinant PVX vectors. The introduced stop codon (*) that replaces the *FT* start codon in the *FT* mutant *mFT* to prevent translation is indicated. Gene of interest (GOI) indicates the position of individual gene insertion in the PVX genome. (**b-d**) Systemic VIGS of GFP expression in leaves and stems of the GFP transgenic *Nicotiana benthamiana* line 16c plants. Plants were mock inoculated (**b**), or inoculated with PVX/GFP (**c**), PVX/FT-GFP (**d**) or PVX/mFT-GFP (**e**). (**f**) VIGS in stem tips of line 16c plants mock inoculated (mock), or infected with PVX/GFP (GFP), PVX/FT-GFP (FT-GFP), or PVX/mFT-GFP (mFT-GFP). (**g-h**) VIGS in shoot apices and surrounding young leaves of line 16c plants mock inoculated (**g**) or infected with PVX/GFP (**h**), PVX/FT-GFP (**i**), or PVX/mFT-GFP (**j**). Photographs were taken at 21 days post-inoculation under long-wavelength UV illumination

through a yellow Kodak No. 58 filter (**b-f**), and using a Zeiss LSM710 Laser Scanning Microscope (**g-j**) through transmitted light (TM) to show the outlines of the shoot apices and their surrounding tissues, or through lasers to show green (GFP) and red (Chlorophyll) fluorescence. The merged images of green and red fluorescence (GFP + Chlorophyll) are displayed (**g-h**). GFP-expressing tissues showed green fluorescence and tissues with silencing of GFP mRNA by VIGS appeared red due to chlorophyll fluorescence. Shoot apices and surrounding tissues are indicated by arrows. Bar = 1 mm.

Figure 2 | *FT* RNA-mediated PVX entry into SAM. *Arabidopsis FT* mRNA enables entry of PVX into the shoot apical meristem (SAM). *Nicotiana benthamiana* plants were infected with PVX/GFP, PVX/FT or PVX/mFT (Fig. 1a). Plant tissues were collected at 12 days post-inoculation and sections were probed with (+) or without (-) an antiserum specifically raised against the PVX coat protein (CP). Pink colour indicates the presence and distribution of recombinant viruses within infected cells and tissues. PVX/GFP (**a**) cannot enter SAM, but PVX/FT (**b**) and PVX/mFT (**c**) overcome SAM exclusion. Bar = 100 µm.

Figure 3 | Viral ectopic expression of the *Arabidopsis* FT protein. Total soluble proteins were extracted from young leaf tissues of *Nicotiana benthamiana* plants mock-inoculated or infected with PVX/GFP, PVX/FT or PVX/mFT at 12 days post-inoculation and analysed by western blot using antibodies specific to PVX CP (**a**), GFP (**b**) and FT (**c**). Coomassie blue-stained gel (**d**) shows equal loading of soluble protein samples. The position and sizes of the protein markers are indicated.

Figure 4 | Viral transient expression of a non-translatable *mFT* RNA triggers early flowering. (**a**) Virus-based flowering assay. MM plants were either mock inoculated (mock) or treated with PVX/mFT (mFT) and grown under SD with a 12-hr photoperiod of light and at 25 °C. An arrow indicates a flower bud at the shoot tip of a mock-inoculated plant whilst a dozen flowers are blossoming in an *mFT* RNA expressing plant. Photographs were taken at 10 weeks post-inoculation (wpi). (**b**) RT-PCR detection of viral transient *mFT* RNA in MM plant treated with PVX/mFT (mFT), but not mock inoculation (mock). The 1-Kb DNA marker (M) (1500, 1000, 750, 500 and 400-bp from top to bottom) is included to show the expected size (684 bp) of the RT-PCR product. (**c**) Direct sequence of the RT-PCR product verified the viral transient mFT RNA, showing the double mutations. Mutation of the native *FT* start codon ATG with TAG (asterisked) and a nucleotide deletion (Δ) are indicated. The codon triplets are underlined. (**d-f**) Western blot detection of the *Arabidopsis* FT protein (**d**) in MM plants treated with PVX/FT (FT), but not with mock inoculation (mock) or PVX/mFT (mFT); of the PVX coat protein (**e**) in plants treated with PVX/mFT or PVX/FT, but not in mock-inoculated plant. Coomassie blue-stained gel indicates the equal loading of protein samples (**f**). The positions and sizes of the protein marker (M) are indicated. (**g-i**) MM plants mock-inoculated (**g**), or treated with PVX/mFT (**h**) or PVX/FT (**i**) were grown under a non-inducing LD 16-hr photoperiod and only plants treated with PVX/FT flowered. Photographs were taken at 10 wpi. (**j-l**) MM plants mock-inoculated (**j**), or treated with PVX/mFT (**k**) or PVX/FT (**l**) were grown in SD. Plants with PVX/FT treatment developed buds at 4 wpi and flowered at 6 wpi whilst plants mock-inoculated or inoculated with PVX/mFT were only bolting during these periods. Photographs were taken at 6 wpi.

Figure 5 | Analyses of budding and flowering time and numbers of floral buds and flowers. (**a**) PVX/mFT-based flowering assays in SD. Seven – nine *N. tabacum* MM plants were either mock-inoculated (mock) or infected with PVX/mFT (mFT) that had the capacity to express a non-translatable *Arabidopsis FT* mRNA, but not the FT protein. Numbers of floral buds and flowers in individual plants were counted weekly starting at 7 weeks post-inoculation (wpi) until 13 wpi. Among PVX/mFT-treated MM plants, first floral buds and flowers appeared at 7 and 10 wpi, respectively. At 11 wpi, more than 45% of floral buds converted into flowers. However, only half of mock-inoculated plants started to produce floral buds at 9 wpi and less than 18% of floral buds converted into flowers at 11 wpi. The average numbers of combining floral buds and flowers per plant [25 ± 7 (n = 7) vs 10 ± 9 (n = 9), p = 0.002; 30 ± 5 vs 18 ± 9, p = 0.005] were significantly higher (Student's *t*-Test) in PVX/mFT- than mock-treated plants at (before) 10 and 11 wpi, but showed no significant difference (33 ± 3 vs 24 ± 11, p = 0.094) at 13 wpi. These data suggest that viral transient expression of the non-translatable *Arabidopsis FT* mRNA induced earlier budding and flowering in MM plants in SD. (**b**) PVX/FT-based flowering assays in SD. Eight MM plants were treated with PVX/FT (FT) that was capable of expressing a wild-type FT gene. Viral transient expression of wild-type *FT* mFT RNA and the FT protein facilitated much earlier development of floral buds and flowers. This important baseline information indicates that the *FT* mRNA and its protein product had a synergetic effect on systemic florigenic signalling.