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Phloem-Borne FT Signals Flowering in Cucurbits

Approximately 45 years ago, Zeevaart (1962) wrote that the identification of florigen, a phloem-borne signaling molecule that acts to initiate photoperiodic-dependent flowering, was the most urgent problem in the study of the physiology of flowering and expressed hope that the recent isolation of a flower-inducing extract from *Xanthium* (Lincoln et al., 1960) would lead to a solution. In the ensuing years, our knowledge of the phenomenon of photoperiodically induced flowering has vastly expanded to include knowledge of the photoreceptors involved and integration with the circadian clock, with other floral induction pathways, and with the downstream genes that control flower morphogenesis in the shoot apex; yet, the nature of florigen has remained a mystery for most of that time. It is only very recently that evidence has begun building for a primary role for *FLOWERING LOCUS T* (*FT*), which encodes a RAF kinase inhibitor-like protein, as a key component of the florigenic signal (reviewed in Corbesier and Coupland, 2006; Imaizumi and Kay, 2006).

In *Arabidopsis*, *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), which encodes a MADS box transcription factor, are considered floral integrator genes because together they receive input from the four main flowering time pathways: the photoperiodic, autonomous, vernalization, and gibberellin pathways. *FT* interacts with the bZIP transcription factor *FD*, which leads to upregulation of *SOC1*. This is one of the early signs of the transition to flowering and in turn leads to activation of the floral initiation genes *LFY* and *AP1* in the shoot apical meristem (SAM) (Borner et al., 2000; Samach et al., 2000).

FT is expressed in the vascular tissue of plant leaves but not in the SAM (Takada and Goto, 2003; An et al., 2004), and *FD* is expressed only in the SAM (Abe et al., 2005; Wigge et al., 2005). This implies that *FT* mRNA, *FT* protein, or both act as florigenic signals, either alone or together with other unknown components of the signal.

FT is a small protein of ~23 kD, which is below the size exclusion limit of plasmodesmata connecting companion cells to the sieve tube system (Imlau et al., 1999), and therefore might move freely through the phloem. An et al. (2004) suggested that the activation of *FT* in the phloem (by photoperiodic or other signals) might precede movement of the *FT* protein to the meristem.

Huang et al. (2005) then reported that *FT* mRNA moves from the leaves to shoot apices and induces flowering in *Arabidopsis*, although this study did not rule out a possible role for transport of *FT* protein. Contrasting data from grafting experiments in tomato revealed that the *FT* ortholog expressed in leaves could induce flowering in a distant shoot meristem but that transcripts of the inducing *FT* gene could not be detected in the meristem (Lifschitz et al., 2006). Corbesier and Coupland (2006) noted that “whether the *FT* mRNA, the *FT* protein or both move in wild-type plants remains to be established, as does the requirement of any movement for flowering.” Recently, the article of Huang et al. (2005) was retracted because the primary RT-PCR data regarding *FT* mRNA movement into the apex was found to be flawed (Böhlenius et al., 2007). At the same time, two new articles appeared, one suggesting that *FT* protein acts as a non-cell-autonomous signal to induce flowering in *Arabidopsis* (Corbesier et al., 2007) and the other that its homolog in rice, called *Hd3a*, has the same activity (Tamaki et al., 2007).

In this issue of *The Plant Cell*, Lin et al. (pages 1488–1506) provide some of the strongest evidence to date that *FT* protein functions as a long-distance florigenic signal, from work performed in cucurbits (squash). The authors used a *Cucurbita moschata* accession responsive to inductive short-day (SD) photoperiods, along with a potyvirus vector, *Zucchini yellow mosaic virus*, to drive *FT* expression under different daylength growing conditions. They also performed grafting experiments between uninduced *C. moschata* and flower-

ing *Cucurbita maxima*, a day-neutral species. Analysis of vascular tissue and phloem sap from photoperiodically induced and uninduced plants by real-time RT-PCR and mass spectrometry showed that the presence in the phloem of *FT*-like (*FTL*) proteins, but not *FTL* mRNA, was highly correlated with the onset of flowering.

THE CUCURBITA EXPERIMENTS

First, most *Cucurbita* species are day-neutral, and the authors screened ~100 accessions to identify one, *C. moschata* PI441726, that behaved as an obligate SD plant, such that flowering could be easily manipulated by altering daylength. They identified two homologs of *Arabidopsis FT* in *C. moschata*, called *Cmo-FTL1* and *Cmo-FTL2*, and two in day-neutral *C. maxima*, called *Cm-FTL1* and *Cm-FTL2*, all of which showed a high level of conservation. The authors tested *Cm-FTL1* and *Cm-FTL2* and found that they had floral-inducing activity similar to that of *At-FT* when expressed in *Arabidopsis*.

Second, instead of a transgenic approach, the authors used a potyvirus vector, *Zucchini yellow mosaic virus* (ZYMV) to drive expression of *At-FT* in nontransgenic *C. moschata* plants. The choice of a potyvirus vector was important as these plant viruses do not produce subgenomic RNA species, whose presence could lead to confounding results. ZYMV-mediated expression of *At-FT* was shown to induce flowering in *C. moschata* grown under long-day (LD) noninductive conditions. Experiments with ZYMV-green fluorescent protein (GFP)-infected plants established that the ZYMV vector appeared to be excluded from apical and lateral meristems.

Next, heterografting experiments with uninduced *C. moschata* scions grafted to flowering *C. maxima* stocks and analysis of phloem sap with mass spectrometry confirmed that long-distance transport of *FT* protein was associated with the induction of flowering. Real-time RT-PCR analysis failed to detect the presence of *FT* mRNA in

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phloem sap of flowering plants, although transcripts for other genes, such as the well-characterized *Cm-RPP16* (Xoconostle-Cázares et al., 1999), could be readily detected.

Finally, Lin et al. conducted additional experiments to determine if there were differences in the amounts of Cmo-FTL transcripts or proteins in vascular tissue and in phloem sap in *C. moschata* plants grown under noninductive LD versus inductive SD conditions. Cmo-FTL mRNA was present in stem vascular tissue of LD- and SD-grown plants (Cmo-FTL1 mRNA was relatively high under both LD and SD conditions, whereas Cmo-FTL2 mRNA was extremely low under LD conditions and increased ~40-fold under SD conditions). By contrast, the Cmo-FTL proteins were undetectable in the phloem sap collected from LD-grown plants and could only be detected in sap collected from SD-grown plants. The proteins were detected at extremely low levels in stem vascular tissue under LD and at significantly higher levels under SD.

These results supported the conclusion that FTL proteins, and not mRNA, play an important role in long-distance florigenic signaling and further suggested that FTL2 might be more important than FTL1 in this role. The observation that FTL proteins were detectable (albeit at extremely low levels) in stem vascular tissue of LD-grown plants, but undetectable in the phloem sap of these plants, led the authors to conclude that an additional mechanism acts to control the trafficking of the proteins from companion cells into the phloem translocation stream and that phloem loading of FT proteins is under photoperiodic control in the obligate SD *C. moschata* accession.

CAVEATS

One of the strengths of the approach taken by Lin et al. in the cucurbit system was the use of nontransgenic plants and monitoring of native proteins and mRNA species. The work by Corbesier et al. (2007) in *Arabidopsis* and Tamaki et al. (2007) in rice was based on expression of *FT:GFP* fusion constructs driven by heterologous tissue-specific promoters in transgenic plants. The use of such transgenic experimental sys-

tems is widespread in the plant science community and has contributed tremendously to the advancement of knowledge in many areas of plant biology. Nevertheless, it is important to recognize two potentially serious complications of using such systems, namely, assumptions about promoter tissue specificity and dramatic overexpression of target genes relative to the wild-type situation.

In the work by Corbesier et al. (2007) in *Arabidopsis*, the main promoter used was that of *SUCROSE TRANSPORTER2* (*SUC2*), and additional experiments were conducted using the *GALACTOL SYNTHASE1* (*GAS1*) promoter. Imlau et al. (1999) presented evidence that the *SUC2* promoter is active specifically in phloem companion cells of major and minor leaf veins, whereas *GAS1* has been shown to be active exclusively in phloem companion cells of minor leaf veins (Haritatos et al., 2000). Although the *SUC2* promoter is often thought of as specific to the loading zone (i.e., companion cells of source leaves), Lin et al. point out that it has recently been shown to be active throughout the transport phloem and in sink tissues (Martens et al., 2006). Indeed, experiments by Corbesier et al. (2007) show FT:GFP mRNA and protein (expressed from the *SUC2* promoter) together in tissues immediately adjacent to the SAM. Therefore, experiments using the *SUC2* promoter, and any other constructs expressed within the phloem, cannot separate long-distance transport of a signal through the phloem from phloem unloading in the sink. In the rice system, Tamaki et al. (2007) used the native *Hd3a* promoter and promoters from *Agrobacterium rhizogenes rolC* and *Rice Phloem Protein16* (*RPP16*), which also appear to be expressed exclusively in the phloem (Guivarc'h et al., 1996; Asano et al., 2002).

Both Corbesier et al. (2007) and Tamaki et al. (2007) provided supporting evidence for the use of the various heterologous promoters. In *Arabidopsis*, the experiments using the *GAS1* promoter were largely supportive of those using the *SUC2* promoter (Corbesier et al., 2007). Tamaki et al. (2007) conducted experiments using the native

Hd3a promoter, which supported the results obtained from the *rolC* and *RPP16* promoters. In addition, they used a Kaede reporter protein, which is retained in the cytoplasm (unlike free GFP, which can freely diffuse to nearby tissues), to provide further evidence that these promoters were not expressed in the SAM. Nonetheless, results obtained using these promoters must still be interpreted with caution because of the dramatic overexpression of the target genes in relation to wild-type levels of expression. One component of the study of Lin et al. (2007), while not using an overexpressing transgene approach, did generate ectopic expression of FTL mRNA following viral infection, and this aspect of their findings is subject to similar qualification.

FT mRNA: STILL IN THE GAME?

The findings of Lin et al. (2007) that mRNA transcribed from the inducing FT gene could not be detected in the induced shoot apex are consistent with those of Lifschitz et al. (2006) in tomato, Corbesier et al. (2007) in *Arabidopsis*, and Tamaki et al. (2007) in rice. So, can a role for mobile FT mRNA be ruled out for most species, a viewpoint which might be further reinforced by the retraction of Huang et al. (2005)? Lin et al. caution against discarding the possibility that FT mRNA may function as a florigenic signal in addition to FT protein. A role for long-distance trafficking of mRNA molecules has been firmly established for a number of developmentally and environmentally regulated gene products in numerous plant species (reviewed in Lough and Lucas, 2006). Lin et al. suggest that different plant species could use various combinations of FT protein and mRNA, and uncovering such differences would be important for understanding the evolution of florigenic signaling.

Two other very recent additions to the FT literature offer further evidence that transport of FT protein, and not FT mRNA, from the phloem to the apical meristem is necessary for floral induction. Similar to the experiments of Corbesier et al. (2007), Jaeger and Wigge (2007) show that an epitope-tagged version of FT (Myc-FT) driven

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by the *SUC2* promoter induces flowering in *Arabidopsis*. In addition, these authors created a nonmobile version of FT by adding a nuclear localization signal to the transgene construct. The results of these experiments suggest that a version of FT targeted to the nucleus produces an active protein that is able to interact with FD and influence downstream gene expression but is no longer able to act as a non-cell-autonomous signal that is transported between cells. Meanwhile, Mathieu et al. (2007) independently report ingenious experiments in *Arabidopsis* wherein FT mRNA and protein effects were uncoupled and also demonstrate that transport of the protein from the phloem to the shoot apex is necessary and sufficient for the induction of flowering.

However, these articles do not absolutely rule out a role for FT mRNA as part of the long-distance signal within the phloem. It is important to remember that there are three aspects of long-distance transport that could require different signaling components: (1) phloem loading in source tissue, (2) long-distance transport through the phloem, and (3) unloading of the signal from the phloem to sink tissue (or SAM). Lin et al. provide the strongest evidence to date that FT protein, and not mRNA, functions as the long-distance trafficking signal in cucurbits, in addition to being the signal that is transported from the phloem into cells at the shoot apex.

FUTURE DIRECTIONS

Zeevaart (1962) posed a central question of flowering physiology: "How does an inductive daylength activate the floral genes"? The *Cucurbita* system represents an excellent model system for further exploration of the nature of photoperiodic control over trafficking of the FTL proteins because proteins and mRNA molecules can be monitored at their normal in vivo concentrations in wild-type plants. The work of Lin et al. suggests that an inductive daylength in the SD plant *C. moschata* triggers uploading of FTL proteins into the phloem in source tissues. The mechanism of this uploading remains unknown, although Lin et al. have some intriguing evidence that there is

a control point in trafficking of the FTL1 and FLT2 proteins from companion cells into the sieve tube system. The mechanism of FT protein unloading in the target tissues is also not known.

Future work in other systems will need to establish more definitively whether or not FT mRNA enters the translocation stream and participates in long-distance florigenic signaling. In addition, *Arabidopsis* is a LD plant, whereas rice and *C. moschata* are SD plants, so it will also be important to determine what causes photoperiod to affect FT proteins (and or mRNA) differently in these and other systems. Comparative approaches will eventually lead us to an understanding of when the FT system evolved and how it has been modified and adapted to regulate the diverse patterns of floral induction observed in flowering plants today.

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