The mRNA of the Arabidopsis Gene FT Moves from Leaf to Shoot Apex and Induces Flowering

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The mRNA of the *Arabidopsis* Gene *FT* Moves from Leaf to Shoot Apex and Induces Flowering

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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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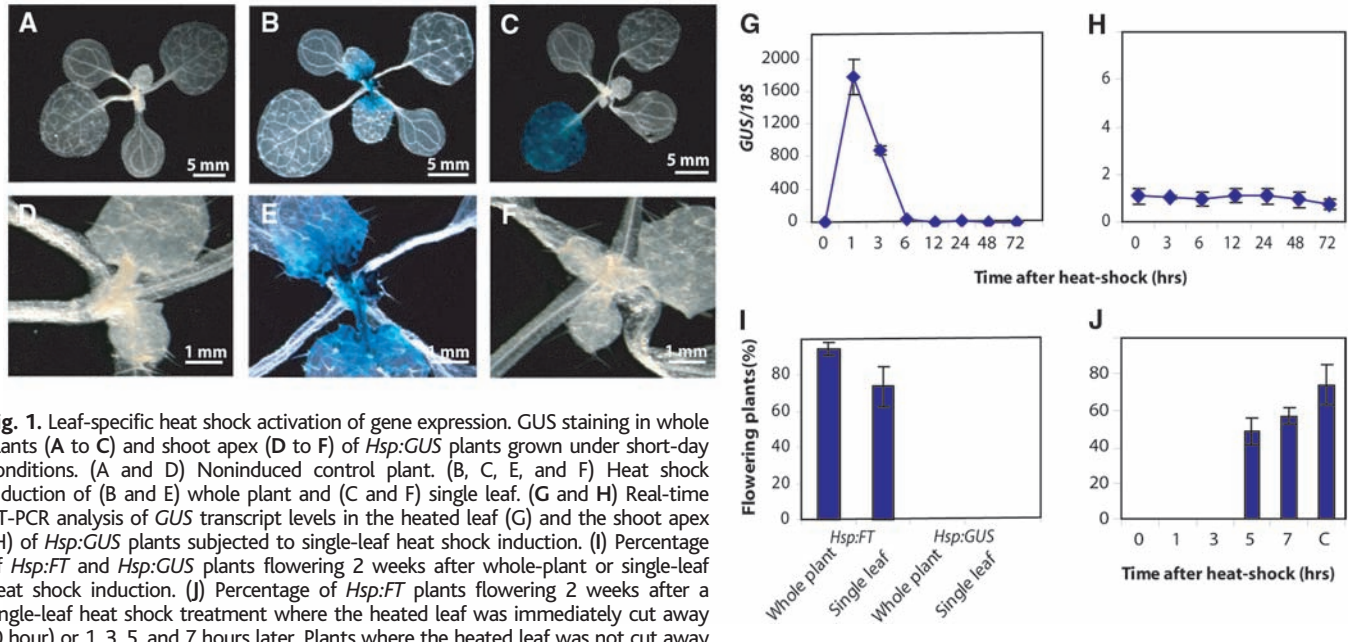

Fig. 1. Leaf-specific heat shock activation of gene expression. GUS staining in whole plants (**A** to **C**) and shoot apex (**D** to **F**) of *Hsp:GUS* plants grown under short-day conditions. (A and D) Noninduced control plant. (B, C, E, and F) Heat shock induction of (B and E) whole plant and (C 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 continues 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* autoregulation 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

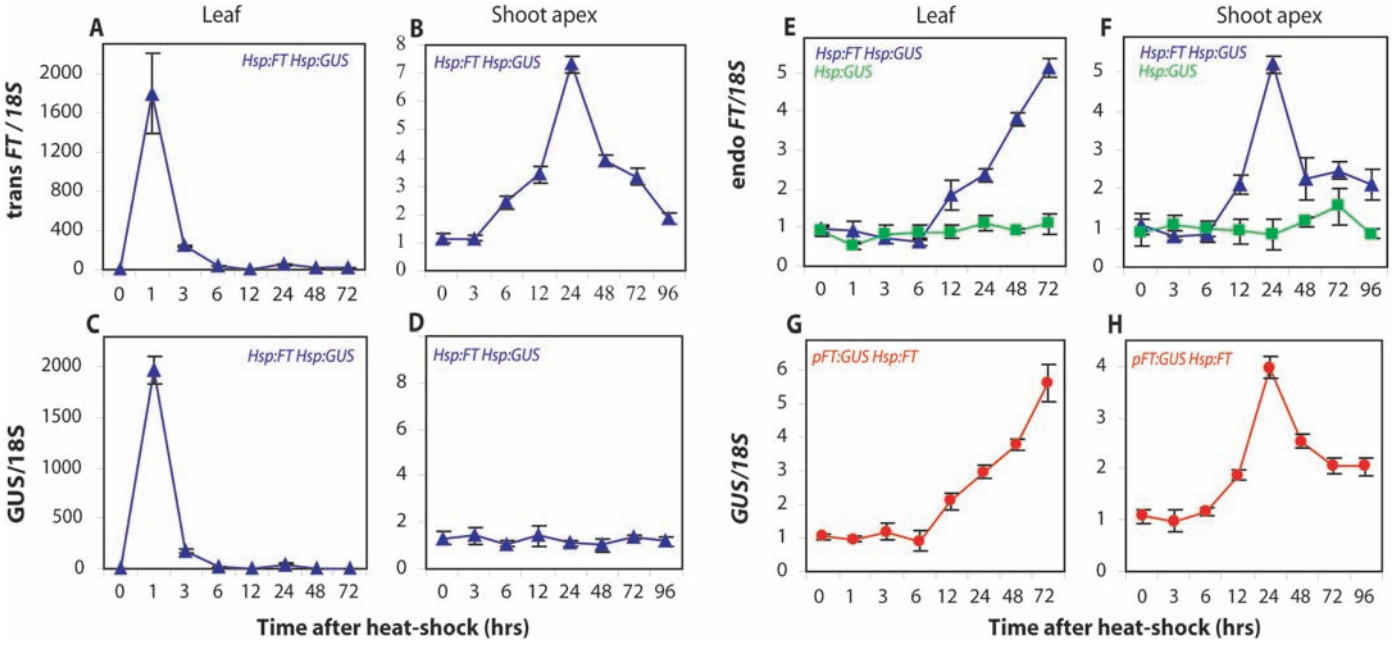

Fig. 2. *FT* transcript levels and *FT* promoter activity in heat shock–induced plants. *FT* and *GUS* transcript levels in the heated leaves (**A**, **C**, **E**, and **G**) and in the shoot apex (**B**, **D**, **F**, and **H**) of 16-day-old short day–grown plants where an individual leaf was subjected 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 (trans *FT*). (C and D) *GUS* transcript levels derived form the *Hsp:GUS* construct. (E and F) *FT* 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*).

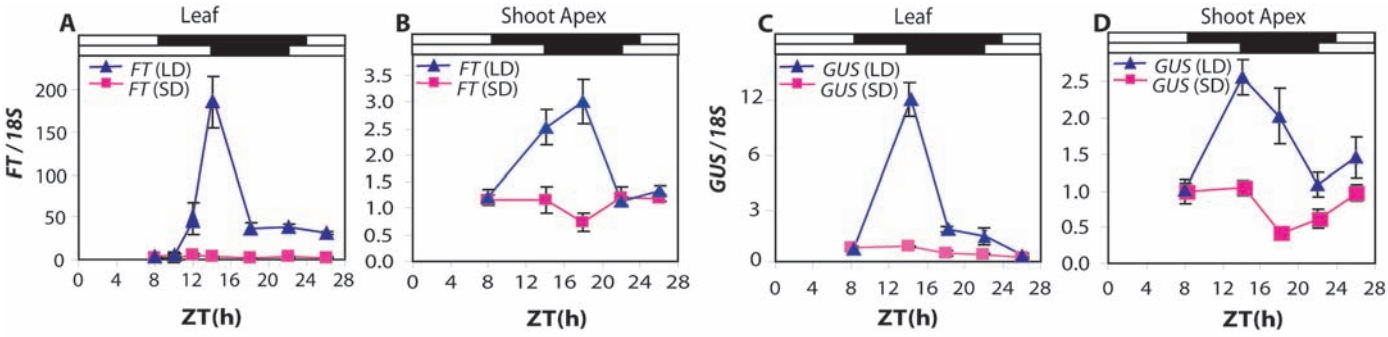

Fig. 3. *FT* transcript levels and *FT* promoter activity in response to a short-day to long-day shift. *pFT:GUS* plants were grown under short days for 4 weeks. At the end of the last short day, half of the plants were shifted to long days (blue triangles) giving a 5-hour extension of that day. The other half of the plants were kept in short days (red squares). The expression levels of the *FT* (**A** and **B**) and *GUS* (**C** and **D**) transcripts in leaf 5 or 6 (A and C) or the shoot apex (B and D) was determined by real-time RT-PCR analysis. Fifteen to 20 shoot apices or leaves were collected at the different time points, with three replicate samples. Error bars, ±SD. Black boxes indicate night and white boxes, light. ZT, zeitgeber time.

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 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 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*).

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Supporting Online Material
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Materials and Methods
Figs. S1 to S3
Table S1
References and Notes

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