

Florigen (II): It is a Mobile Protein

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Abstract

The true identity of florigen – the molecule(s) that migrates from leaves to apical meristem to initiate flowering – was notoriously elusive, having made it almost the “Bigfoot” of plant biology. There was never a lack of drama in the field of florigen study, and florigen researchers have once again experienced such a swing in the last two years. We wrote a minireview last year in this journal (Yu et al. 2006) to excitedly salute, among other discoveries, the notion that the flowering locus T (*FT*) mRNA might be the molecular form of a florigen. However, this hypothesis was challenged in a little less than two years after its initial proposition, and the original paper proposed that the *FT* mRNA hypothesis was retracted (Huang et al. 2005; Bohlenius et al. 2007). Interestingly enough, the *FT* gene previously proposed to encode a florigen was never challenged. Rather, the *FT* protein, instead of the *FT* mRNA, is now believed to migrate from leaves to the apical meristem to promote floral initiation. In this update, we will share with our readers some entertaining stories concerning the recent studies of florigen in five different plant species. In addition to the published reports referenced in this update, readers may also refer to our previous minireview and references therein for additional background information (Yu et al. 2006).

Key words: *Arabidopsis*; florigen; flowering; photoperiod.

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Rescue of the Maryland Mammoth Tobacco by the Graft-transmissible Tomato *SFT* Protein

The hypothesis that the flowering locus T (*FT*) mRNA may be the florigen was first challenged by Eliezer Lifschitz and colleagues at the Technion Institute of Technology (Haifa, Israel) in a paper published in the 18 April 2006 issue of the *Proceedings of the National Academy of Sciences USA* (Lifschitz et al. 2006). In this paper, Eliezer and his co-workers reported a study of the functional homolog of *FT* in tomato called *SFT* (*SINGLE-FLOWER TRUSS*). Tomato *sft* is a late-flowering

mutant isolated long ago and mapped to the same chromosome position as that of a homologous gene of *Arabidopsis FT*. Sequence analyses of multiple *sft* alleles revealed missense or deletion mutations of this *FT*-like gene in those *sft* alleles, indicating that *SFT* is most likely the *FT* ortholog in tomato.

Transgenic tomato and tobacco plants constitutively expressing either the *Arabidopsis FT* gene or the tomato *SFT* gene under the 35S promoter (35S::*FT* or 35S::*SFT*) flower earlier, although the wild type tomato is day-neutral and insensitive to photoperiod. The researchers then tested whether the *SFT* signal is graft transmissible. Although the wild type donor failed to promote flowering in the receptor *sft* mutant, a transgenic donor expressing 35S::*SFT* rescued the mutant *sft* receptor. The interpretation was that the wild type donor may not have enough *SFT* to donate to the receptor *sft* mutant, that the transgenic 35S::*SFT* grafting stock expressed *SFT* at a higher concentration, and that the transgenic *SFT* mRNA or protein was systemically transmitted across the grafting union to promote flowering in the *sft* mutant receptor. Interestingly, the authors could detect the *SFT* protein, by immunoblot, in the receptor samples. In contrast, no transgenic *SFT* mRNA was detected, by nested RT-PCR, in the RNA samples isolated from the grafting receptor samples, although *SFT* mRNA was detected in 1/2500 as much RNA isolated from the donor samples. These results demonstrated that at least in tomato,

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SFT protein is more likely than the *SFT* mRNA to act as the graft transmissible signal that promotes flowering.

The researchers went further and used the heterograft technique to test whether *SFT* synthesized in tomato may cross the species boundary to activate flowering in a tobacco variety called Maryland Mammoth. Maryland Mammoth is a short-day tobacco strain that is probably a spontaneous mutant arisen from the day-neutral tobacco population. Maryland Mammoth tobacco is of historical significance, as it was used, along with Biloxi soybean, in the original study by Garner and Allard that lead to the experimental demonstration of photoperiodism (Garner and Allard 1920; 1931). Garner and Allard showed, by moving the plants into a dark room in the afternoon to artificially shorten the day length and by comparing them with the controls grown in the field under the natural long-day condition (Figure 1A), that both Biloxi soybean and Maryland Mammoth tobacco treated with the short-day condition flowered earlier than the control grown in long-day conditions. This explained why the sowing date had little influence on the flowering time of soybeans in the field (because an SD plant starts to flower in response to short day length regardless of the extent of vegetative growth) and why Maryland Mammoth tobacco failed to flower in the field (in Maryland, USA) during the normal growing season in long days (because an obligated SD plant does not flower until the day length is shorter than a threshold). In the experiment by Lifschitz and his colleagues, a donor shoot of transgenic tomato overexpressing *SFT* was grafted onto a receptor leaf petiole of the Maryland Mammoth tobacco, and the grafted plant was left to grow in long days. Sure enough, the Maryland Mammoth tobacco receptor plant grown in long days flowered (Figure 1B), whereas the control Maryland Mammoth never flowered in the same long-day condition. Therefore, a signal apparently moved across the grafting boundary from tomato to tobacco to accelerate flowering in the latter. Taken together with the results of the other experiments in their study, the authors concluded that this signal was most likely the *SFT* protein.

FT Protein is Transmitted through the Vascular Bundles from Leaves to the Shoot Apical Meristem in *Arabidopsis* and Rice

Soon after the discovery of the *SFT* gene in tomato, four papers were published (Corbesier et al. 2007; Jaeger and Wigge 2007; Lin et al. 2007; Mathieu et al. 2007), whereby each used different approaches to re-examine the role of *Arabidopsis* FT gene products in photoperiodic control of floral initiation. In the first study, George Coupland's group at the Max Planck Institute for Plant Breeding Research (Colgone, Germany) investigated the consequences of transgenic expression of the fusion protein CO-GR, and the behaviors of another fusion protein FT-GFP (green fluorescent protein) (Corbesier et al. 2007). CO-GR is a fusion

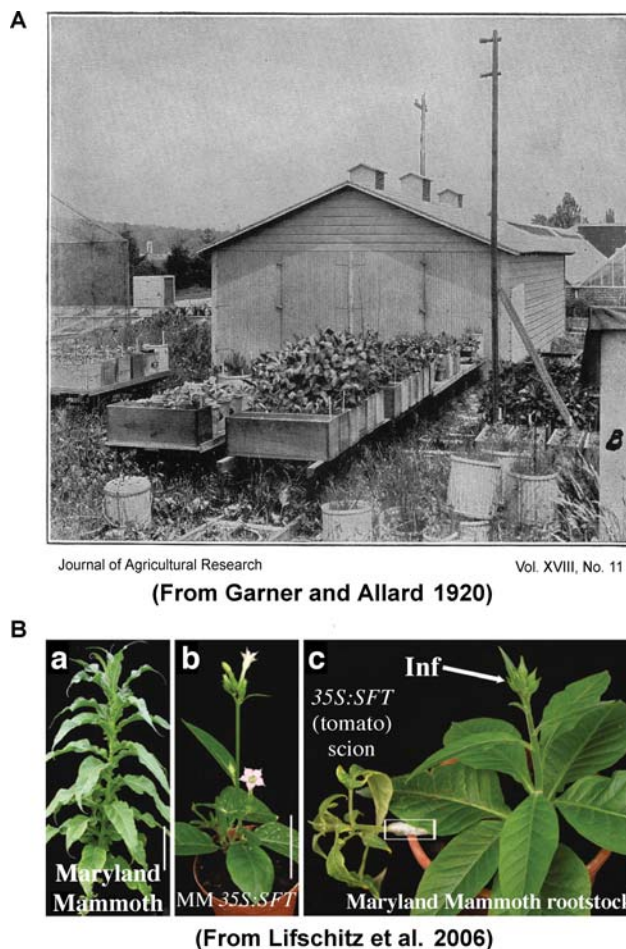


Figure 1. Maryland Mammoth in 80 years.

(A) The experimental set-up of Garner and Allard, by which soybean and Maryland Mammoth tobacco plants were manually moved into a dark room in the afternoon to artificially shorten the day length. The photograph was reproduced from (Garner and Allard 1920; 1931).

(B) Graft-transmission of *SFT* (SINGLE-FLOWER TRUSS) signal from tomato to promote flowering in Maryland Mammoth tobacco (Lifschitz et al. 2006). (a) A Maryland Mammoth plant grown under long (18-h light/6-h dark) days, which did not flower. (b) Early flowering in long-day grown Maryland Mammoth plants expressing the *35S:SFT* transgene. (c) Tomato *35S:SFT* donor scions grafted onto leaf petioles (boxed) of a Maryland Mammoth receiver induced flowering in the tobacco receiver (arrow) grown under long-day conditions. The photographs were reproduced from (Lifschitz et al. 2006).

protein of CO (CONSTANS) and the rat glucocorticoid receptor (GR). GR is well known for its conditional nuclear localization activity. Transgenically expressed GR-fusion proteins reside in the cytosol of *Arabidopsis* cells, whereas they are translocated into the nucleus in the presence of corticosteroid hormones or the synthetic analog, dexamethasone (dex) (Lloyd et al. 1994). As

expected, transgenic expression of CO-GR under the control of the native *CO* promoter rescued the late-flowering phenotype of the *co* mutant only in the presence of dex, as CO activates flowering in the nucleus. Application of dex to a single leaf induced *FT* mRNA expression only in the leaf that dex was applied to.

Importantly, the dex treatment to the single leaf induced flowering, demonstrating that nuclear translocation of the CO-GR fusion protein in a single leaf was sufficient to activate *FT* mRNA expression and floral initiation. However, no *FT* mRNA was detected in leaves not treated with dex, indicating that *FT* mRNA did not migrate into the leaf where it was not expressed.

One technical difficulty in the study of *FT* is that it expresses at a relatively low level, making it difficult to analyze expression and activity in wild-type plants. To overcome this problem, the authors next prepared transgenic plants (in the *ft* mutant background) expressing the FT-GFP fusion protein under control of the *SUC2* promoter. The *SUC2* promoter has been previously shown to specifically (and strongly) expressed in the phloem companion cells (Imlau et al. 1999). The *SUC2::FT-GFP* transgene rescued the late-flowering phenotype of the *ft* mutant parent, demonstrating that *FT* expressed in the phloem companion cells was sufficient to promote flowering. The authors then examined where the *FT-GFP* mRNA and FT-GFP protein could be found in the transgenic plants. An *in situ* hybridization study failed to detect the *FT-GFP* mRNA in the shoot apical meristem. However, GFP fluorescence was clearly detected in the shoot apical meristem. The GFP fluorescence found in the shoot apical meristem was clearly derived from the FT-GFP fusion rather than a proteolytic product, because free GFP protein was never detected in the transgenic plants on western blots. Therefore, it was concluded that the FT-GFP protein moved from leaves through the phloem cells to the apical tissue, and was then unloaded from the phloem cells into the apical meristem cells, where it activated flowering. Using a grafting method developed for *Arabidopsis* (Turnbull et al. 2002; An et al. 2004) and expression analyses, the authors were also able to show that the FT-GFP fusion protein, but not the mRNA, migrated from the donor expressing *SUC2::FT-GFP* to the *ft* mutant receiver to activate flowering of the receiver.

Based on cell-specific expression studies, Ko Shimamoto's laboratory at the Nara Institute of Science and Technology (Ikoma, Japan) also showed that the *FT* ortholog in rice (called Hd3a) worked in the same way as its *Arabidopsis* counterpart (Tamaki et al. 2007).

Only the FT Protein that Moves Freely Intracellularly and Intercellularly was Able to Promote Floral Initiation

For a molecule to move from leaves to apical meristem through the vascular tissue, it has to be within certain size limits and it must be able to move intercellularly through the plasmodes-

mata, which are small pores of approximately 40 nm diameter connecting adjacent cells (Wu et al. 2002; Lucas and Lee 2004). The other two studies addressed the question whether the *FT* protein may be such a molecule, using some creative experimental designs (Jaeger and Wigge 2007; Mathieu et al. 2007). In one study (Mathieu et al. 2007), researchers in Markus Schmid's laboratory at the Max Planck Institute for Developmental Biology (Tubingen, Germany) asked the question: how could cell-specific suppression of *FT* mRNA expression affect flowering time? These researchers used an artificial microRNA (*amiR*) strategy (Schwab et al. 2006) to achieve targeted destruction of the *FT* mRNA in specific cells of the transgenic plants. They prepared transgenic plants expressing the *amiR-FT* transgene driven by either the vascular-specific *SUC2* promoter or by the shoot apical meristem-specific *FD* promoter. It was shown that plants expressing the *amiR-FT* transgene driven by the vascular-specific *SUC2* promoter, but not by the shoot apical meristem-specific *FD* promoter, caused delayed flowering. Because it has been previously shown that the *FT* protein physically interacts with *FD* in the shoot apical meristem to activate flowering (Abe et al. 2005; Wigge et al. 2005), these results argued strongly that *FT* mRNA accumulated in the vascular tissue is necessary to activate floral initiation in the shoot apical meristem cells. In contrast, the targeted destruction of *FT* mRNA in the shoot apical meristem failed to affect flowering, suggesting that accumulation of the *FT* mRNA in the apical meristem is insufficient to activate flowering. If a presumed destruction of the *FT* mRNA in the shoot apical meristem cells of the late-flowering transgenic plants expressing *FD::amiR-FT* had been shown (Mathieu et al. 2007), it would have constituted the most compelling evidence to argue against the hypothesis of *FT* mRNA being a florigen.

In another elegantly designed experiment (Mathieu et al. 2007), Markus Schmid and his colleagues prepared two transgenic lines. One line expresses a fusion protein, FT-TEV-3xYFP, in which the *FT* coding sequence was connected, via a cleavage site for the viral tobacco etch virus (TEV) protease, to three copies of the yellow fluorescent protein (YFP) coding sequence. Another line expresses the viral TEV protease. Both transgenes were driven by the *SUC2* promoter to express only in the phloem companion cells. Neither transgenic line showed accelerated flowering, but the F1 progenies of the cross between the two transgenic parents showed accelerated flowering. These results suggest that FT-TEV-3xYFP accumulated in the phloem companion cells are either biochemically inactive or too big (112 kD, compared with *FT* of 20 kD) to move out from the phloem companion cells to exert its activity. In a control experiment, it was found that constitutive expression of the FT-TEV-3xYFP protein under the control of the 35S promoter resulted in accelerated flowering. Therefore, FT-TEV-3xYFP must be biochemically active and FT-TEV-3xYFP accumulated in the vascular cells must be too big to move and activate floral initiation.

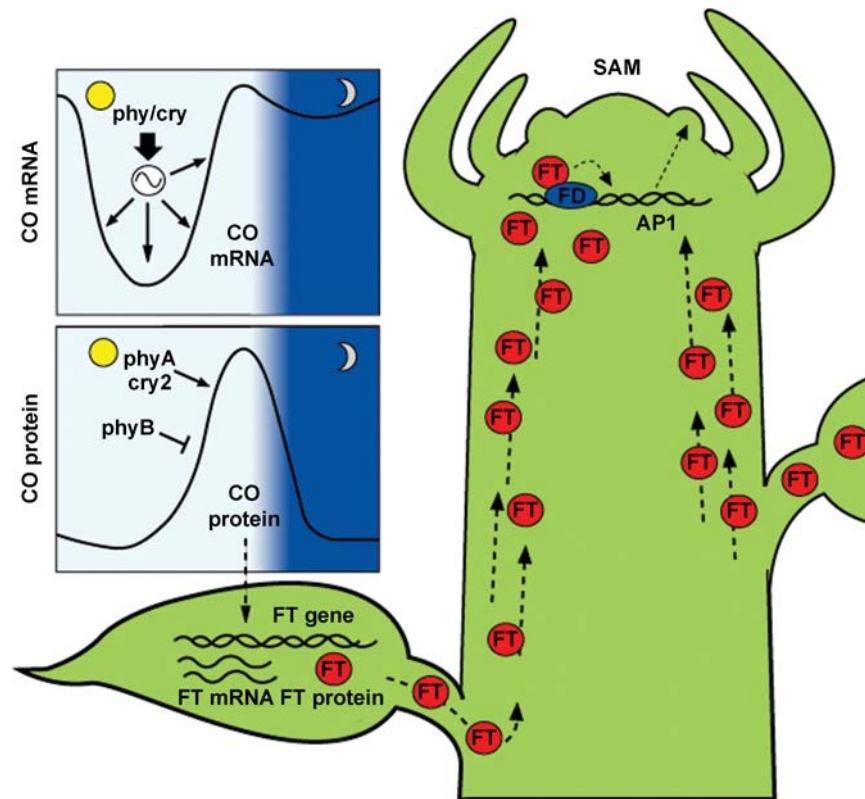


Figure 2. A model of photoperiodic floral induction in *Arabidopsis*.

FT mRNA expression is upregulated in leaves by the CO protein, which accumulates to significant levels only under long day (LD) conditions. CO mRNA expression is regulated by the circadian clock so that peak expression is in the early morning and late afternoon in LD (top left panel). The CRY2 and PhyA photoreceptors antagonize the degradation promoting effect of the PhyB photoreceptor to stabilize the CO protein at the end of the day (bottom left panel). The FT protein (represented by the red disks) produced in the leaves travels through vascular tissues to the shoot apical meristem, whereby it interacts with FD at the promoters of floral meristem identity genes such as *AP1* to induce their transcription and floral initiation.

What might happen if the FT protein expressed in the phloem cells was constitutively restrained in the nucleus to prevent its intercellular movement? This question was addressed by the study of Markus Schmid and his colleagues (Mathieu et al. 2007), and by another study from Philip Wigge's laboratory at the John Innes Center (Norwich, UK) (Jaeger and Wigge 2007). In order to keep a protein constitutively in the nucleus, one can attach a NLS (nuclear localization signal) sequence to the target protein. These researchers prepared transgenic lines expressing the *SUC2::Myc-FT* transgene or *SUC2::NLS-Myc-FT* transgene, respectively (Jaeger and Wigge 2007). They found that the NLS-Myc-FT fusion protein constitutively accumulated in the nucleus was incapable of activating flowering, whereas the My-FT fusion protein that can freely move intracellularly and intercellularly was able to activate floral initiation. In addition, those authors also showed by tissue immunostaining that Myc-FT fusion proteins expressed in the phloem companion cells could be detected in the shoot apical meristem cells. In contrast,

in situ hybridization failed to detect the *Myc-FT* mRNA in the same cells.

FT Protein but not *FT* mRNA was Detected in Pumpkin Phloem Sap

To unequivocally demonstrate that FT protein is the florigen, one would like to detect the movement, or at least existence, of the FT protein in the phloem sap, by which most metabolites are transmitted. For the same reason, one would also like to know whether the *FT* mRNA can be detected in the phloem sap. These tests were carried out by the Bill Locus laboratory at UC Davis (Davis, California) (Lin et al. 2007), which has been studying plasmodesmata and intercellular trafficking for decades (Lucas and Lee 2004). In this study, the authors used the short-day plant pumpkin species (*Cucurbita* sp.) as the model system (Lin et al. 2007). Pumpkin has several technical

advantages for florigen study, including a well-established viral expression system, simple graft procedures, and abundant phloem sap that is relatively easy to collect. This study first tested ZYMV (*Zucchini mosaic virus*) viral-derived expression of *Arabidopsis* FT. It was shown that pumpkin plants infected with the ZYMV virus modified to encode *Arabidopsis* FT could induce flowering in the pumpkin plants grown in long days. Because ZYMV does not produce subgenomic RNAs and the ZYMV viral infection domain does not include the shoot apical meristem, only FT protein would be free to move into the shoot apical meristem. Therefore, the FT protein appeared to act as a florigen in pumpkin as well. These researchers then cloned the two FT-like genes (FTL1 and FTL2) in pumpkin, and examined the contents of the phloem sap collected from pumpkin plants grown in the inductive short days or non-inductive long days. No FTL mRNA was detected in the phloem sap by Q-PCR analyses, although some other RNAs, including *rbcS* and *PP16* were readily detected. When proteins around the size of FTL were collected from the pumpkin phloem sap and analyzed using mass spectrometry, the FTL peptides were detected. Importantly, the FTL protein was detected only in the phloem sap collected from plants grown in short days, but not from plants grown in long days. These studies demonstrated that the FTL protein accumulates in the phloem sap in a photoperiod-dependent manner, which seems to close the remaining gap for the hypothesis that FT protein acts as a florigen.

Conclusion

There is little doubt that the previous hypothesis that the FT mRNA might act as a mobile florigen is most likely incorrect, although it is always difficult to prove a “negative”. On the other hand, given that (i) FT is generally expressed in vascular tissue; (ii) FT protein, but not FT mRNA, expressed in phloem companion cells can be detected in the shoot apical meristem cells; (iii) only the FT protein that freely moves intercellularly can activate flowering; and (iv) FT protein, but not FT mRNA, was detected in the phloem sap in response to photoperiodic induction; it appears quite clear now that FT protein is a florigen (Figure 2). The observation that FT protein acts as a florigen in five different plant species indicates a universal mechanism used by plants to regulate flowering time in response to photoperiods.

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