The mRNA of the Arabidopsis Gene FT Moves from Leaf to Shoot Apex and Induces Flowering
Tao Huang, et al.
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Day length controls flowering time in many plants. The day-length signal is perceived in the leaf, but how this signal is transduced to the shoot apex, where floral initiation occurs, is not known. In Arabidopsis, the day-length response depends on the induction of the FLOWERING LOCUS T (FT) gene. We show here that local induction of FT in a single Arabidopsis leaf is sufficient to trigger flowering. The FT messenger RNA is transported to the shoot apex, where downstream genes are activated. These data suggest that the FT mRNA is an important component of the elusive "florigen" signal that moves from leaf to shoot apex.

In many plant species, the length of the day is a major environmental determinant controlling the time of flowering. For instance, Arabidopsis thaliana is a facultative long-day plant. Although it can flower in short days, it flowers much faster when days are long. The day-length signal is perceived in the leaf, where it induces a graft-transmissible signal that moves through the phloem sieve elements to the shoot apex, where flowering is initiated (1–3). This signal is sometimes referred to as the floral stimulus or florigen (1, 3–5).

In Arabidopsis, two genes have been shown to be central for the photoperiodic response. Mutations in the genes CONSTANS (CO) and FLOWERING LOCUS T (FT) lead to late flowering under inductive long-day conditions, whereas flowering under noninductive short-day conditions is only slightly affected (6). CO is expressed mainly in the leaf, where the CO protein is responsible for sensing the day-length signal (7–10). CO then induces FT in the leaf phloem (8, 9). CO appears to act upstream of the graft-transmissible floral stimulus, but neither CO nor FT appears to be expressed in the shoot apex, where floral initiation occurs (8, 9). CO can induce FT expression in the leaf phloem, but not when expressed from shoot apex–specific promoters (9, 11). In contrast, FT expression in both the leaf and the shoot apex can trigger floral initiation (9). These data raise the possibility that the FT mRNA or the FT protein could be a part of the floral stimulus that moves from the leaf to the shoot apex.

**Local induction of FT.** To separate the role of FT from that of other genes that are induced in response to an increase in day length, we determined whether a local induction of FT in a single leaf is sufficient to induce flowering under noninductive short-day conditions. To test this, we constructed transgenic plants expressing FT and the reporter gene GUS under control of a heat shock–inducible promoter (Hsp) from soybean (12, 13). We then heated to 37°C a single Arabidopsis leaf attached to a plant grown under short-day conditions (fig. S1); this heating induced a local activation of the Hsp promoter. We then monitored gene expression in the induced leaf and in microdissected shoot apices (13). Although heating the whole plant induced GUS transcription in the young leaf and the shoot

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**Fig. 1.** Leaf-specific heat shock activation of gene expression. GUS staining in whole plants (A to C) and shoot apex (D to F) of Hsp:GUS plants grown under short-day conditions. (A and D) Noninduced control plant. (B, C, E, and F) Heat shock induction of (B and E) whole plant and (C and F) single leaf. (G and H) Real-time RT-PCR analysis of GUS transcript levels in the heated leaf (G) and the shoot apex (H) of Hsp:GUS plants subjected to single-leaf heat shock induction. (I) Percentage of Hsp:FT and Hsp:GUS plants flowering 2 weeks after whole-plant or single-leaf heat shock induction. (J) Percentage of Hsp:FT plants flowering 2 weeks after a single-leaf heat shock treatment where the heated leaf was immediately cut away (0 hour) or 1, 3, 5, and 7 hours later. Plants where the heated leaf was not cut away were used as positive controls (C in [J]). For the Hsp:GUS control plants in (I) and (J), the first floral buds are visible after 7 weeks, at the same time as for wild-type plants grown in short days. Abscissa indicates chronological time after the start of the heat shock. Error bars ±SD. Details of these experiments are described in (13).
apex (Fig. 1, A, B, D, and E), heating a single leaf induced strong transient GUS transcription in this leaf (Fig. 1, C and G), but no increase in GUS activity or mRNA level could be detected in the shoot apex (Fig. 1, F and H). This confirms that this heat shock system can be used to induce genes locally and that the GUS transcript appears incapable of moving from leaf to shoot apex.

We then used Hsp:FT transgenic plants to test whether a single pulse of FT transcription in leaves was sufficient to trigger flowering. Heating the whole plant caused 95% of the plants to develop visible flower buds 2 weeks after induction; control plants remained vegetative for another 3 weeks (Fig. 1I). However, the local heating of a single leaf was almost as efficient in inducing early flowering, leading to visible flower buds in 75% of the treated plants (Fig. 1I). We also tested whether this response was dependent on the presence of the endogenous FT gene. In the ft mutant background, there was no difference in the efficiency in which Hsp:FT-induced flowering when the whole plant was heated, and only a slight reduction in efficiency when a single leaf was heated (Fig. S2). Thus, a single pulse of FT induction in an individual leaf was sufficient to trigger flowering.

**Movement of the FT mRNA.** Because earlier studies suggested that FT might contribute to the mobile floral stimulus and that small RNA molecules can enter and move through the phloem of several plant species (14–17), we tested whether the FT transcript can travel. In the Hsp:FT Hsp:GUS plants, the single-leaf heat shock treatment induced a strong transient expression of the transgenic FT transcript and the GUS transcript in the leaf (Fig. 2, A and C). An increase in the shoot apical levels of transgenic FT transcript could be detected 6 hours after the start of the leaf induction (Fig. 2B). This increase was not due to activation of the Hsp promoter by FT, because Hsp:GUS is not induced in the same transgenic plants (Fig. 2D). This suggests that the transgenic FT transcript, but not the GUS control transcript, can move from leaf to shoot apex. The heat shock treatment did not affect the expression of the endogenous FT gene in a transgenic Hsp:GUS plant (Fig. 2, E and F). However, in the Hsp:FT:Hsp:GUS plants the levels of the endogenous FT transcript started to increase 6 to 12 hours after the leaf induction, both in leaves and in the shoot apex (Fig. 2, E and F). This suggests the existence of a positive autoregulatory loop where FT can induce, directly or indirectly, its own expression. Unlike transgenic FT, endogenous FT expression continues to increase in the induced leaf, even 3 days after induction, in spite of the fact that the plants are maintained in noninducing short-day conditions (Fig. 2E). This suggests that, once induced, FT can stably maintain its expression irrespective of day-length conditions. This finding explains classical experiments in which a leaf that has received a floral inductive signal stably continued to generate a graft-transmissible signal after up to seven successive graftings on multiple plants under noninductive conditions (1, 18). Our finding of positive FT auto-regulation also explains the phenomenon of indirect induction of flowering, that is, shoots induced to flower by grafting to donor shoots can themselves act as donors in subsequent grafts (1), which suggests that the floral stimulus can act in the leaves of these species to trigger its own synthesis. Our observations, therefore, give further support to the idea that FT is, at least partly, involved in the production of the classical floral stimulus.

The increased levels of endogenous FT transcript in the shoot apex could be due to transport of the leaf-induced FT and to de novo transcription in the apex. In order to distinguish between these two possibilities, we analyzed the activity of the FT promoter in heat shock–induced Hsp:FT pFT:GUS plants. FT promoter activity followed closely the levels of the endogenous transcript in the heat-shocked leaves (Fig. 2G). In the shoot apex, the GUS transcript levels increased between 6 and 12 hours (Fig. 2H), as did the levels of the endogenous FT transcript (Fig. 2F). Because the GUS transcript cannot move from leaf to shoot apex (Fig. 2, C and D), this result shows that a pulse of FT transcription in the leaves can induce FT promoter activity in the shoot apex. However, it does not exclude a contribution from transport of leaf-induced endogenous FT transcript.

To confirm movement of the FT-induced signal from the leaf to apex and to more closely analyze the kinetics of this movement, we removed the heat-shocked leaf at various

![Fig. 2. FT transcript levels and FT promoter activity in heat shock–induced plants. FT and GUS transcript levels in the heated leaves (A, C, E, and G) and in the shoot apex (B, D, F, and H) of 16-day-old short day–grown plants where an individual leaf was subjected to heat shock induction. Chronological time after the start of the heat shock is shown on the x axis. (A and B) FT transcript levels derived from the Hsp:FT construct (transcript levels derived from the endogenous FT gene (endo FT) in Hsp:FT Hsp:GUS plants (blue triangles) and in Hsp:GUS control plants (green squares). (G and H) GUS transcript levels derived from the pFT:GUS construct. Error bars, ±SD. Details of these experiments are described in (13).](www.sciencemag.org)
times after the heat shock induction. The induced leaf needed to be attached to the plant for more than 3 hours, but less than 5 hours, in order to induce an early flowering response (Fig. 1J). This correlates well with the first detectable occurrence of the transgenic FT transcript in the shoot apex 5 hours after heating (Fig. 2B). This also correlates well with previous estimates of the velocity of movement of the leaf derived from floral stimulus (2.4 to 3.5 mm/h (3)]. The heat-induced leaf blade is 6 to 7 mm from the shoot apex in our system, and the FT transcript has reached the apex 2 to 5 hours after maximum leaf induction (Fig. 2B); we calculate a velocity between 1.2 and 3.5 mm/h.

**FT mRNA in the apex.** To confirm that the levels of FT transcript are also increased in the apex of long-day-induced “wild-type” plants, and to confirm the FT autoregulation, we analyzed the levels of FT and GUS transcript in a transgenic pFT:GUS plant after a short-day to long-day shift. At the end of the first long day, the levels of both the FT transcript and the GUS transcript had increased significantly in the leaf (Fig. 3, A and C). Likewise, both transcripts were increased in the shoot apex (Fig. 3, B and D), although at this resolution we could not determine whether the levels of the FT transcript increased before the FT promoter was activated in the shoot apex. Nevertheless, these results show that in normal long-day-induced flowering, FT transcript levels increase in the shoot apex, and the FT promoter is induced. The most parsimonious explanation for these results is that this induction is at least partly caused by a transport of the FT transcript from leaf to shoot apex, followed by a positive autoregulation of the FT gene. Previous studies have failed to detect FT expression in the shoot apex (8, 9); we attribute this discrepancy to the higher sensitivity of our reverse-transcription polymerase chain reaction (RT-PCR) assay on microdissected shoot apices.

**Downstream targets.** To further characterize FT-induced floral induction, we looked at the activation of putative downstream targets. APETALA 1 (AP1) expression is an early marker for reproductive development, already expressed at stage 1 of the induced floral primordium (19). The earliest signs of AP1 induction at the apex could be seen 48 hours after Hsp:FT induction in the leaf (fig. S3A). This fits well with the in situ hybridization data showing the first signs of AP1 induction 48 to 72 hours after a short-day to long-day shift (20). APETALA 3 (AP3) expression is induced at stage 3 of flower development (21). Consequently, we could detect the first signs of AP3 induction 72 hours after FT induction (fig. S3B). However, several genes showed a more rapid response to FT induction: LEAFY (LFY) (fig. S3C), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) (fig. S3D), CAULIFLOWER (CAL) (fig. S3E), and FRUITFULL (FUL) (fig. S3F) were all induced between 6 and 12 hours, suggesting that these genes are early targets in FT-induced floral induction. This is consistent with in situ hybridization data showing that an increase in LFY and FUL (AGL8) transcript levels can be detected 16 hours after a short-day to long-day shift (20).

**Summary.** Our data indicate that the FT mRNA is part of the mobile floral stimulus. However, we cannot exclude the possibility that the FT protein could also be moving and could be responsible for the floral induction. Neither can we exclude that FT may induce another gene or compound in the leaf that moves together with the FT transcript to induce flowering. In addition, the FT autoregulation demonstrated here could help to transduce the FT signal with a relay mechanism involving subsequent FT reinductions. Nevertheless, the simplest explanation of our data is that the FT mRNA constitutes an important part of the floral stimulus that moves from leaf to shoot apex.

**Note added in proof:** It has now been shown that FT can act in the shoot apex by controlling the activity of the shoot apex–expressed transcription factor FD (22, 23).

**References and Notes**
13. Material and methods are available as supporting online material on Science Online.
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**Supporting Online Material**
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Materials and Methods
Figs. S1 to S3
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References and Notes
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