Systemic movement of *FT* mRNA and a possible role in floral induction

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FLOWERING LOCUS T (FT) protein is known to be part of the mobile flowering inducing "florigen" signal in plants, but it may not be acting alone. This article reviews the data that *FT* mRNA can also move systemically throughout the plant and into the shoot apical meristem (SAM) independently of the FT protein. There is a promotion of flowering when increased levels of virally expressed *FT* mRNA are present together with endogenously produced FT protein in inducing conditions, even if the additional *FT* mRNA is non-translatable and thus not increasing the overall levels of FT protein. A specific sequence, or "zip code" of the *FT* mRNA is required for systemic movement and this sequence binds a specific protein(s) in plant extracts. This raises the possibility the *FT* mRNA may be moving systemically through the plant and into the SAM as an RNA–protein complex, whether FT protein is also a component of this mobile complex remains to be determined.

Keywords: flowering locus T, FT, mRNA, flowering, tobacco

In *Arabidopsis* the *FLOWERING LOCUS T* (*FT*) gene plays a key role in the induction of flowering (Abe et al., 2005; Wigge et al., 2005). The *Arabidopsis* FT protein (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007) and its orthologs from rice (Tamaki et al., 2007), cucurbit (Lin et al., 2007), and tomato (Lifschitz et al., 2006), have been shown to be a component of a systemic flowering signal that moves through the phloem from the leaves to the shoot apical meristem (SAM) to induce the switch from vegetative growth to flower formation. However more recently it has been demonstrated that, in addition to FT protein, *FT* RNA is also able to move systemically through the plant from leaves to the SAM (Li et al., 2009, 2011) and here we review the evidence that systemic movement of *FT* mRNA may have a role to play in floral induction.

To analyze the movement of *FT* RNA an RNA movement assay was developed based upon a virus expression system. In this assay a PVX virus which had its coat protein gene deleted from its viral genome, and which because of the lack of coat protein was therefore was unable to move from cell to cell, was used to express *Arabidopsis FT* RNA fused downstream of a *GFP* coding sequence. As a control the same viral vector was used that expressed *GFP* RNA alone. It was found that both the viral RNA and *GFP* RNA from this control virus vector was only detectable in those leaves of the tobacco plants that had been inoculated with the virus construct, and not in any other leaves of the plants. This is because the lack of viral coat protein prevented intercellular and systemic movement of the viral RNA and associated *GFP* RNA. In plants inoculated with the virus vector expressing the *Arabidopsis FT* RNA, however, both virus RNA and *GFP-FT* RNA were detected in inoculated leaves, and in systemic young leaves of the tobacco plant which had grown after the viral inoculation had taken place (and thus the presence of viral derived RNA in these samples could not be due to accidental

cross-contamination during inoculation; Li et al., 2009). In addition to the full genomic RNA, the PVX virus expresses two sub-genomic RNAs from internal promoters in its genome, which because the *GFP-FT* sequence replaced the coat protein gene at the 3′ end of the viral genome, both contain the *GFP-FT* RNA sequence and thus were mobile and detectable in systemic leaves. Thus the addition of the *FT* RNA sequence to the *GFP* RNA sequence in the viral RNA genome/sub-genomes enabled it to overcome the movement deficiency caused by the absence of the coat protein gene.

Expression of *FT* RNA from a PVX viral vector with a functional coat protein resulted in the production of *Arabidopsis* FT protein throughout the plant, and the presence of this FT protein was able to induce flowering in the short-day (SD) requiring Maryland Mammoth tobacco plants even in non-inducing long day (LD) photoperiods. To show that the movement of the *FT* RNA was a property of the RNA itself and not due to the phloem-mobile FT protein that was being produced from the viral construct, another viral construct was tested that expressed a mutated *FT* gene. The mutation converted the ATG start codon to a stop codon thus preventing translation of FT protein from this mutant *FT* (*mFT*) RNA. Plants inoculated with virus constructs expressing *mFT* were not induced to flower in non-inducing LD photoperiods and remained vegetative the same as mock inoculated control plants. Interestingly, movement of the *mFT* associated RNAs was unaffected and they were still detectable in systemic leaves even without FT protein production (Li et al., 2009). To show that this movement ability was not due to any associated PVX viral sequences, a different viral vector (Turnip Crinkle Virus, TCV) was used to express *GFP*, *GFP-FT*, or *GFP-mFT* in *Arabidopsis*. Confirming previous results, only viral RNAs associated with the *FT* and *mFT* sequences were able to move systemically though the *Arabidopsis* plants from inoculated leaves
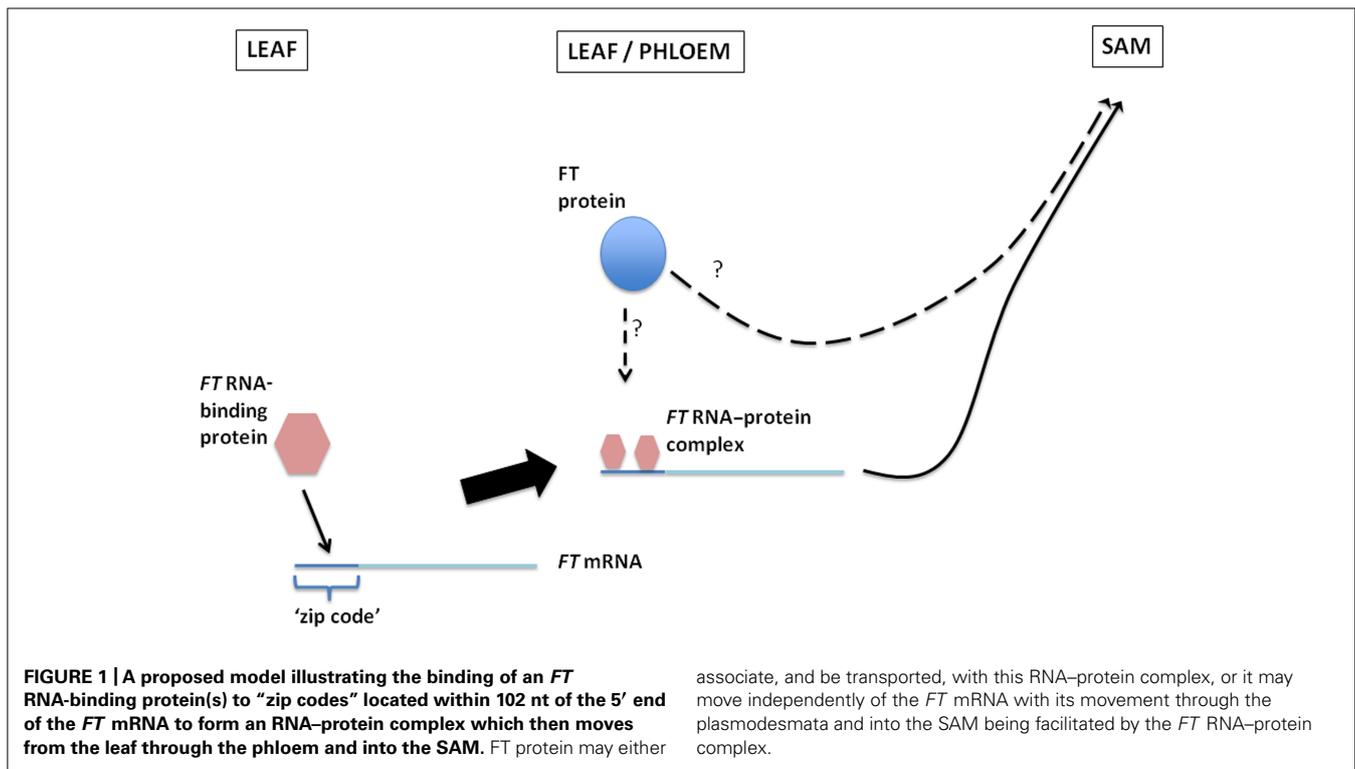
to non-inoculated newly formed systemic leaves. Furthermore, *FT* mRNA was shown to be able to move in the complete absence of any viral RNA. This was demonstrated in a transient assay where tobacco leaves were infiltrated with *Agrobacterium* that were expressing *GFP-FT* or *GFP-mFT* fusion constructs. Both RNAs were subsequently detected in both infiltrated, and non-infiltrated systemic leaves showing that the *FT* and *mFT* RNA could move through the plant independently of any viral RNA sequences (Li et al., 2009).

If *FT* mRNA movement is involved in the induction of flowering then the *FT* mRNA must be able to move into the SAM. Evidence that this is the case was obtained from *in situ* immune-detection and RNA silencing assays which showed that viruses expressing *FT* or *mFT* were able to enter the SAM, unlike viruses expressing *GFP* alone which were prevented from entering the SAM by the normal meristem exclusion process (Li et al., 2011). Thus *FT* mRNA is able to overcome the selective meristem exclusion system that plants have evolved to prevent entry of viral and other endogenous RNAs into the SAM. As both *mFT* RNA as well as *FT* RNA are able to direct entry into the SAM it shows that the FT protein is not involved in overcoming meristem exclusion. Indeed, whilst the mechanism by which *FT* and *mFT* RNA overcome meristem exclusion is not yet understood, it may be that this function of the *FT* mRNA is necessary in order for the FT protein to enter the SAM to induce flowering and thus that *FT* mRNA may be a necessary component of the florigen signal. This is a hypothesis that deserves further examination and we will consider the arguments both for and against in more detail.

In rice, both the Hd3a protein and low levels of *Hd3a* mRNA have been detected in the SAM, although in *Arabidopsis* only FT protein and no *FT* mRNA was detected in the SAM (Corbesier et al., 2007; Tamaki et al., 2007), this difference may be explained by differences in the sensitivity of the methods of detection used in these analyses. There have been elegant experiments designed to try to uncouple the effects of *FT* mRNA and protein on floral induction (Jaeger and Wigge, 2007; Mathieu et al., 2007; Notaguchi et al., 2008). In these cases movement of FT protein, but not *FT* mRNA, was inhibited by either attachment of a nuclear localization signal and/or fusion of multiple YFP proteins. As these prevented movement of the FT protein out of phloem companion cells into the SAM then flowering was inhibited demonstrating that movement of the FT protein into the SAM is required to induce flowering. In those cases where movement of FT protein out of the phloem is completely prevented then any effect of *FT* mRNA in facilitating entry into the SAM would not be observed. In other experiments the levels of *FT* mRNA in different parts of the plant where reduced through the expression of artificial microRNAs against *FT* mRNA (*amiR-FT*). Expression of *amiR-FT* throughout the plant, or in phloem companion cells, inhibited flowering presumably because it prevented production of FT protein, whereas expression of *amiR-FT* in just the SAM did not inhibit flowering indicating that *FT* mRNA does not play any role within the SAM itself (Mathieu et al., 2007). However, this is not inconsistent with the hypothesis that *FT* mRNA may be involved in facilitating movement of the FT protein into the SAM as *FT* mRNA does not have to be acting inside the SAM to have this effect.

Evidence that *FT* mRNA itself has an effect in promoting flowering is difficult to obtain because active FT protein needs to be present in order for flowering to occur and it is hard to distinguish what contribution to the induction of flowering is made by the *FT* mRNA alone. However it has been successfully demonstrated that *FT* mRNA does have a promotive effect on floral induction using tobacco plants that are induced to flower. These plants were Maryland Mammoth tobacco plants that were grown in SD inducing photoperiods, and were thus producing endogenous FT protein which would move to the SAM to induce flowering. Some of these plants were inoculated with a virus expressing the *mFT* RNA (hence no further FT protein was produced), and this promoted earlier flowering over the mock inoculated controls which flowered at the normal time in SD (Li et al., 2011). This demonstrates that *FT* mRNA (or *mFT* RNA) itself is able to enhance the induction of flowering by the FT protein which was being produced endogenously in the plant in SD conditions.

What enables *FT* mRNA to move and have its effect on flowering time? It is apparent that some feature of the *FT* mRNA not only enables this RNA to move, and confers this on associated RNA molecules (e.g., *FT-GFP* RNA moves but *GFP* RNA alone does not), but it may also enhance the movement (or activity) of the FT protein. A deletion analysis of the *FT* mRNA showed that a short 102 nucleotide sequence at the 5′ end of the *FT* mRNA was all that was necessary to direct systemic RNA movement, all *FT* RNA sequences containing this 102 nucleotide domain were able to move within the plant whereas *FT* RNAs lacking this domain were unable to move (Li et al., 2009). Further deletion analysis of this 102 nucleotide domain indicated that there may be more than one sequence in this domain involved in promoting *FT* RNA movement (Li, unpublished results), these cis-acting sequences may be "zip code" sequences that Lucas et al. (2001) proposed may interact with zip code binding proteins to form RNA–protein complexes that are able to be transported through plasmodesmata. It has been well established that such zip codes in the 3′ UTR are important for intracellular trafficking of mRNA molecules such as β-actin (St Johnston, 2005), it is now apparent that similar sequence motifs are also involved in the intercellular movement of mRNA molecules. Viruses have specific sequences that promote viral RNA movement (Wang and Ding, 2010), and this has also been shown to be the case for endogenous plant mRNAs such as *GIBBERELLIC ACID INSENSITIVE* (*GAI*) mRNA which has specific motifs that enable it to move systemically throughout the *Arabidopsis* plant (Huang and Yu, 2009). In pumpkin (*Cucurbita maxima*), the *GAI* mRNA forms an RNA–protein complex with about 17 proteins present in the phloem sap one of which is RBP50, a phloem-mobile polypyrimidine tract binding (PTB) protein that selectively binds the *GAI* mRNA (Ham et al., 2009). It is not known if *FT* mRNA binds to PTB proteins, however both the *FT* mRNA and the 102 nucleotide movement sequence were found to bind specifically to a protein(s) of around 20 kDa in *Arabidopsis* and 24 kDa in tobacco in protein extracts made from vegetative tobacco plants (Li et al., unpublished). No binding was detected to proteins extracted from leaves of flowering *Arabidopsis* or tobacco plants indicating that the expression of these *FT* mRNA-binding proteins might be developmentally regulated, being switched off once floral induction has

FIGURE 1 | A proposed model illustrating the binding of an *FT* RNA-binding protein(s) to "zip codes" located within 102 nt of the 5′ end of the *FT* mRNA to form an RNA–protein complex which then moves from the leaf through the phloem and into the SAM. FT protein may either associate, and be transported, with this RNA–protein complex, or it may move independently of the *FT* mRNA with its movement through the plasmodesmata and into the SAM being facilitated by the *FT* RNA–protein complex.

been initiated. This could potentially add another layer of control in the regulation of flowering.

Notaguchi et al. (2008) argued that the *FT* mRNA sequence is not important for the long distance promotion of flowering by the FT protein. This is based on experiments where the sequence of the *FT* mRNA but not the protein was altered by synonymous substitutions in 171 of the 175 codons, and replacement of the 5′ and 3′ untranslated regions, to create a synonymous *FT* (*synFT*) mRNA which shared only 63% identity to the ORFs in the original *FT* sequence. All these changes did not affect the long distance and graft-transmissible ability of the FT protein to induce flowering. As the specific protein binding sites in the *FT* mRNA sequence (specifically within the 102 nucleotide domain) have not yet been identified, it is not yet known which nucleotides are essential for binding and which ones can be varied without affecting binding. What has been demonstrated is that a non-translatable *FT* mRNA (*mFT*) is capable of enhancing the promotion of flowering by an endogenous FT protein, that a 102 nucleotide sequence at the 5′ end of the *FT* mRNA binds a specific protein(s), and that deletion of this 102 nucleotide sequence prevents movement of the *FT* mRNA. This raises the possibility that protein binding to the 102 nucleotide region of the *FT* mRNA may be necessary for *FT* mRNA

movement and the resulting enhancement of floral induction by the FT protein (**Figure 1**).

The systemic movement of RNA molecules is tightly regulated, not all RNA molecules can move (e.g., GFP mRNA) although many do as thousands of mRNA transcripts have been detected in the phloem of *Arabidopsis* (Deeken et al., 2008). Of those viral and cellular-derived RNA molecules that are present in the phloem only a few are able to enter into the meristem, these include the mRNAs of *FT*, *GAI*, *CmNACP*, and *LeT6* a tomato KNOX gene (Ruiz-Medrano et al., 1999; Kim et al., 2001; Haywood et al., 2005; Li et al., 2011). Viral RNAs are prevented from entering the meristem by a selective surveillance mechanism (Foster et al., 2002) which involves RNA silencing as well as other mechanisms such as selective transport through plasmodesmata mediated by proteins with trafficking signal domains (Kim et al., 2005; Qu et al., 2005; Schwach et al., 2005). It is known that *FT* mRNA is able to enter the meristem. It remains to be determined whether the protein(s) that bind to the first 102 nucleotides of the *FT* mRNA have trafficking signals that enable it to do this and whether they also facilitate the entry of FT protein, perhaps as part of a RNA–protein complex, into the meristem to promote flowering at the same time.

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