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·Minireview·

Florigen: One Found, More to Follow?

Xuhong Yu, John Klejnot and Chentao Lin*

(*Department of Molecular, Cell and Developmental Biology, University of California,* Los Angeles, CA 90095-1606, USA)

Abstract

Florigen(s) are molecules that are synthesized in response to appropriate photoperiods and transmitted from leaves to shoot apices to promote floral initiation. It has been recently discovered in Arabidopsis that mRNA of the *FT* gene acts as a florigen. In Arabidopsis, cryptochromes and phytochromes mediate long-day promotion of CO protein expression, which activates *FT* mRNA expression in leaves. *FT* mRNA is transmitted to the shoot apex, where it acts together with FD to activate transcription of floral meristem identity genes, resulting in floral initiation. The discovery of the molecular nature of a florigen was a major scientific breakthrough in 2005.

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

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Plants characteristically flower during a particular time of the year, in response to the most predictable seasonal change on earth, photoperiod. Long-day (LD) plants, such as Arabidopsis, garden pea, and wheat, tend to flower from late spring to early summer as the days lengthen, whereas short-day (SD) plants, such as rice, soybean, and corn, prefer to flower in the autumn (fall), when nights become longer. This daylength-sensing phenomenon or photoperiodism has fascinated biologists for almost a century (Garner and Allard 1920; Zeevaart 1976; Bernier et al. 1993; Hayama and Coupland 2004).

The major question concerning photoperiodic flowering is the molecular nature by which the photoperiodic signals are perceived and executed to eventually trigger or suppress flowering. We now know that phytochromes and cryptochromes are the photoreceptors that perceive photoperiodic signals in plants (Lin 2000). It was also found as early as in the 1930s that photoperiodic signals can induce synthesis of molecule(s) in leaves that are then transmitted to the shoot apex to promote floral initiation (Knott 1934; Chailakhyan 1936). Those photoperiod-responsive, inter-organ transmittable, and flowering-stimulating molecules are referred to as florigen(s) (Chailakhyan 1936; Zeevaart 1976; Bernier et al. 1993; Thomas and Vince-Prue 1997). It may be familiar to most students of plant physiology that leaves of an SD plant, such as *Perilla crispa*, grown under inductive SD conditions can be grafted onto a plant grown under non-inductive LD conditions to promote floral initiation of the latter.

The molecular nature of florigen(s) has remained a mystery for the past 70 some years, despite extensive investigations (Zeevaart 1976). This mystery has been recently solved, at least in part, by three reports published in two consecutive issues of the journal *Science* (Abe et al. 2005; Huang et al. 2005; Wigge et al. 2005). The first two papers, by Detlef Weigel's laboratory at the Salk Institute and Max Planck Institute, and Takashi Araki's laboratories at Kyoto University, reported identification and characterization of the *FD* gene, one of the flowering-time loci discovered by Maarten Koornneef more than 15 years ago (Koornneef et al. 1991). Using different approaches, these two groups arrived at the same conclusion, namely that FD, a bZIP transcription factor preferentially expressed in the shoot apex, interacts with FT, which is expressed primarily in leaves in response to photoperiodic signals to promote flowering. The third report, by Ove Nilsson's group at the Swedish University of Agriculture Sciences, demonstrated that mRNA of the *FT* gene moves from the leaf to the shoot apex (Huang et al. 2005), thus closing the remaining loop of a florigen mystery. Identification of the molecular nature of a florigen is regarded by the journal *Science* as one of the 10 scientific breakthroughs in 2005.

By the early 21st century, control of flowering time has become one of the best understood developmental processes in

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*Author for correspondence. E-mail: <clin@mcdb.ucla.edu>.

plants. Several major players in the photoperiodic-sensing pathway have been identified and their roles in photoperiodic flowering elucidated (Yanovsky and Kay 2003; Komeda 2004; Searle and Coupland 2004). Two of those genes are *CO*, which encodes a B-box zinc finger protein, and *FT* which encodes a RAF-kinase inhibitor-like protein (Putterill et al. 1995; Kardailsky et al. 1999; Kobayashi et al. 1999). It is likely that CO is a transcriptional regulator, although it may not directly bind to DNA (Hepworth et al. 2002). The *CO* gene activates the expression of *FT* and both are positive regulators of flowering (Kardailsky et al. 1999; Kobayashi et al. 1999; Samach et al. 2000). Plants impaired in the *CO* or *FT* gene flower later in LD, whereas plants overexpressing *CO* or *FT* flower early, regardless of daylength. The mRNA expression of *CO* is controlled by the circadian clock in a photoperiod-dependent manner. The *CO* mRNA level peaks only in the night in SD, but remains high at both dawn and dusk in LD. On the other hand, the CO protein undergoes ubiquitin/proteosome-dependent degradation in early morning or in the dark, but CO degradation is suppressed by the action of photoreceptors phyA and cry2 in the daytime. Therefore, LD-grown plants that express high levels of *CO* mRNA in the daytime can accumulate relatively higher levels of CO protein, whereas SD-grown plants that express high levels of *CO* mRNA primarily in the night accumulate little CO protein (Suarez-Lopez et al. 2001; Yanovsky and Kay 2002; Valverde et al. 2004; Figure 1). These observations have answered, at least in part, the first question in the florigen hypothesis: which molecule is responsive to photoperiodic signals and responsible for the stimulation of flowering?

The CO protein itself was once suspected to be a florigen, because CO acts non-cell autonomously and CO activity is graft transmittable (An et al. 2004; Ayre and Turgeon 2004). However, another observation, namely that CO expressed in phloem can activate *FT* expression and floral initiation whereas CO expressed in shoot apex cannot, suggests that CO is unlikely to be a florigen itself (An et al. 2004).

Could the CO-target gene, *FT*, be a candidate for a florigen? A study using DNA microarray analysis indicated that *FT* mRNA expression is extremely sensitive to the level of CO, as well as to photoperiodic signals (Wigge et al. 2005). Using yeast two-hybrid assay and bimolecular fluorescence complementation (BiFC) analyses, it was found that FT protein physically interacts with FD, a bZIP transcription factor (Abe et al. 2005; Wigge et al. 2005). The original *fd* mutant locus was cloned using the positional cloning method and several new *fd* alleles were identified as T-DNA insertion mutants. It was shown that the *fd* mutation is a strong suppressor of the early flowering phenotype caused by transgenic overexpression of *FT*. Consistent with this result, the *ftfd* double mutant showed synergistic delay of floral initiation. The *FT* and *FD* genes act on the same target genes, including the floral meristem identity gene *AP1*. The *FD* gene can activate *AP1* expression, but only in the presence of *FT*. In addition, an FD-responsive promoter region of the *AP1* gene was identified using reporter gene and chromatin-immunoprecipitation assays. Taken together, these results establish that FT and FD interact to activate floral meristem identity genes required for floral initiation in the shoot apex. However, in contrast with *FD*, which is expressed mainly in the shoot apex, *FT* is expressed primarily in cotyledons and hypocotyls and not in shoot apex of 6-day-old seedlings grown in LD (Kobayashi et al. 1999). Because 7-day-old Arabidopsis seedlings grown in LD are already committed to flower (Bradley et al. 1997; Mockler et al. 1999), how could FT and FD proteins, which are spatially separated at about this time, meet at the shoot apex to promote floral initiation? In other words, can *FT* mRNA or protein act as a florigen, moving up from the leaves to the shoot apex to activate *FD*? The authors of both papers raised this intriguing question (Abe et al. 2005; Wigge et al. 2005).

This question leads to the third paper of our discussion (Huang et al. 2005). In this report, Tao Huang and colleagues specifically tested the hypothesis that *FT* may act as a florigen, using a series of elegantly designed experiments. They prepared transgenic Arabidopsis lines that allowed the expression of *FT* only in the leaves and followed closely the consequences of the localized *FT* expression in the leaves. Localized *FT* expression was accomplished by transgenic expression of the *Hsp : FT* transgene, for which transcription of *FT* is driven by a heat-inducible promoter. Controls for this experiment included a glucuronidase (GUS) reporter gene driven by either the heat-inducible promoter (*Hsp : GUS*) or the *FT* promoter (*FT : GUS*). It was shown that when a single leaf from a plant was placed on a copper plate connected to a water bath heated to 37 °C, the *Hsp : GUS* transcripts could be detected in the heated leaf but not in other parts of the plant. Heat induction of *Hsp : FT* in leaves could cause accelerated flowering and complementation of the *ft* mutation, confirming that heat-induced *FT* in leaves can activate floral initiation in the shoot apex. However, this result may be interpreted as either that *Hsp : FT* expressed in the leaf migrates to the shoot apex to promote flowering or that *Hsp : FT* expressed in the leaf triggers a synthesis of an unknown molecule migrating to the shoot apex to promote flowering. These two possibilities were discriminated by following levels of the transgenic *FT* mRNA or the control GUS mRNA in leaves and apices.

It was found that the transgenic *FT* mRNA derived from the *Hsp : FT* transgene and the GUS mRNA derived from the *Hsp : GUS* transgene started to accumulate in the heat-treated leaves almost immediately after heat treatment and that peak levels were detected approximately 1 h after the heat treatment. Importantly, the transgenic *FT* mRNA, but not the reporter GUS mRNA, also started to appear in the shoot apex approximately 3 h after heat treatment. Approximately 24 h after heat treatment, the *FT* mRNA peaks in the apex. Because the transgenic *FT*

mRNA was not detected in the apex in the absence of heat treatment of leaf and the promoter of *Hsp : FT* is unlikely to be regulated by the FT protein, the transgenic *FT* mRNA in the shoot apex must come from the heat-treated leaf. That is, the transgenic *FT* mRNA must migrate from heat-treated leaf to the untreated shoot apex. Based on the distance between the heated leaf and unheated apex and the time difference between the peaks of *Hsp : FT* mRNA in the heated leaf and unheated apex, the speed of *FT* mRNA transmission was calculated to be approximately 1.2–3.5 mm/h. The velocity of *FT* mRNA transportation correlates well with the speed of 2.4–3.5 mm/h estimated previously for a florigen.

Interestingly, transcription of the endogenous *FT* gene is apparently activated by the *Hsp : FT* transgene products, because plants expressing *Hsp : FT* started to also accumulate the endogenous *FT* mRNA in both leaf and apex after heat treatment of leaves. This was confirmed by detection of the GUS mRNA in both the apex and the heat-treated leaf in a transgenic plant co-expressing *Hsp : FT* and *FT : GUS*. This autoregulatory activity of FT may explain a phenomenon observed many decades ago, namely that the graft-transmittable florigen molecules appeared capable of "self-renewal" during repeated grafting experiments without losing their ability to promote flowering (Zeevaart 1976).

Is the *FT* mRNA the only florigen? It remains unclear, but chances are that there may be more than one florigen in plants, or even in Arabidopsis. First, despite the fact that *FT* is known to act as a floral activator in dicot Arabidopsis and in monocot

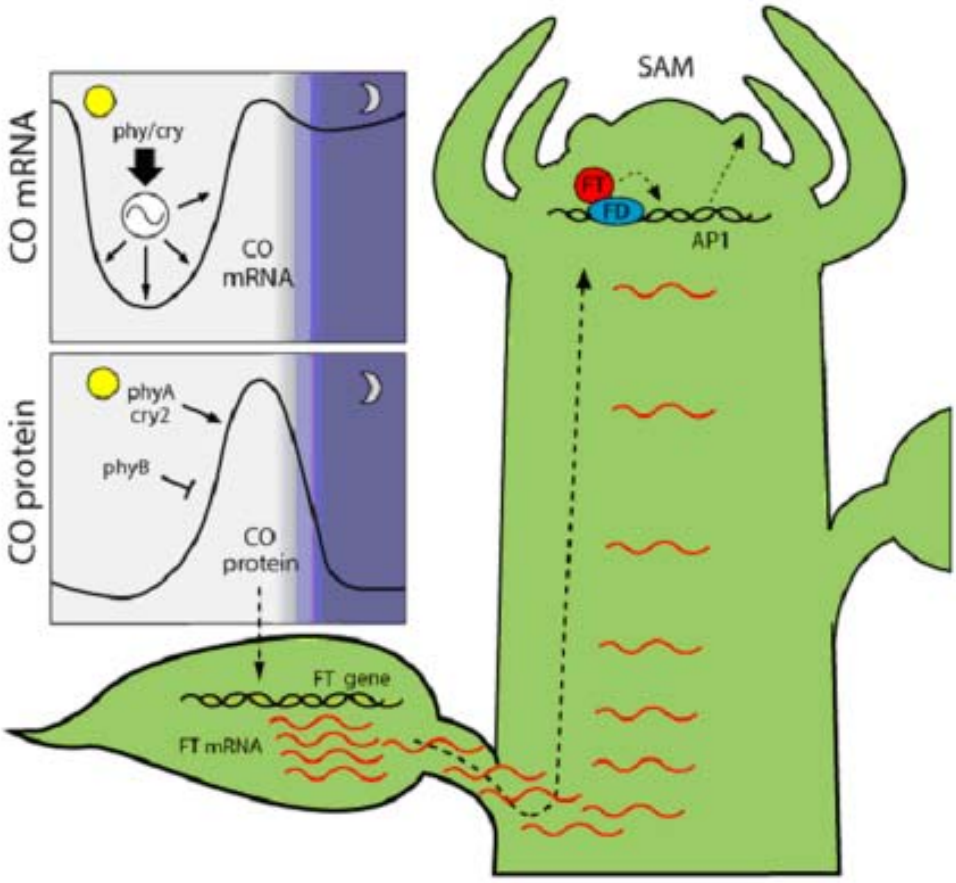

Figure 1. Movement of *FT* mRNA from leaf to shoot induces flowering.

FT mRNA expression is up-regulated in leaves by the CO protein, which accumulates to significant levels only under 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). *FT* mRNA produced in the leaves travels to the shoot apical meristem (SAM) or apex where it is translated. FT protein interacts with FD at the promoters of floral meristem identity genes such as AP1 to induce expression and initiate the floral transition.

rice (Hayama et al. 2003), other plants may still use different strategies to regulate photoperiodic flowering. For example, the *id1* gene of corn encodes a zinc finger protein that acts as a positive regulator of floral transition in a non cell-autonomous manner, similar to that of *CO* in Arabidopsis. It is possible that *id1* target genes may act as florigens, just like the *CO* target gene *FT*, but it is not clear what the *id1* target genes are (Colasanti et al. 1998). Second, the fact that the *ftfd* double mutant is only incompletely insensitive to photoperiods suggests the involvement of additional genes in the process. Indeed, it is known that *CO* activates floral initiation via not only the FT-dependent pathway, but also via an FT-independent pathway (An et al. 2004). Third, *FT* mRNA is apparently not the only one that can travel from leaves to shoot via the vascular system to affect floral initiation at shoot apex. For example, it has been reported recently that the mRNA of the *GAI* gene, which is critical for gibberellin (GA) signal transduction, also undergoes long-distance trafficking (Haywood et al. 2005). GA is a major regulator of flowering time, especially in SD. It has been proposed that certain 2-oxidase-resistant isoforms of GA may travel to the shoot apex to affect flowering (King and Evans 2003). Therefore, it remains a formal possibility that GA or its signaling molecules may act as additional florigens. Finally, in addition to mRNAs, various small RNAs, including miRNAs and siRNAs, have been detected in the phloem sap of pumpkin and other plants (Yoo et al. 2004). The miRNAs are known to regulate various developmental processes in plants, whereas siRNA-mediated gene silencing has been associated with systemic signaling (Voinnet and Baulcombe 1997; Baulcombe 2004; Dugas and Bartel 2004). Interestingly, at least one of the miRNAs detected in the phloem sap of pumpkin, miR159, has been shown to affect GA signal transduction and photoperiodic flowering (Achard et al. 2004; Yoo et al. 2004). It remains to be seen whether any of the plant small RNAs may act as a florigen.

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