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 and initiate long-distance signaling that induces flowering at the shoot apex. The identity of the long-distance signal is unknown. In Arabidopsis activation of FLOWERING LOCUS T (FT) transcription in leaf vascular tissue (phloem) induces flowering. We show that FT mRNA 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, was 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 defined a regulatory pathway that promotes flowering in response to long days (LDs), and 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). Since FT is expressed in the leaves in response to photoperiod, but FT protein acts in the meristem to promote gene expression, 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 were recently retracted (12). Furthermore, the floral stimulus, but no detectable mRNA of FT-like genes 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.

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). SD-grown Arabidopsis plants exposed to 3 LDs and then returned to SDs flowered much earlier than plants exposed only to SDs (Fig. 1A; SOM text). FT expression rises during the first LD after a shift from SDs (16). 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; fig. S1A). In control plants grown only in SDs, FT mRNA abundance remained low (Fig. 1B). In contrast, in plants exposed to 3 LDs, FT mRNA abundance was increased in each of the three LDs. However, after return to SDs, FT mRNA levels fell after one 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 leaves of plants in which FT is widely overexpressed from a transgene (Fig. 1C). We conclude 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 non-induced 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 introduced this into the co-2 mutant. In these plants CO activity is induced by addition of the steroid dexamethasone (dex) only under LDs when the CO mRNA accumulates in
the light (17–19). Application of dex to a single leaf induced flowering and increased FT mRNA level 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, 20). The use of well-characterized heterologous promoters avoids 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 (21), whereas the promoter of the KNAT1 gene is active in the shoot apical meristem and expression of FT from these promoters complements the co-2 mutation (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 significantly 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 have not undergone the transition to flowering, FT:GFP was detected in the vascular tissue of the shoot (Fig. 2F). However, in 10-day old plants, which are about to undergo the floral transition and have 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 is detected in provasculature and apical tissues in which FT:GFP mRNA is not detected (compare Fig. 2, D and E). These results suggest that FT:GFP protein moves from the phloem companion cells to the meristem (SOM text). Such movement could occur through symplastic downloading from the phloem into the apical meristem region (22).

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 rootstock, which represents a strong sink for contents of the phloem (Fig. 3, A and B). No FT:GFP mRNA could be detected in these rootstocks by RT-PCR after 40 cycles of amplification (Fig. 3C). A SUC2:FT:GFP shoot was then grafted as a donor to 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-1 mutants (23), 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, 24, 25). Our data support movement of the protein. Alternatively, FT expression in the leaves may activate a second messenger, which is transmitted to the apex and induces flowering, perhaps through activation of FT or FT-like genes in the meristem. We refer to this third 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 (26), and not in the companion cells of the shoot or major veins of the leaf. GAS1:CO complements the co-1 mutation (27). 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 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 (28). 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 to 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 that during floral induction of Arabidopsis transient expression of FT in a single leaf is sufficient to induce flowering and 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, because 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 FT-like proteins in the phloem of Brassica napus plants (29). The data in the accompanying paper demonstrate this function of FT is highly conserved in rice. The presence of a wide-range of different proteins in phloem sap may suggest that long-distance movement of proteins is the basis of other signaling processes in plants (22), in addition to the shorter distance movement of proteins between neighboring cells (30) and previous indications of the significance of long-distance mRNA movement (31, 32).

References and Notes
33. This work was funded by the DFG through SFB 572 and by a core grant from the Max Planck Society to G.C. We thank P. Schulze-Lefert, S. Davis, F. Turck, and A. de Montaigu for valuable comments, and K. Shimamoto for providing results prior to publication.

Supporting Online Material
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Materials and Methods
SOM Text
Figs. S1 to S3
References
26 February 2007; accepted 6 April 2007
Published online 19 April 2007; 10.1126/science.1141752
Include this information when citing this paper.
Fig. 1. Regulation of FT mRNA in leaves during flowering. (A) Flowering time of wt Ler and ft-7 plants grown for 2 weeks under SD and exposed to 3 inductive LDs before return to SDs. (B) Expression of FT mRNA during 7 days comprising one SD followed by 3 LDs and then 3 subsequent SDs. FT mRNA expression in the SD-grown controls is also shown. RNA was tested every 4 hours. White bars illustrate duration of day, black bars duration of night. Top bars represent the shift experiment, lower bars the control experiment. (C) Endogenous FT mRNA (FT 3'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 non-treated (F) leaves of CO:CO:GR plants.

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 to 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 DNA fragment spanning the junction between FT and GFP in FT:GFP. The hybridization signal is restricted to the mature phloem (arrows). (E) In situ hybridization of a 12 ESD old SUC2:CO co-2 apex probed with FT. (F) 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 (right-hand pictures show GFP signals separated from background emissions). (F) 6 day old vegetative plant and (G) 10 day old plant which is induced to flower (fluorescence is detected in the provascular tissue and at the base of the SAM; arrowhead). In (H) a leaf primordium flanking the SAM was removed to facilitate visualization. SAM, shoot apical meristem, Lp, leaf primordium, IM, inflorescence meristem. Bars = 50 µm in (C) to (E), (G), and (H); Bar = 25 µm in (F).

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). In (A) and (B), the right-hand pictures 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 respectively. (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 to Ler and ft-7 grown in LDs. (F) FUL expression in leaves of the same plants.