FT Protein Movement Contributes to Long-Distance Signaling in Floral Induction of Arabidopsis
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27. The change in Coulomb failure stress is defined as $\Delta CFS = \Delta\sigma_s + \mu\Delta\sigma_n$, where $\Delta\sigma_s$ is the change in shear stress, $\Delta\sigma_n$ is the change in effective normal stress, and μ is the coefficient of effective internal friction (*28*). We use $\mu = 0.4$.
28. G. C. P. King, R. S. Stein, J. Lin, *Bull. Seismol. Soc. Am.* **84**, 935 (1994).
29. S. Owen *et al.*, *Geophys. Res. Lett.* **27**, 2757 (2000).
30. S. Jonsson *et al.*, *Geophys. Res. Lett.* **26**, 1077 (1999).
31. Y. Fukushima, V. Cayol, P. Durand, *J. Geophys. Res.* **110**, B03206 (2005).
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FT Protein Movement Contributes to Long-Distance Signaling in Floral Induction of *Arabidopsis*

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

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

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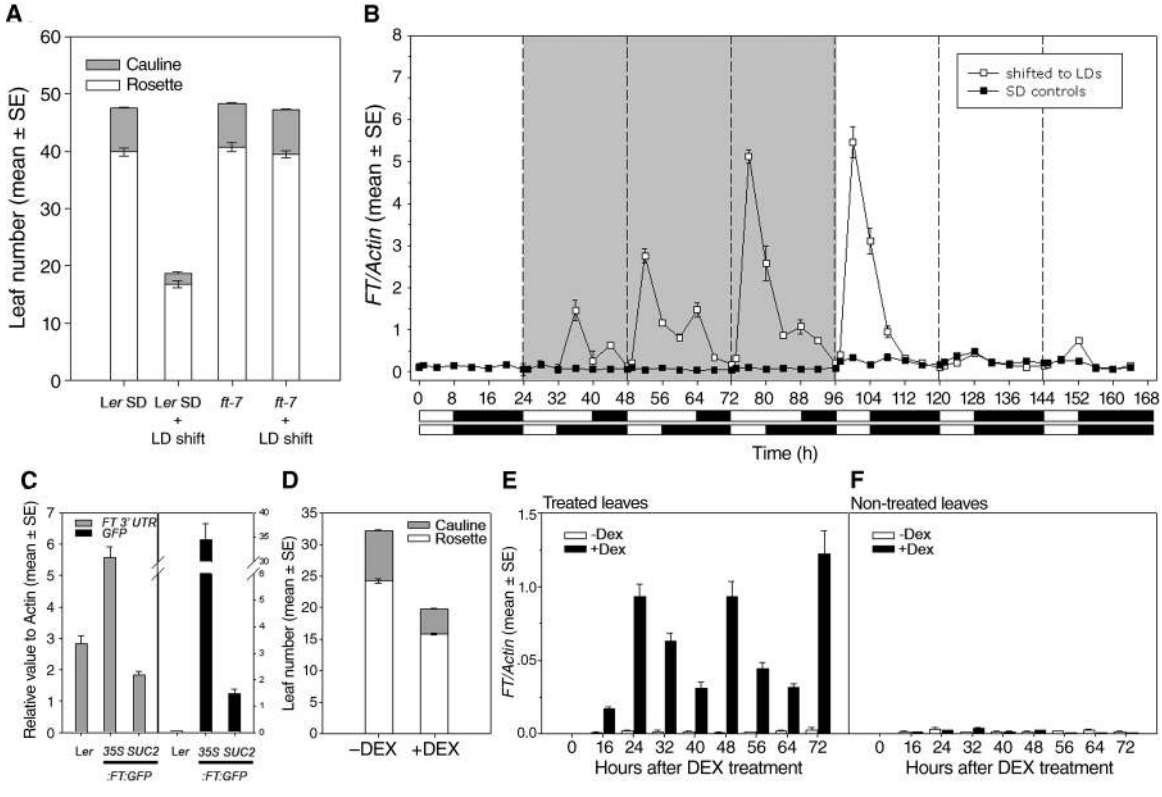


Fig. 1. Regulation of *FT* mRNA in leaves during flowering. (**A**) Flowering time of wild-type *Ler* and *ft-7* plants grown for 2 weeks under SD and exposed to three inductive LDs before return to SDs. (**B**) Expression of *FT* mRNA during 7 days comprising one SD followed by three LDs and then three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

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

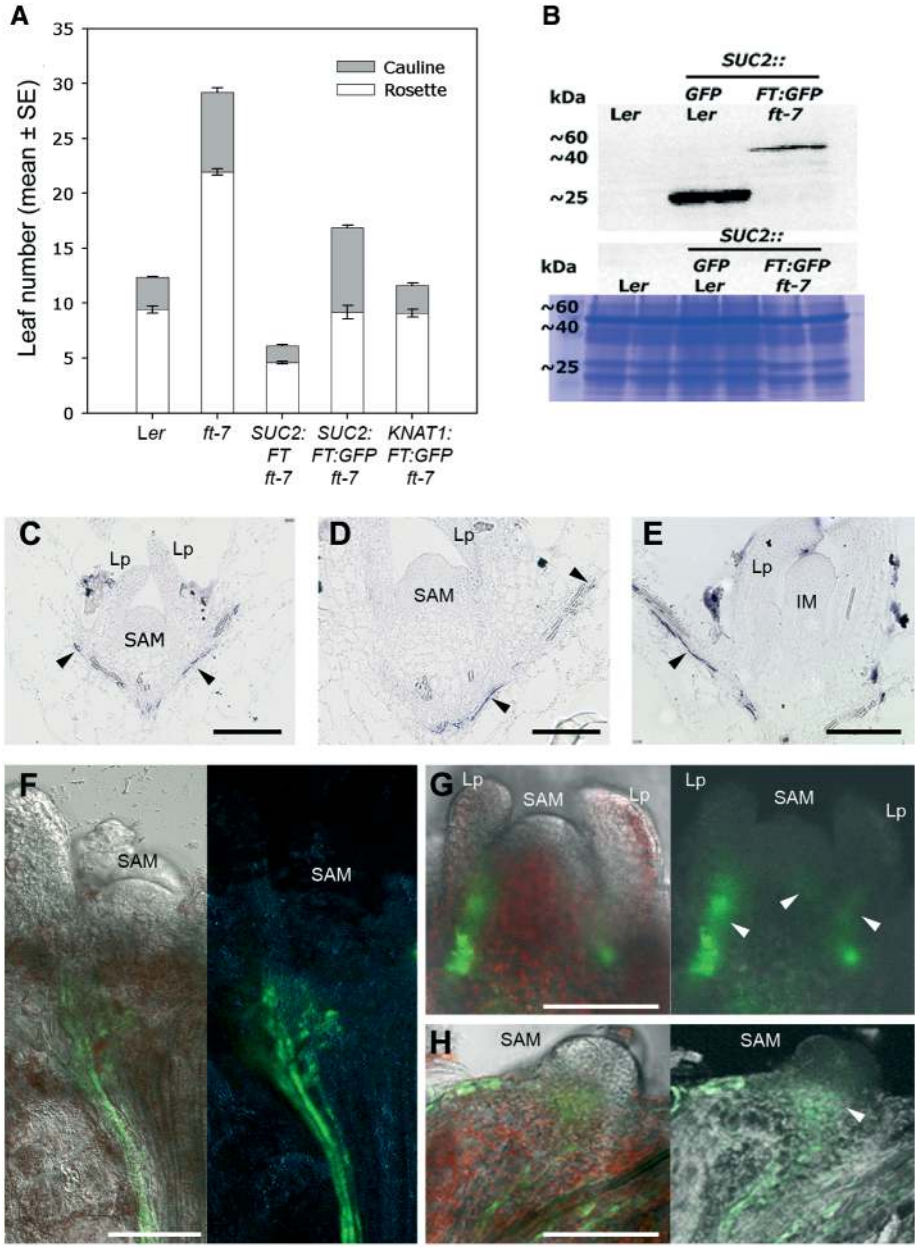


Fig. 2. Analysis of FT:GFP protein distribution in *SUC2:FT:GFP ft-7*. (**A**) Flowering time expressed as total leaf number (rosette and cauline) of representative transformants grown in LDs and compared with *Ler* and *ft-7*. (**B**) Western blot analysis showing expression of the intact FT:GFP fusion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

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

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

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

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

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

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

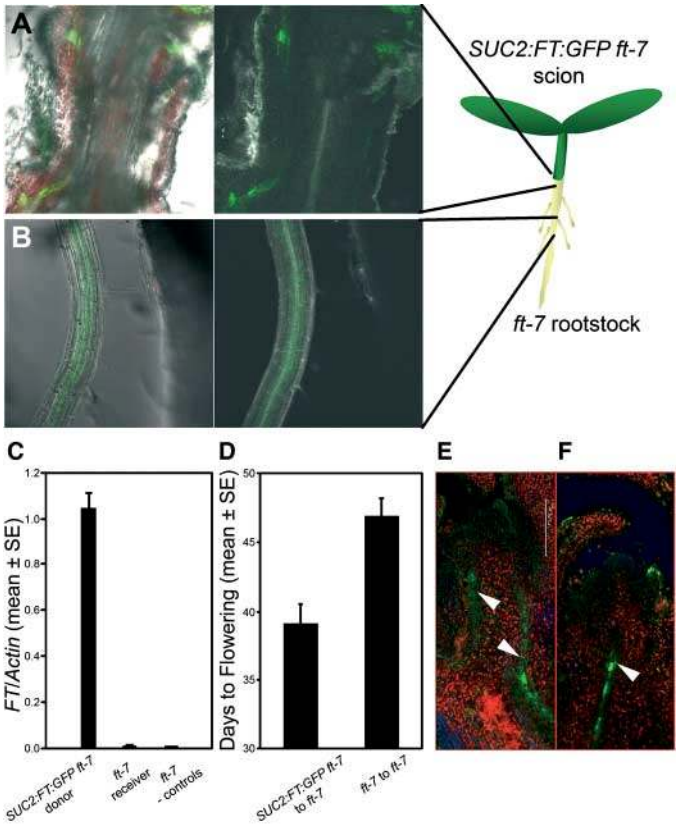
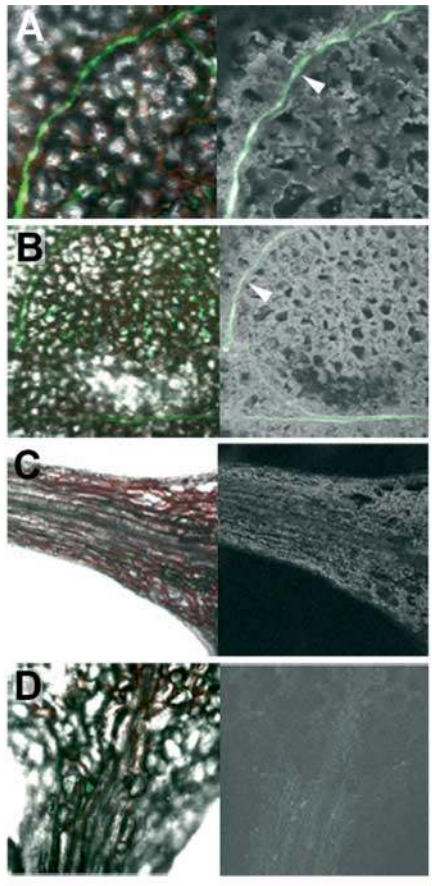


Fig. 3. Grafting of *SUC2:FT:GFP ft-7* plants to *ft-7* mutants. (**A** to **C**) Root grafting: Distribution of the FT:GFP fusion protein and *FT:GFP* mRNA. Confocal analysis of the distribution of FT:GFP fusion protein demonstrates that the protein is able to cross a graft junction (A) and can be detected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



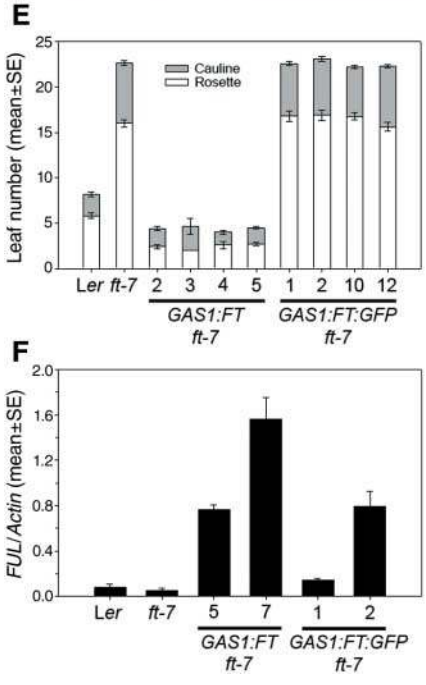


Fig. 4. Expression of FT:GFP in the minor veins alters gene expression patterns but does not induce flowering. (**A** to **D**) Confocal images of leaves expressing *GAS1:FT:GFP:GFP ft-7*. The GFP signal is detected in the minor veins [arrows in (A) and (B)] but not in the petiole (C) or the midrib (D).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

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

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

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

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

References and Notes

1. J. E. Knott, *Proc. Am. Soc. Hort. Sci.* **31**, 152 (1934).
2. J. A. D. Zeevaart, *Annu. Rev. Plant Physiol.* **27**, 321 (1976).
3. I. Searle, G. Coupland, *EMBO J.* **23**, 1217 (2004).
4. T. Imaizumi, S. A. Kay, *Trends Plant Sci.* **11**, 550 (2006).
5. I. Bäurle, C. Dean, *Cell* **125**, 655 (2006).
6. H. An *et al.*, *Development* **131**, 3615 (2004).
7. P. A. Wigge *et al.*, *Science* **309**, 1056 (2005).
8. A. Samach *et al.*, *Science* **288**, 1613 (2000).
9. M. Abe *et al.*, *Science* **309**, 1052 (2005).
10. Y. Kobayashi, H. Kaya, K. Goto, M. Iwabuchi, T. Araki, *Science* **286**, 1960 (1999).
11. I. Kardailsky *et al.*, *Science* **286**, 1962 (1999).
12. H. Böhlenius, S. Eriksson, F. Parcy, O. Nilsson, *Science* **316**, 367 (2007).
13. E. Lifschitz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6398 (2006).
14. A. Lang, in *Encyclopedia of Plant Physiology*, W. Ruhland, Ed. (Springer-Verlag, Berlin, 1965), vol. XV/1, pp. 1380–1536.
15. J. A. D. Zeevaart, *Meded. Landbouwhogesch. Wageningen* **58**, 1 (1958).
16. Materials and methods are available as supporting material on *Science* Online.
17. T. Imaizumi, H. G. Tran, T. E. Swartz, W. R. Briggs, S. A. Kay, *Nature* **426**, 302 (2003).
18. P. Suarez-Lopez *et al.*, *Nature* **410**, 1116 (2001).
19. F. Valverde *et al.*, *Science* **303**, 1003 (2004).
20. M. J. Yanovsky, S. A. Kay, *Nature* **419**, 308 (2002).
21. S. Takada, K. Goto, *Plant Cell* **15**, 2856 (2003).
22. A. Imlau, E. Truernit, N. Sauer, *Plant Cell* **11**, 309 (1999).
23. T. J. Lough, W. J. Lucas, *Annu. Rev. Plant Biol.* **57**, 203 (2006).
24. C. G. N. Turnbull, S. Justin, *Flower. Newsl.* **37**, 3 (2004).
25. I. Searle *et al.*, *Genes Dev.* **20**, 898 (2006).
26. M. Schmid *et al.*, *Development* **130**, 6001 (2003).
27. E. Haritatos, B. G. Ayre, R. Turgeon, *Plant Physiol.* **123**, 929 (2000).
28. B. G. Ayre, R. Turgeon, *Plant Physiol.* **135**, 2271 (2004).
29. P. Teper-Bamnolker, A. Samach, *Plant Cell* **17**, 2661 (2005).
30. P. Giavalisco, K. Kapitza, A. Kolasa, A. Buhtz, J. Kehr, *Proteomics* **6**, 896 (2006).
31. S. Tamaki, S. Matsuo, H. L. Wong, S. Yokoi, K. Shimamoto, *Science* **316**, 1033 (2007).
32. K. L. Gallagher, P. N. Benfey, *Genes Dev.* **19**, 189 (2005).
33. M. Kim, W. Canio, S. Kessler, N. Sinha, *Science* **293**, 287 (2001).
34. V. Haywood, T.-S. Yu, N.-C. Huang, W. J. Lucas, *Plant J.* **42**, 49 (2005).

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Hd3a Protein Is a Mobile Flowering Signal in Rice

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

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

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